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Blaxter M. 1976. Social class and health inequalities. In: Carter C and Peel J, editors. **Equalities and Inequalities in Health**. London: Academic Press, pp. 369-80.

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Evaluation Anti-hyperglycemic and antihyperlipidaemic activities of *Andrographis lineata* Nees on Streptozotocin induced diabetic Rats

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Abstract

The present study was performed to find out the antihyperglycemic and antihyperlipidaemic effects of methanol and aqueous extracts of *Andrographis lineata* (Acanthaceae) in normal and streptozotocin (STZ) induced diabetic rats. Diabetes was induced by injecting streptozotocin (STZ, 50 mg/kg) intraperitoneally in adult male albino Wistar rats. The methanol and aqueous extracts of *A. lineate* as well as the standard antidiabetic drug Glibenclamide were administered orally to different group's diabetic rats once a day for fifteen days in the dosages of 400 mg/kg b.wt of individual extracts and 500 µg/kg b.wt of standard drug glibenclamide, respectively. Blood glucose levels in all the rats (Both normal and diabetic) of different groups were determined on the 1st, 4th, 7th, 10th and 15th days after standard and sample drugs administration. The serum lipid profile like total cholesterol (TC), triglycerides (TG), phospholipids (PL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) were also determined in all the rats administration. Both extracts exhibited significant reduction in BGL as well as TC, LDL, VLDL and an increase in HDL in diabetic rats when compared to the standard drugs. The above results indicate that the plant is capable of ameliorating hyperglycemia in STZ induced diabetic rats. Hence this plant may be a potential source for the isolation of new orally active agent(s) for diabetic mellitus. The present investigation established pharmacological evidence to support the folklore claim of this plant being used as an antidiabetic.

Keywords: *Andrographis lineata*, Glibenclamide, Hyperglycemia, Streptozotocin.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 10% of the global population. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major causes of morbidity and death (Bennet and Joslin's, 1998). Currently, the available therapy for diabetes includes insulin and various oral anti-diabetic agents such as sulfonylureas, metformin, etc. These drugs are used as monotherapy or in combination to achieve better glycemia control. Each of the above oral antidiabetic agents suffers of a number of serious adverse effects (Moller, 2001). Plants have played a major role in the introduction of new therapeutic agents. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin (Aiman, 1970). Despite the considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times, there has been a renewed interest in the plant remedies (Dinesh puri and Mohapatra, 1997; Ratnakar and Murthy 1996).

Andrographis lineata Nees (Fam. Acanthaceae) is a small plant found in and around Salem district, Tamil Nadu, India. All parts of this plant are medicinally important in the traditional system of medicine in India and have been used extensively in snake bite and as anti-pyretic (Alagesaboopathi, 1999). It is also used as blood purifier and also in veterinary medicine. Three Flavonoids were isolated from the leaf extract (Hari kishore *et al.*, 2003). Leaves are used as hepatoprotective (Sangameswaran *et al.*, 2007) and they exhibit diuretic activity (Sangameswaran *et al*., 2007).

In present work, the plant selected is locally available in Salem district and has been used for a long time in local folklore medicine for the treatment of diabetes. Since not much study had been done to evaluate the pharmacological activity of this plant, the present study is focused on evaluating the anti-diabetic activity of the leaves of *A. lineata.*

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2. Materials and Methods

2.1. Plant material

Leaves of *A. lineata* were collected from foot hill of Yercaud, Salem, Tamil Nadu, India and were authenticated by Dr P. Jayaraman, Director of Plant anatomy Research Centre, Chennai, Tamil Nadu, India. Voucher specimens (AL/088) were deposited at our College Museum for future reference.

2.2. Preparation of the extract

The powdered material (500g) of leaves of *A. lineata* was extracted separately using methanol (1000ml) by Soxhlet technique and water by cold maceration (Evans, 1989). The extracts were dried under reduced pressure. The dried extracts were stored in desiccator and were subjected to further studies.

2.3. Preliminary phytochemical screening

The methanol and aqueous extracts were subjected to preliminary phytochemical screening to find out the presence of active constitution such as alkaloid, glycosides, flavonoids, tannins etc.

2.4. Animals

Male albino Wistar rats, 9-12 weeks old with average weight of 150-180 g were purchased from M/S Venkateshwara enterprises (P) Ltd, Bangalore and used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by Institutional Animal Ethical Committee (No: P.Cog-12/07).

2.5. Acute toxicity studies

The animals were divided into five groups separately and were treated orally with aqueous and methanol extracts of *A. leneata* at 100mg, 200, 300 400 and 500 mg/kg, body weight doses. The animals were continuously observed for 1 hr., then frequently for 12 days. The animals were observed continuously for the initial 4 h and intermittently for the next six h and then again at 24 h and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate, and convulsion

2.6. Glucose tolerance test

Overnight fasted rats were divided into 4 groups. First group was kept as normal control which received 5% Tween 80 (0.5 ml, p.o), second group received standard drug Glibenclamide (500 µg/kg) third and fourth groups received methanol and aqueous extracts of *A. lineata* 400 mg/kg, respectively. The rats of all the groups were loaded with glucose (3 g/kg, p.o) after the administration of the sample: blood samples were collected at 0, 30, 60 and 120 min after the glucose loading.

2.7. Streptozotocin-induced diabetic rats

Streptozotocin (STZ), purchased from Sigma Aldrich chemical Co., Bangalore, was dissolved in ice-cold normal saline immediately before use. Diabetes was induced in rats by intraperitoneal (i.p) injection of streptozotocin at a dose of 50 mg/kg (Pulok K Mukarjee, 2002). Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels were determined to confirm the on set of diabetes. The diabetic rats that exhibited blood glucose levels higher than 300 mg/dL were selected for the study. The rats were divided into 4 groups as follows: the first group served as normal control, received food and water; the second group served as (Group II to IV) diabetic control, received 0.5 ml of 5% Tween 80; the third group received glibenclamide (500 µg/kg); and the fourth and fifth groups received 400 mg/kg of methanol and aqueous extracts of *A. lineata*, respectively. The treatment was continued daily for 15 days. Blood drop was collected from the tail for glucose estimation, just before drug administration on 1st day and 1 h after sample administration on days 4, 7, 10 and 15 (Table 1).

Table 1. Effect of extracts of *A. lineata* and glibenclamide on oral glucose tolerance test

Treatment	Changes in blood Glucose levels (mg/dl)			
	0 min	30 min	60 min	120 min
Normal	68.42 ± 3.88	65.82 ± 3.20	66.52 ± 1.20	68.20 ± 0.20
Glibenclamide 500 µg/kg	73.50 ± 1.66	74.82 ± 0.82*	72.20 ± 3.98**	85.50 ± 0.82**
Methanol extract 400 mg/kg	68.46 ± 2.66	81.82 ± 1.42*	70.48 ± 1.32**	64.52 ± 1.20**
Aqueous extract 400 mg/kg	69.82 ± 2.30	77.63 ± 0.32	62.82 ± 0.2**	61.48 ± 0.82**

Values are mean ±SEM, n= 6. When compared with diabetic control *= p<0.05, **p<0.01
(One way ANNOVA Followed by Dunnette multiple comparison tests).

2.8. Anti-hyperlipidaemic activity

At the end of the experiment, the animals from each group were sacrificed by cervical dislocation for biochemical and histological studies. Blood was collected from the heart and allowed to clot and the serum was separated by centrifuged at 3500 rpm for 10 minutes. Serum was assayed either immediately or stored at -20^0 C. The tissue like pancreas was collected and used for histological studies.

Serum samples were analyzed spectrophotometrically for triglycerides, total cholesterol, high density lipoprotein (HDL-C), using their respective kits UV- visible spectrophotometer (Shimadzu-1601, Japan), VLDL-C and LDL-C were calculated as per Friedwald's equation (Richterich, 1981).

VLDL was calculated using the formula, $VLDL = \frac{\text{Triglycerides}}{5}$

LDL cholesterol was calculated as

$LDL = \text{Total Cholesterol} - HDL - \frac{\text{Triglycrides)}}{5}$.

2.9. Estimation of biochemical parameters

Serum lipid profiles like low density lipids (LDL), very low density lipids, high density lipids, triglycerides, and total cholesterol were determined standard procedures in an auto analyzer using Ecolin kits (E. Merck, Mumbai, India).

2.10. Statistical evaluation

All the data are presented as mean ±SEM. The differences between group were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. P<0.01 was considered to be significant.

3. Results and Discussion

3.1. Preliminary chemical test

Our phytochemical studies indicated that methanol and aqueous extracts of leaves of *A. lineata* contain alkaloids, flavanoids, glycosides, saponins, terpenes and steroids.

3.2. Toxicity studies

In performing preliminary test for pharmacological activity in rats, aqueous and methanol extracts did not produce any significant changes in the behavioral or neurological responses up to 5000 mg/kg b. wt. Toxicity studies revealed the non-toxic nature of the aqueous and ethanol extracts of *A. lineata*. The result obtained from the LD_{50} study indicates that both methanol and aqueous extracts of leaves of *A. lineata* are safer to use in animals even at a dose of 500 mg/kg p.o.

3.3. Oral glucose tolerance test

Effect of methanol and aqueous extracts of *A. linetata* (each 400 mg/kg) and glibenclamide (500µg/kg) on glucose tolerance has been shown in Table 1. At 30 min after glucose administration, the blood glucose concentration increased rapidly from the fasting value and then attains nearly the same value at the end of the study.

3.4. Antihyperglycemic activity

The effects of extracts of *A. lineata* on blood glucose levels in normal and diabetic rats are reported in Table 2. Blood glucose level of the diabetic rats was significantly higher than those in normal rats. A significant decrease in blood glucose levels was observed in the rats treated with methanol extract of *A. lineata* from an initial level of 366 to 192 mg/dl. The present experiment was conducted to study the anti-diabetic effect of *A. lineata* in normal as well as streptozotocin induced diabetic rats.

Table 2. Anti-hyperglycemic activity of extracts of *A. lineata* on STZ induced diabetic rats

Treatment/ Dose	Changes in blood glucose level in mg/dl				
	1st Day	4th Day	7th Day	10th Day	15th Day
Normal control	96.50 ± 2.88	97.24 ± 2.24	96.00±2.62	96.08±2.42	96.60±2.81
Diabetic control	376.72 ± 0.25	380.62±1.66	386.00±1.24	390.16±1.42	396.22 ± 1.2
Glibenclamide 500 µg/kg	363.70±0.16	322.82±0.12	280.64± 0.42	242.00± 0.26	127.60±0.14**
Methanol extract 400 mg/kg	362.71±0.26	338.82±0.46	296.48±0.42	258.22±0.22	222.10±0.22**
Aqueous extract 400 mg/kg	366.5 ± 0.20	325.62±0.44	288.46± 0.48	246.00± 0.24	192.1 ± 0.10**

The values are mean ±SEM, n=6, When compared with diabetic control

** = p<0.001, (One way ANOVA followed by Dunnett's, multiple comparison test.

In group II (Diabetic control), the BGL significantly increased from 376.72 ± 0.25 to 396.22 ± 1.2 mg/kg. Methanol and aqueous extracts (400 mg/kg) treatment (Group IV and V) showed decreased blood glucose levels significantly from 362.71±0.26 to 222.10±0.22 and 366.5 ± 0.20 to 192.1 ± 0.10 mg/dl, where as in glibenclamide standard drug (500 µg/kg) treated diabetic rats (Group III), the BGL significantly decreased from 363.70±0.16 to 127.60±0.14 mg/dl, respectively.

3.5. Anti-hyperlipidaemic activity

The lipid profiles in the experimental rats are depicted in Table 3. In STZ induced diabetic rats, there was a significant (P<0.001) increase of total cholesterol, triglycerides, phospholipids, and low density lipoproteins (LDL) and very low density lipoprotein (VLDL) cholesterol and significant (p<0.001) decrease in high density lipoprotein (HDL) cholesterol in serum when compared with normal control. The extracts treated rats were significantly (p<0.001) decreased the total cholesterol, triglycerides, phospholipids and LDL and VLDL cholesterol and significantly (p<0.001) increased HDL cholesterol.

Table 3. Anti-hyperlipidaemic effects of extracts of *A. lineata* on STZ induced diabetic rats.

Treatment (mg/kg body wt)	Changes in mg/dl					
	TC	TG	HDL	LDL	VLDL	PL
Normal 10 ml/kg p.o	84.5 ± 7.9	87.3 ± 7.5	23.8 ± 2.1	23.8 ± 2.1	13.3 ± 1.7	144.3 ± 7.7
Diabetic control	192.3 ± 11.6	169.3 ± 5.1	12.3 ± 3.1	69.7 ± 8.3	28.3 ± 1.1	250.8 ± 10.6
Glibenclamide 500 µg/kg	131.7 ± 9.2**	112.7 ± 2.8**	17.2 ± 1.0*	38.2 ± 4.5**	15.8 ± 2.0**	174.8 ± 7.6**
Methanolic extract 400	152.7 ± 10.4*	125.0 ± 4.6**	16.2 ± 0.9*	44.2 ± 6.7*	18.7 ± 2.1**	190.0 ± 10.4**
Aqueous extract 400	146.2 ± 9.5**	118.5 ± 3.2**	18.9 ± 0.9*	42.2 ± 5.3*	17.0 ± 2.0**	184.2 ± 8.4**

The values are mean ± SEM n= 6, when compared with diabetic control, * = p<0.05, ** = p<0.01 (One way ANOVA followed by Dunnestt's, multiple comparison tests)

The present experimental result indicated that methanol and aqueous extracts exhibited a potent blood glucose lowering properties in STZ diabetic rats. A further exploration of the bioactive molecule responsible for the activity is under investigation in our laboratory.

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Nutrients in Water and Sediments of King Talal Dam-Jordan.

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Abstract **الملخص**

Samples of water and sediments were collected from three sites along the main body of King Talal Dam (KTD) in the period between Spring 2007 and Winter 2008. The sites represent the middle point of KTD (site A), its deepest area (site B) and its outlet or intake point (site C). Samples were collected from surface and near-bottom water during four seasons within the study period, while the sediment samples were collected during dry (summer) and wet (winter) seasons within the same study period. In addition to temperature, dissolved oxygen (DO) and pH, water samples were analyzed for nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), dissolved inorganic phosphate-phosphorus; DIP (PO_4^-), total nitrogen (TN), and total phosphorus (TP) contents. Sediments samples were analyzed for grain size distribution, organic matter, total phosphorus (TP), and total nitrogen (TN). Nutrients in water fluctuated in space, depth and time. Nitrogen species concentrations were highest during spring, while higher phosphorus species concentrations were recorded during winter. However, nutrients showed significant differences between seasons and insignificant differences between sites and between depths. The concentrations of nutrients in the water were generally less than their concentrations in the sediments. Nutrients in sediments showed more or less similar general trend of spatial distribution, where maximum values occurred in site A and site B during winter, and most of the pollutants decreased towards the far end of the dam at its outlet or intake point (site C). The concentrations of nutrients were higher in the clay–silt size fraction (<63μm), which has higher organic matter contents compared to the larger size fractions. The results of the present study agree with and confirm the results of many previous studies which indicated that water and sediments of KTD are polluted due to many anthropogenic and natural sources. The results and its relationships with these sources are discussed in details.

Keywords: Nutrients, Water, Sediments King Talal Dam, Jordan.

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1. Introduction

As with most arid and semi-arid countries Jordan suffers from water shortages in domestic, agricultural and industrial demands. These shortages stem from the fact that the major supply is ground water, which is in turn dependent for its recharge, on the rates of rainfall, which vary from year to year (Stanley Consultants, 1978). The total area of Jordan is about 89,000 km², only 8.5% of this area receives more than 200 mm of annual rainfall (Rawajfih et al., 1988). The average annual rainfall ranges from 50 to 600 mm/year (Al-A´raj., 1997). The scarcity and uneven distribution of precipitation over Jordan results in limited surface and groundwater resources available for domestic, agricultural and industrial uses. Rapid population growth coupled with increased urbanization and industrialization have lead to the over-exploitation of aquifers and the contamination of diminishing supplies through inadequate industrial and municipal wastewater treatment capacities. Setting of industrial plants near or immediately upstream from potable supplies; and overuse and misuse of pesticides, insecticides, fungicides and fertilizers are among the factors that have lead to pollution of the limited ground and surface water resources (Al-Jayyousi and Shatanawi, 1995).

Realizing all these conditions, Jordan has built many dams of various storage capacities in order to store it for different purposes such as domestic, agriculture and industry uses and to control floods, improve drainage, collect water from rivers and streams. Among these artificial surface water bodies is King Talal Dam (KTD); it is an earth-fill dam that was constructed in the period 1972-1978. The total storage capacity of the dam is 85 million cubic meters (MCM). The main lake behind the dam is 7.50 kilometers long and 450 meters wide (area 33.75 km^2) (RSS reports, 1984-2005, Numayr, 1999). The dam which was constructed within the main course of Zarqa River at a discharge of 40 kilometers northwest of Amman, receives its major inflow as natural run-off from Zarqa River and major springs. The Zarqa River is the third largest river in the region in terms of its annual discharge and its waters are extensively used for irrigation and industrial needs. The area of the Zarqa River catchment is about 3468 Km² (RSS, 1981). Other inflow comes from small springs, and effluents from Al-Samra, Jerash and Baqa'a sewage treatment plants, and from industrial plants between Amman and Zarqa. The plants effluents contribute about 50% of the water reaching the dam where the average annual inflow is about 113 MCM (Bandel and Salameh, 1979, 1981; Salameh, 1991; RSS, 1984-2004; Numayr, 1999).

Wadi Rmemeen drainage which is composed basically from the wastewater from AlBaqa'a treatments plant in addition to some water springs that are spread all over the valley represents the second important source of water discharged into the dam (RSS, 2005). Khirbet Assamra effluent and the industrial wastewater discharged into the dam via Zarqa River has relatively high concentration of heavy metals, phosphorus and ammonia which have adversely affected the quality of water in King Talal Dam (Salameh, 1980; Sigg et al., 1987; Britton, 1999; Alawneh, 1997; Mahasneh and Soub, 1983; Hashwa, 1985; Al-Soub, 1981; Al-Jassabi and Khalil, 2006; RSS, 2005), and consequently limited the use of its water for irrigation purposes (Saqqar, 1994). It has been reported that the dam is suffering from sedimentation problems and the sediments entering the dam contain nutrients and heavy metals, and from the cyanobacteria *Microcystis aeruginosa* which forms extensive summer blooms in its water (Numayer, 1999).

Phosphorus (P) and nitrogen (N) are essential nutrients necessary for the growth of plants in water bodies. Of these two nutrients, phosphorus is most often considered to be the nutrients that regulate the production of algae in water bodies and is most amenable to control (Schindler, 1977).

Anthropogenic activities, including fertilizer application, in combination with channelization of streams in the agricultural areas, have lead to increased N and P loads carried by streams, with their concentrations in water bodies often exceeding drinking water standards (David et al., 1997) and thus contributed to a number of human health problems (Townsend et al., 2003), eutrophication of surface waters (Carpenter et al., 1998), and seasonal hypoxia in many regions (Goolsby et al., 2001). This undesirable process can lead to deterioration of water quality and significant economic repercussions (Cooke and Kennedy, 2001).

The growth and development of phytoplankton communities and consequently water bodies productivity depend on the recycling of nutrients already incorporated in living organisms, or the supply of new nutrients (Reynolds, 1997). In addition, nutrients may be adsorbed to the sediments accumulated on the bottom of rivers or lakes and other water bodies. These sediments can act as new nutrient sources to the overlying water (Masunaga et al., 1993; Abrams and Jarrell, 1995).

Many studies have been carried out on KTD environment during the past two and half decades. Most of these studies have focused on measuring the level of various pollutants in KTD water and sediments. However, little attention was devoted to the distribution and relationship of nutrients with other environmental parameters in water and sediments of the dam. Therefore, this study was initiated to fill a gap in the information on this relationship, to examine the factors affecting the environmental conditions of the dam, and to provide useful and usable information about nutrients and other pollution indicators in the dam environment.

2. Materials and Methods:

2.1. Sampling Sites:

Samples of water and sediments were collected from three sites along the main body of King Talal Dam (Fig. 1): Site (A) which is located at latitude N 32º 19' 001" and longitude E 35º 80' 887" represents a middle point in King Talal Dam, Site (B) is located at latitude N 32º 19' 025" and longitude E 35º 80' 519" and represents the deepest area in the main body of King Talal Dam, and Site (C) at latitude N 32º 19 193' and longitude E 35º 80 343' and represents the outlet of King Talal Dam and the intake point that feeds King Abdullah Canal (RSS reports, 1984-2005).

2.2. Samples Collection, Transportation, Storage and Treatment:

Surface and near-bottom water samples were collected from the selected locations by the use of a 10 liter PVC Niskin water sampler on seasonal basis between April 2007 and April 2008. Sub-samples were taken from the sampler and put in a prelabeled, precleaned acid washed polyethylene bottles. The bottles were also washed with water from the samples before they were filled with sampled water. The bottles were kept in an ice box and covered with crushed ice until they were transported to the laboratory. Dissolved oxygen samples were transferred into clean dissolved oxygen bottles and treated properly with 2 ml of manganese sulfate solution $MnSO_4.H_2O$ and 2 ml of alkaline iodide reagent (NaOH+NaI) were added below the surface of the water samples.

In the laboratory, water samples were treated, preserved and stored as recommended by and described in the APHA (1998). Sediments samples were collected from the same sampling points of water by the use of a stainless steal grab sampler. Collected samples were put in acid-washed polyethylene bags and kept in an ice box that contain crushed ice until they were transported to the laboratory. In the laboratory, parts of the sediments samples were wet sieved on a 63 µm sieve. Sediment samples were then dried in an oven at 105 Cº. After drying, samples were homogenized by the use of agate pestle and mortar before being split into sub-samples for future use and analysis**.**

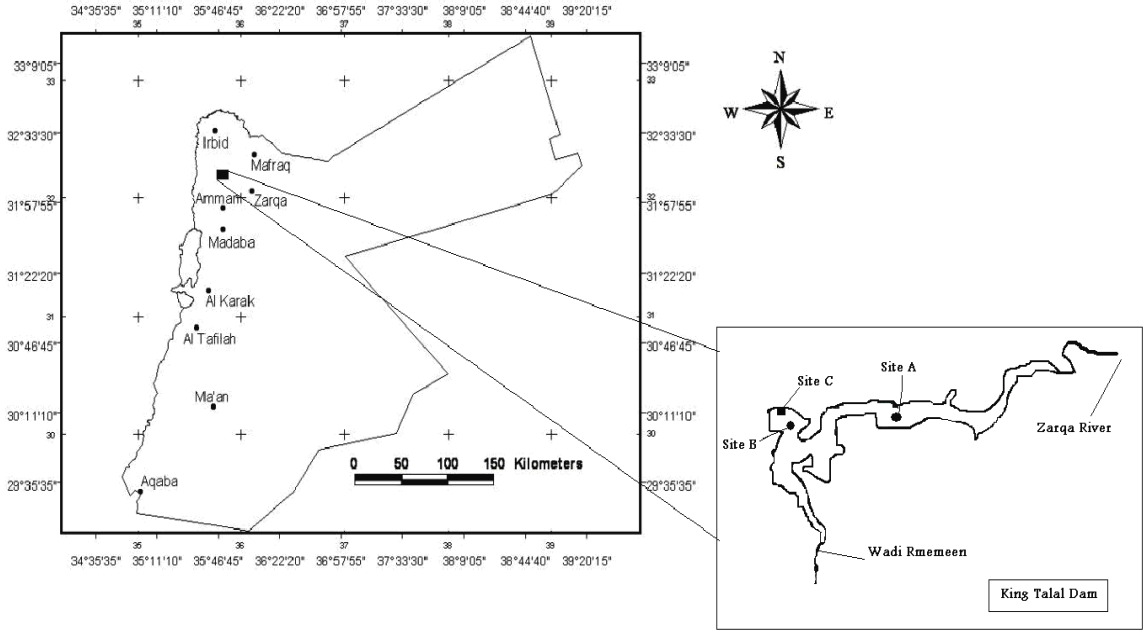

Figure 1: Locations of sampling sites in the main body of King Talal Dam.

2.3. Water Analysis:

Temperature of water was measured *in situ* by the use of a portable thermometer. The pH of water was determined by the use of a pH meter equipped with a standard hydrogen electrode and a reference electrode. Transparency was measured by the use of a 30 cm diameter Secchi disc, which was lowered on the shaded side of the boat. The average depth of two measurements was recorded as the disc transparency. Dissolved oxygen (DO) was determined by the use of the Winkler method as described in the APHA (1998). All nutrients were determined as described in the APHA (1998). Nitrite (NO_2^-) was determined by the diazotisation of sulphanilamide solution by nitrates in the sample to form diazo compound. The compound couples with N-(1-naphthyl)-ethylene diamine solution to form a purplish-pink dye. Nitrate (NO_3^-) was determined by the use of the ultraviolet spectrophotometric screening method. Ammonia (NH_3) was determined by the use of the Nesslerization method. Phosphate (PO_4^-) was determined by the use of the ammonium molybdate-ascorbic acid method. Total phosphorus (TP) and total nitrogen (TN) in water samples were determined as dissolved (reactive) phosphate and as nitrate (APHA, 1998) after digestion with alkaline potassium persulphate solution.

a) Oxidizing Reagent:

50 g of potassium persulphate and 30 g boric acid were dissolved in 250 ml of 1 M sodium hydroxide solution. Then solution was made up to 1000 ml with deionized water and stored in a brown bottle at room temperature, protected from direct light.

b) Digestion and Analysis Procedures:

4 ml of the oxidizing reagent were added to 30 ml of sample in 50 ml autoclaved bottles. Samples were boiled in a stainless steel pressure cooker for 20 minutes.

The bottles were allowed to cool. The volume of the digest was adjusted to 40 ml with distilled water. For the determination of total nitrogen as nitrate, 1 ml of the 2.5 % buffer solution (ammonium chloride) were added to 10 ml of digested sample and the volume were adjusted to 50 ml with distilled water. The procedure was continued as described in the APHA (1998). Aliquots of 25 ml of the digested samples were used to determine the concentration

of total phosphorus as dissolved inorganic phosphorus by the use of the ammonium molybdate-ascorbic acid method as described in the APHA (1998).

2.4. Sediments Analysis:

The analysis of sediments was performed on the bulk sediments, fractions larger than 0.063 mm (>63 µm) and less than 0.063mm (mud). These fractions were analyzed for organic matter, total phosphorus, total inorganic phosphorus and total nitrogen. Organic matter was determined according to the method of Walkley and Black (1934) as described in the APHA (1998). Total phosphorus (TP) was determined by use of the perchloric acid method as described in the APHA (1998). Total inorganic phosphorus (TIP) was determined according to (Andersen, 1976): 1 g of sediments was ignited in a muffle furnace at a temperature of 550 Cº for 1 hour, dissolved in 25 ml of 1 M HCl solution and determined as total inorganic phosphorus according to Strickland and Parsons (1972). Total Nitrogen was determined as ammonia: 1 g of each sediment sample was treated with 2 ml of sulphuric acid. The sample was heated on a hotplate for 2-3 hours. Aliquots of 50 ml of deionized water were added to each sample. The sample was filtrated through No. 41 Whatman filter paper. The filtrate of each sample was made up to 250 ml with deionized water and 55 ml of 1 M sodium hydroxide solution. An aliquot of the digest was used for the determination of total nitrogen as ammonia using the spectrophotometric method as described in APHA (1998).

2.5. Statistical analysis:

Results are presented as means ± standard deviation (S.D.) and the one way ANOVA and multiple comparison tests were used to evaluate differences between means (confidence interval=95%). Also results were analysed by analysis of correlations and relationships between different factors.

3. Results and Discussion:

3.1. Water/ Spatial and Temporal Effects:

3.1.1. pH, Temperature and Dissolved Oxygen:

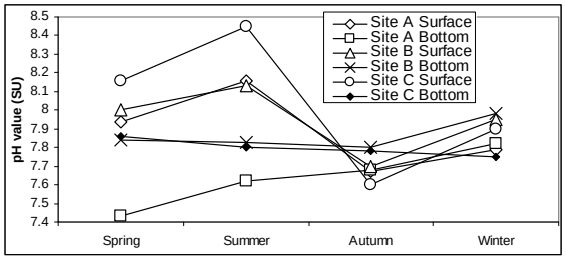

Figure 2: Mean value of pH (SU) in KTD surface and near-bottom water during the four seasons.

The examination of the results in figure 2 indicate that the pH of the surface water ranged between 7.60 and 8.45 with an overall mean value of 7.96 while pH of the near-bottom water ranged between 7.40 and 7.98 with an overall mean value of 7.77. The highest value was recorded in the surface water of the Intake Area of the Dam (site C), while the lowest values were recorded in the near-bottom water of the Mid Point of the Dam (site A). Seasonally, the distribution of pH in the four seasons was in the following order: summer > spring > winter > autumn (Fig. 2). The one way ANOVA test (Table 3) showed significant differences between sites, between depths and between seasons.

The higher values of pH in the surface water compared to those of the near-bottom water can be attributed at least partially to the CO_2 consumption by photosynthetic activity of algae and other flora in the surface water and in the euphotic zone. In addition, Zarqa River and Wadi Rmemeen receives combined industrial and waste water discharges of alkaline nature. According to Hellawell (1986) discharges of alkaline nature may increase the pH values of the surface waters above 8 to 9 or 10. The lower pH values in the near-bottom water can be attributed to lower levels of DO in the near-bottom water compared to the surface water, due to decomposition and higher levels of OM, and lower rate of photosynthetic activity in this water. Higher pH values were recorded during summer and spring, which represent the periods of low water flow, and the increased percentage of the alkaline industrial wastewater in the total flow into the reservoir. High summer pH could also be caused by CO_2 removal via algal photosynthesis which may counter balance the effect of increasing water temperature.

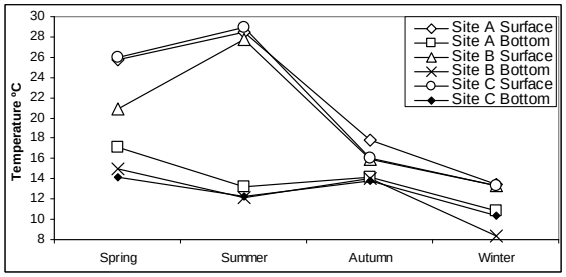

Figure 3: Mean value of temperature in KTD surface and near-bottom water during the four seasons.

Mean temperature of the surface water samples ranged between 13.30 and 28.90 ˚C with an overall mean value of 20.70 ˚C while, the mean temperature of the near-bottom water ranged between 8.30 and 17.10 ˚C with an overall mean value of 12.90 ˚C. The highest value was recorded in the surface water of site (A) followed by site (C). The lowest value was recorded in the near-bottom water of site (B). The seasonal distribution of temperature was in the following order: summer > spring > autumn > winter (Fig. 3). The one way ANOVA test (Table 3) showed significant differences between depths and between seasons and in significant differences between sites.

It is known that water temperature depends on many factors that include climate, altitude, air temperature, seasons of the year, the ratio of the water's surface area compared to its depth, input of discharge, and water flow rate (Best, 1975; USEPA, 1976). The high surface temperature is attributed to the direct contact with sun light. Temperature value fluctuated seasonally and has a general seasonal trend similar to that of air temperature. As expected, the lowest temperature was recorded during winter, while the high temperature was recorded in summer due to the effect of solar heating on the surface water of KTD.

In summer, sunlight heats the surface layer of the water (temperature of surface layer begins to rise) but the

temperature in deep layer remains low compared to the surface layer. Such thermal stratification results in low dissolved oxygen (DO) concentrations in the hypolimnion during the summer time. This stratification is considered an essential element for very high density blooms of algal growth in King Talal Dam (KTD). When these algae and other phytoplankton and zooplankton settle into the deeper water where they are decomposed, decomposition takes up oxygen and gives off carbon dioxide and produces the anaerobic products such as H_2S and NH_4. It has been reported that KTD as a warm monomictic waterbody, experiences overturn between surface and bottom waters once a year during autumn (RSS, 2005; Numayr, 1999).

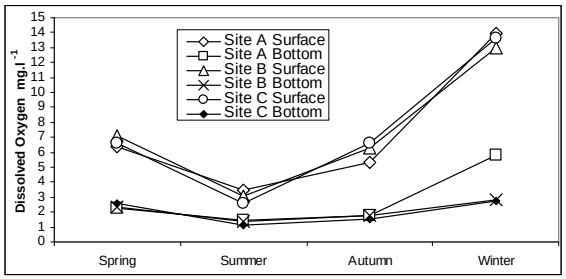

Figure 4: Mean concentration of DO in KTD surface and near-bottom water during the four seasons.

The results (Fig. 4) show that the DO in the surface water varied widely in the range of 2.60 -13.90 mg/l, with an overall mean value of 7.30 mg/l while the mean concentration of DO in the near-bottom water varied in the range of 1.10-5.80 mg/l with a much lower overall mean value (2.3 mg/l) as compared to the surface water. The highest mean concentration of DO was recorded in the surface water of site (A) while the lower mean values were recorded in the near-bottom water of sites (C) and (B). The seasonal distribution of DO concentration was in the order: winter > spring > autumn > summer. The one way ANOVA test (Table 3) showed significant difference in DO between depths and between seasons and insignificant difference between sites. High values of DO in surface water are not unusual and are mainly attributed to the direct contact of surface water with air and to the relatively higher photosynthetic activity compared to that in the near-bottom water. In contrast, lower DO values in the deeper water are attributed to the degradation of organic substances that are accumulated in the deeper water (USEPA, 1976). The high DO values in winter is attributable to relatively low temperature of water (higher solubility of gases) and high input of flood water that contains high oxygen levels due to the possibility of mixing with atmospheric oxygen. Low values of DO during summer can be attributed to the relatively high water temperature, higher oxidation rate of NH_4 (nitrification), and other biodegradable organic material which consumes oxygen during its oxidation.

3.2. Nitrate, Ammonia, Nitrite and Total Nitrogen:

Nitrate (NO_3^-), ammonia (NH_3), nitrite (NO_2^-) and total nitrogen (TN) concentrations in the water of the three sampling sites (A, B, C) within the main body of King Talal Dam are presented in Table (2) and represented in Figures (5), (6), (7) and (8).

The results (Fig. 5) indicate that nitrate concentration in the surface water ranged between 0.002 and 38.60 mg/l with overall mean value of 16.20 mg/l, while the concentration in the near-bottom water ranged between 0.15 and 36.3 mg/l with overall mean value of 15.7 mg/l. Relatively higher mean concentrations of nitrate in surface and near-bottom water were recorded in site (A) and site (C) compared to site (B).

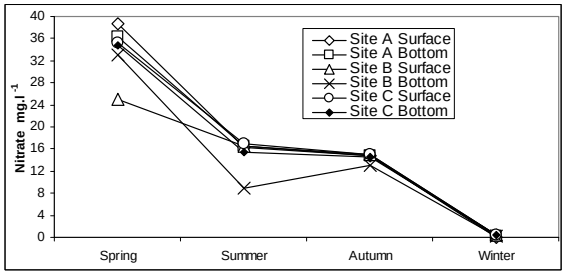

Figure 5: Mean concentration of nitrate in KTD surface and near-bottom water during the four seasons.

Seasonally, the levels of nitrate concentration were in the order: spring > summer > autumn > winter. The one way ANOVA test (Table 3) showed significant differences between nitrate concentration in different seasons and insignificant differences between sites and between depths.

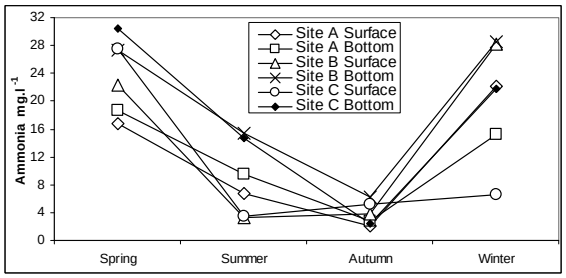

Figure 6: Mean concentration of ammonia in KTD surface and near-bottom water during the four seasons.

The results of ammonia (Fig. 6) indicate that the mean concentrations in the surface water ranged between 2.12 and 28.20 mg/l, with overall mean value of 12.33 mg/l while, ammonia in the near-bottom water ranged between 2.51 and 30.50 mg/l, with overall mean value of 16.10 mg/l, the highest mean concentrations were recorded in the near-bottom and surface water of site (B) followed by site (C). Seasonally, the levels of ammonia concentration were in the order: spring > winter > summer > autumn. Similar to nitrate, the one way ANOVA test (Table 3) showed significant differences in ammonia concentrations between seasons and insignificant differences between sites and between depths.

Figures 5 and 6 show that the concentrations of nitrate were generally higher in the surface water, while ammonia concentrations were generally high in deeper water. This distribution can be attributed to the more oxic conditions in the surface water which enhances the nitrification process compared to less oxic or anoxic conditions in the deeper near-bottom water that enhances the production of ammonia (denitrification) in this type of water (Van Den Bos, 2003).

Figure 6 shows also that the concentration of ammonia was generally low in the dry seasons (summer and autumn) because biota are more efficient in utilising ammonia under dry conditions and because dry weather, sunshine and higher water temperature enhances the

process of oxidation and nitrification of ammonia (Seager et al., 1988). In contrast, during rainy or wet seasons the temperature of water is low, biological growth is minimal and nitrification process is slow which give rise to high concentration of ammonia (Seager et al., 1988).

The results as presented in Figure 7 show that the mean concentration of nitrite in the surface water ranged between 0.09 and 2.23 mg/l with overall mean value of 1.44 mg/l, while its mean concentration in the near-bottom water ranged between 0.10 and 3.14 mg/l, with overall mean value of 1.46 mg/l. Similar to nitrate, higher mean concentrations of nitrite were recorded in the near-bottom water of site (A) and site (C) compared to site (B). Seasonally, the levels of nitrite concentration were in the order: autumn > spring > summer > winter. Similar to the case of nitrate and ammonia, the one way ANOVA test (Table 3) showed significant differences in nitrite concentrations between different seasons and insignificant differences between sites and between depths.

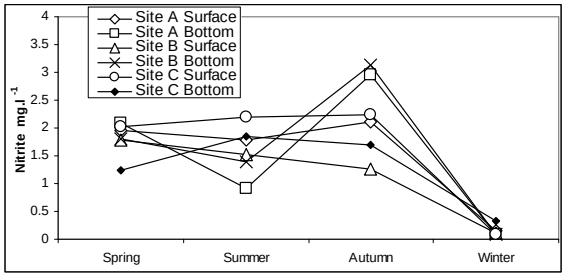

Figure 7: Mean concentration of nitrite in KTD surface and near-bottom water during the four seasons.

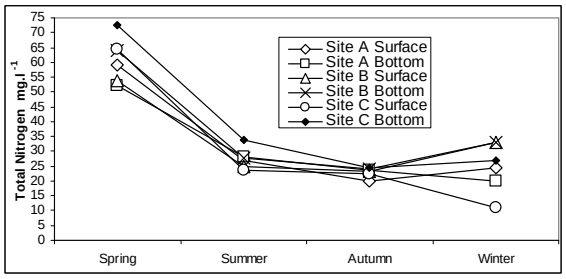

Figure 8: Mean concentration of total nitrogen in KTD surface and near-bottom water during the four seasons.

The results (Fig. 8) indicate that mean total nitrogen concentration (TN) in the surface water samples ranged between 11.18 and 64.40 mg/l with overall mean value of 32.30 mg/l, while its mean concentration in the near-bottom water ranged between 20.10 and 72.60 mg/l with overall mean value of 35.90 mg/l. The high mean concentrations were recorded in sites B and C compared to site A. Seasonally, the levels of total nitrogen were in the order: spring > summer > winter > autumn. The one way ANOVA test (Table 3) showed significant differences in total nitrogen concentrations between different seasons and insignificant differences between sites and between depths. The concentration of inorganic nitrogen which includes ammonia, nitrite and nitrate is usually affected by many factors that include water temperature and pH, in addition to surface runoff from the surrounding catchment area, streams, discharge of effluent from wastewater treatment plants, agricultural fertilizers and industrial wastes which are considered major sources of inorganic nitrogen entering aquatic systems (Anon, 1996). Ammonia (NH_3) and ammonium (NH_4^+) are the reduced forms of inorganic nitrogen and their relative proportions are controlled by water temperature and pH. This explains the low levels of ammonia in the surface water during summer which is characterized by higher temperature, pH and dissolved oxygen as compared with the near-bottom water and with winter time. Nitrite (NO_2^-) is the intermediate inorganic species of the interconversion of nitrate and ammonia. In the aquatic ecosystem, nitrite is converted to nitrate rapidly under oxidizing conditions. Therefore, nitrate is usually far more abundant in the aquatic environment (Anon, 1996). This explains the relatively high concentrations of nitrate (Fig. 8) compared to nitrite concentration (Fig. 7) during the whole study period.

The relatively high concentrations of nitrate, ammonia and TN in KTD during spring has been attributed to the high amount of the nutrients that enter KTD during this season from Zarqa River and Wadi Rmemeen effluents (RSS, 2005), and to the wastewater that comes from As-Samra and Al Baqa´a treatment plants (Numayr, 1999).

3.3. Phosphorus and Total Phosphorus:

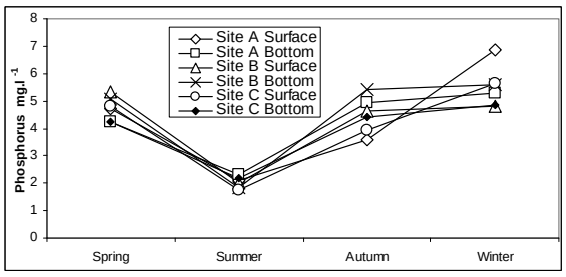

Figure 9: Mean concentration of phosphorus in KTD surface and near-bottom water during the four seasons.

The results (Fig. 9) indicate that the dissolved inorganic phosphorus in the surface water ranged between 1.80 and 6.90 mg/l with overall mean value of 4.19 mg/l, while its mean concentration in the near-bottom water ranged between 1.80 and 5.60 mg/l, with overall mean value of 4.21 mg/l. Higher mean concentrations of phosphorus were recorded in sites B and A as compared with those recorded in site C. Seasonally, the levels of phosphorus concentration was in the following order: winter > spring > autumn > summer. As in the case of the nitrogen species, the one way ANOVA test (Table 3) showed significant difference in phosphorus concentrations between seasons, and insignificant differences between sites and between depths.

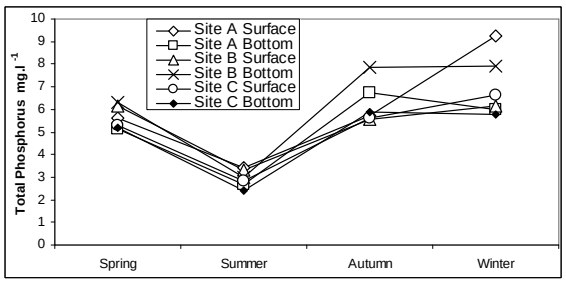

Figure 10: Mean concentration of total phosphorus in KTD surface and near-bottom water during the four seasons.

Figure 10 indicates that the total phosphorus in the surface water ranged between 2.90 and 9.20 mg/l, with overall mean value of 5.50 mg/l, while its mean concentration in the near-bottom water ranged between 2.40 and 7.60 mg/l, with overall mean value of 5.40 mg/l. Comparatively, high mean concentrations of total phosphorus were recorded in site A and site B, compared to those recorded in site C. Seasonally, the distribution of total phosphorus was in the following order: winter > autumn > spring > summer. Similar to dissolved inorganic phosphorus and all other nutrients the one way ANOVA test (Table 3) showed no significant difference in the concentration of total phosphorus between sites and between depths, and significant difference between seasons.

The high (TP) concentration at site A is in agreement with what has been reported by the RSS and has been attributed to the industrial wastewater as well as to the municipal wastewater reaching the dam from Khirbet As-Samra treatment plant (RSS, 2005). By comparison, the high phosphorus (PO_4-P) concentration at site B can be attributed to the agricultural activities and related fertilizers and to the wastewater from Al-Baqa´a sewage treatment plants that enters the dam via Wadi Rmemeen (RSS, 2005).

Seasonally, higher phosphorus and total phosphorus concentrations were recorded during winter while lowest concentrations were recorded during summer. Ekholm et al. (2000) suggests that the rainfall during winter is able to carry soil erosion products and agricultural waste such as chemicals fertilizers as well as wastewater from the treatment plants. Algal growth and phosphorus uptake by plankton and algae is triggered in spring and continue in a higher rate during summer. This uptake and growth process explains why the phosphorus concentration in the water is reduced to lower levels during summer. Later in the year plankton begins to dye off due to the lack of nutrients. The dead plankton containing the assimilated phosphorus falls through the water column and the degradation of plankton releases phosphorus back to the water column during autumn. This will result in an increase in the phosphorus concentration in water column during autumn. The process continues and phosphorus concentration increases and reaches maximum levels in winter when there is minimal photosynthesis activity by the plankton (Dojlido and Best, 1993; Harris, 1986; HMSO, 1980; Golachowska, 1979).

Table (1): Average concentrations of pH, temperature and dissolved oxygen in surface and near-bottom waters of King Talal Dam during spring, summer, autumn and winter.

Water	Site	Season		pH (SU)	Temp. (°C)	D.O (mg/l)
Surface Water	Mid Point of the Dam (A)	Spring	$\bar{X}$ ±S.D	7.95±0.07	25.80±0.60	6.35±0.20
		Summer	$\bar{X}$ ±S.D	8.16±0.01	28.50±0.40	3.50±0.40
		Autumn	$\bar{X}$ ±S.D	7.67±0.01	17.80±0.50	5.20±2
		Winter	$\bar{X}$ ±S.D	7.79±0.03	13.40±0.20	13.90±0.70
	Deepest Point of the Dam (B)	Spring	$\bar{X}$ ±S.D	8.04±0.03	20.90±5	7.10±0.50
		Summer	$\bar{X}$ ±S.D	8.13±0.01	27.83±0.30	3.10±0.40
		Autumn	$\bar{X}$ ±S.D	7.70±0.02	15.88±0.10	6.30±0.08
		Winter	$\bar{X}$ ±S.D	7.95±0.02	13.30±0.50	13±0.70
	Intake Point of the Dam (C)	Spring	$\bar{X}$ ±S.D	8.16±0.03	26±0.20	6.60±0.01
		Summer	$\bar{X}$ ±S.D	8.45±0.01	28.90±0.40	2.60±0.80
		Autumn	$\bar{X}$ ±S.D	7.58±0.10	16.10±0.30	6.60±0.09
		Winter	$\bar{X}$ ±S.D	7.88±0.01	14.30±0.20	13.60±0.40
Near-Bottom Water	Mid Point of the Dam (A)	Spring	$\bar{X}$ ±S.D	7.43±0.01	17.13±0.20	2.30±0.10
		Summer	$\bar{X}$ ±S.D	7.62±0.03	13.20±0.20	1.45±0.80
		Autumn	$\bar{X}$ ±S.D	7.68±0.02	14.10±0.10	1.80±0.20
		Winter	$\bar{X}$ ±S.D	7.82±0.01	10.80±0.40	5.78±1.90
	Deepest Point of the Dam (B)	Spring	$\bar{X}$ ±S.D	7.84±0.10	15.10±0.06	2.35±0.10
		Summer	$\bar{X}$ ±S.D	7.84±0.03	12.10±0.20	1.35±0.30
		Autumn	$\bar{X}$ ±S.D	7.80±0.010	14±0.20	1.77±0.30
		Winter	$\bar{X}$ ±S.D	7.98±0.05	8.33±0.30	2.83±0.20
	Intake Point of the Dam ©	Spring	$\bar{X}$ ±S.D	7.86±0.08	14.20±0.08	2.56±0.01
		Summer	$\bar{X}$ ±S.D	7.80±0.08	12.19±0.10	1.09±0.20
		Autumn	$\bar{X}$ ±S.D	7.78±0.07	13.80±0.30	1.52±0.01
		Winter	$\bar{X}$ ±S.D	7.75±0.03	10.40±0.50	2.76±0.07

$\bar{X}$: Mean. S.D: Standard Deviation.

Table (2): Average concentrations of nutrients (mg/l) in surface and near-bottom waters of King Talal Dam during spring, summer, autumn and winter.

Water	Site	Season		NO_3^-	NO_2^-	NH_3	TN	P	TP
Surface Water	Mid Point of the Dam (A)	Spring	$\bar{X}$ ±S.D	38.60±5.50	1.95±0.2	16.70±4.10	59.20±6.90	4.73±0.25	5.64±0.50
		Summer	$\bar{X}$ ±S.D	16.40±3.30	1.79±0.37	6.80±0.81	26.90±4.60	2.08±0.18	3.41±1.50
		Autumn	$\bar{X}$ ±S.D	14.70±2.40	2.12±0.28	2.12±0.33	20±2.30	3.60±0.30	5.70±1.90
		Winter	$\bar{X}$ ±S.D	0.002±1^{-1}	0.14±5^{-1}	22.10±5.30	24.50±2.60	6.88±0.29	9.23±1.65
	Deepest Point of the Dam (B)	Spring	$\bar{X}$ ±S.D	25±4	1.78±0.34	22.30±1.80	53.80±8.30	5.33±0.40	6.13±0.40
		Summer	$\bar{X}$ ±S.D	16.50±3.20	1.53±0.24	3.40±0.31	25±1.80	2±0.22	3.33±0.26
		Autumn	$\bar{X}$ ±S.D	15.10±4.10	1.27±0.16	3.80±0.30	23.30±2.40	4.66±0.21	5.56±0.38
		Winter	$\bar{X}$ ±S.D	0.33±0.02	0.11±0.01	28.20±6.50	32.90±5.80	4.83±0.32	6.17±0.42
	Intake Point of the Dam (C)	Spring	$\bar{X}$ ±S.D	35.12±6	2±0.29	27.50±4	64.40±9.50	4.81±0.37	5.32±0.45
		Summer	$\bar{X}$ ±S.D	17±5.70	2.19±0.3	3.42±1.10	23.60±2.70	1.77±0.14	2.86±0.37
		Autumn	$\bar{X}$ ±S.D	14.95±3.10	2.23±0.26	5.24±0.40	22.20±1.50	3.95±0.36	5.64±0.50
		Winter	$\bar{X}$ ±S.D	0.49±0.02	0.09±1^{-1}	6.45±1	11.20±1.40	5.63±0.40	6.61±0.82
Near-Bottom Water	Mid Point of the Dam (A)	Spring	$\bar{X}$ ±S.D	36.30±4.90	2.08±0.33	18.70±4.10	52±2	4.26±0.20	5.16±0.35
		Summer	$\bar{X}$ ±S.D	16.60±3.20	0.91±0.25	9.61±1.60	28.30±3.50	2.30±0.25	2.70±0.62
		Autumn	$\bar{X}$ ±S.D	14.80±3.30	2.95±0.3	2.83±0.25	23.70±2.30	4.94±0.34	6.73±1.70
		Winter	$\bar{X}$ ±S.D	0.19±5^{-3}	0.10±2^{-3}	15.30±3.30	20.10±1.70	5.30±0.24	6±1.60
	Deepest Point of the Dam (B)	Spring	$\bar{X}$ ±S.D	33.10±6.50	1.81±0.2	27.30±2.40	64.20±8.10	5.07±0.41	6.30±0.66
		Summer	$\bar{X}$ ±S.D	8.95±2.80	1.39±0.25	15.40±4.30	27.70±2.60	1.82±0.17	2.99±0.17
		Autumn	$\bar{X}$ ±S.D	13±2.40	3.14±0.4	6.21±0.37	24.10±3.30	5.40±0.12	7.87±0.67
		Winter	$\bar{X}$ ±S.D	0.15±0.01	0.09±1^{-1}	28.50±6.60	33±3.40	5.60±0.14	7.91±0.41
	Intake Point of the Dam (C)	Spring	$\bar{X}$ ±S.D	34.80±3.40	1.24±0.25	30.50±4.20	72.60±14.50	4.26±0.21	5.21±0.37
		Summer	$\bar{X}$ ±S.D	15.50±40	1.85±0.6	14.60±1.70	33.80±3.60	2.18±0.15	2.43±0.04
		Autumn	$\bar{X}$ ±S.D	14.60±3.30	1.69±0.1	2.51±0.15	24.30±3.10	4.43±0.35	5.88±0.30
		Winter	$\bar{X}$ ±S.D	0.46±0.02	0.32±0.45	21.80±3.20	27.10±2.50	4.84±0.33	5.78±0.60

$\bar{X}$: Mean. SE: S.D: Standard Deviation.

Table (3): P-Values of nutrients, pH, temperature and dissolved oxygen in KTD Waters. (confidence interval 95%)

	Between sites	Between depths	Between seasons
Nitrite	0.89 (N.S)	0.88 (N.S)	<0.0001 (S)
Ammonia	0.13 (N.S)	0.06 (N.S)	<0.0001 (S)
TN	0.67 (N.S)	0.31 (N.S)	<0.0001 (S)
Phosphorus	0.54 (N.S)	0.89 (N.S)	<0.0001 (S)
TP	0.33 (N.S)	0.55 (N.S)	<0.0001 (S)
Nitrate	0.57 (N.S)	0.86 (N.S)	<0.0001 (S)
pH	0.01 (S)	<0.0001 (S)	<0.0001 (S)
Temperature	0.55 (N.S)	<0.0001 (S)	<0.0001 (S)
DO	0.92 (N.S)	<0.0001 (S)	<0.0001 (S)

(S): Significant (N.S): Not Significant

3.4. Sediments/ Spatial and Temporal Effects:

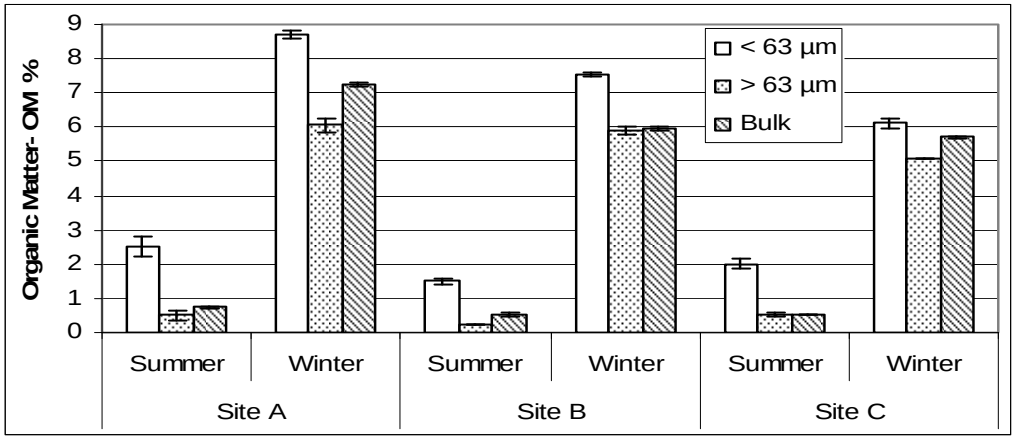

Figure 11: Mean percentage of OM ± SD in KTD sediments during dry (summer) and wet (winter) seasons.

Organic matter (OM) contents % in KTD sediments ranged between 0.25 and 8.7% with overall mean value of 3.70% (Table 4 and Figure 11). Highest overall mean values during winter and summer occurred at site A (mid point of the dam) followed by site B (deepest site of the dam) with mean values of 4.29 and 3.61 %, respectively. The lowest overall mean values were found at site C (intake point of the dam) with a value of 3.33 %. The less than 63 µm sediment fraction showed the highest OM contents which is in agreement with what has been widely reported, that OM contents of the sediments are predominantly associated with the fine fraction, through adsorption at clay surfaces (Greenland and Hays, 1981). Particulate matter from surface runoff, decay of plants especially in winter and municipal and industrial wastewater can be considered important sources for OM in the sediments of the dam. The one way ANOVA test (Table 5) showed significant difference in the OM between seasons and between fractions and insignificant difference between sites.

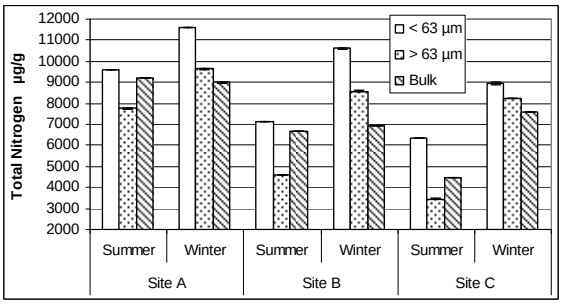

Figure 12: Mean concentration of TN ± SD in KTD sediments during dry (summer) and wet (winter) seasons.

The results (Fig. 12) show that the concentrations of total nitrogen (TN) in KTD sediments ranged between 3459 and 11604 µg/g (Table 4), with overall mean value of 7797 µg/g. Higher overall mean concentrations in winter and summer were recorded in site A followed by site B with concentrations of 9464 and 7422 µg/g, respectively. Relatively lower overall mean concentrations were recorded in site C (6506 µg/g). The highest concentrations at site A are attributed to the effluents of As-Samra wastewater treatments plants as well as to the runoffs from the agricultural land around the dam. The results as presented in the figure show clearly that the less than 63 µm sediment fraction has the highest contents of TN. Higher values were recorded in winter, which can be attributed to the fact that during this season the dam receives wastewater effluents and runoffs from agricultural land that contains high concentration of nutrients. The ANOVA test (Table 5) showed the presence of significant differences in TN contents between sites, between sediment fractions and between seasons. Table (4) and Figures (13a and 13b) show the concentration of total phosphorus (TP) and inorganic phosphorus (IP) in the sediments of King Talal Dam (sites A, B and C). The concentrations of TP (Fig. 13a) ranged between 1857 and 3312 µg/g with overall mean value of 2437 µg/g. The high overall mean concentrations during winter and summer were recorded in site A followed by site B with values of 2740 and 2509 µg/g, respectively. Relatively, lower overall mean concentrations were recorded in site C with a value of 2062 µg/g.

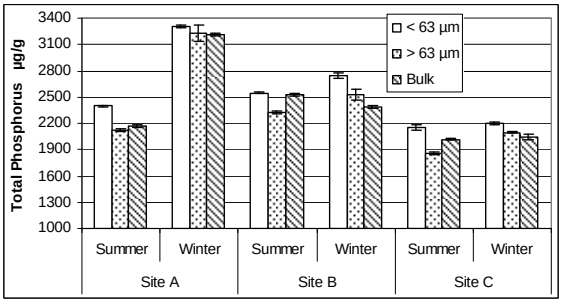

Figure 13a: Mean concentrations of TP ± SD in KTD sediments during dry (summer) and wet (winter) seasons.

The results (Fig. 13b) show also that the inorganic phosphorus (IP) ranged between 346 and 947 µg/g with overall mean value of 597µg/g. Similar to TP, The higher overall mean concentrations were recorded in the site A followed by site B with values of 615 and 606 µg/g, respectively. Comparatively lower overall mean concentrations were recorded in site C with a mean concentration of 571 µg/g. Phosphate anions can be sorbed on freshly precipitated ferric and aluminum hydroxides that may occur in the sediments (Bache and Williams, 1971). It may be transported also to sediments by settling of phytoplankton (organic particulates), settling of solid inorganic phosphorus (apatites), and settling of clays

containing adsorbed phosphorus (Furumai, 1989). According to Brady (1990), 98-99% of phosphorus is associated with primary or secondary minerals and with organic matter. This can explain the presence of the relatively high concentrations of TP and IP in the sediments of sites A and B which contain high concentration of OM as compared with site C.

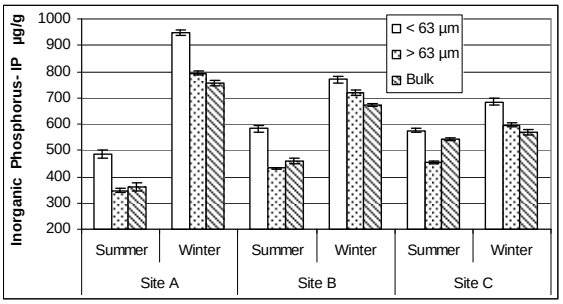

Figure 13b: Mean concentrations of IP ± SD in KTD sediments during dry (summer) and wet (winter) seasons.

The less than 63 µm sediment fraction contained the higher concentrations of IP and TP which can be at least partly attributed to its higher contents of organic matter compared to the >63 µm fraction. Seasonally, highest values of IP and TP were recorded in winter which can be attributed to the weathering of rocks and erosion of soils of the catchment area, agricultural runoffs, and industrial effluents which normally contain high concentrations of phosphorus. The ANOVA test (Table 5) showed significant differences in TP between sites and between seasons. By comparison, there were no significant differences in IP concentration between sites, but there was significant difference between seasons. The difference in TP and IP concentrations between fractions was significant in summer but not in winter. A result that need more investigation.

Table (4): Mean concentration of nutrients (µg/g) and organic matter (%) in the sediments of King Talal Dam during summer and winter time.

Site	Fraction	Season		Organic Matter	Total Nitrogen	Total Phosphorus	Inorganic Phosphorus	Organic Phosphorus
Mid Point of the Dam (A)	<63 µm	Summer	$\bar{X}$ ±S.D	2.5±0.30	9603±8	2400±7.10	485±14.80	1916±3.60
		Winter	$\bar{X}$ ±S.D	8.7±0.10	11604±5	3312±16.30	947±10.40	2365±15
	>63 µm	Summer	$\bar{X}$ ±S.D	0.5±0.15	7762±15	2120±14	346±7.80	1775±5
		Winter	$\bar{X}$ ±S.D	6.1±0.20	9642±28	3233±62	794±6.10	2439±11
	Bulk	Summer	$\bar{X}$ ±S.D	0.75±0.03	9189±10	2168±10.60	361±16.30	1807±10
		Winter	$\bar{X}$ ±S.D	7.25±0.07	8982±14	3211±15.60	756±8	2455±14
Deepest Point of the Dam (B)	<63 µm	Summer	$\bar{X}$ ±S.D	1.5±0.08	7128±11.50	2548±10.70	583±12.80	1965±10.50
		Winter	$\bar{X}$ ±S.D	7.5±0.05	10626±38	2753±31	769±11.30	1984±9.10
	>63 µm	Summer	$\bar{X}$ ±S.D	0.25 ± 4^{-1}	4580±13	2320±15	432±2.90	1888±8
		Winter	$\bar{X}$ ±S.D	5.9±0.10	8591±3	2528±67.20	718±11.30	1810±4.20
	Bulk	Summer	$\bar{X}$ ±S.D	0.51±0.06	6658±12	2523±17.70	461±10.20	2062±3.40
		Winter	$\bar{X}$ ±S.D	5.95±0.07	6947±6.60	2383±17	674±5.80	1708±18
Intake Point of the Dam (C)	<63 µm	Summer	$\bar{X}$ ±S.D	2±0.16	6344±7	2155±28.30	576±6.40	1580±14
		Winter	$\bar{X}$ ±S.D	6.1±0.14	8940±53	2195±14	686±13.40	1510±12.20
	>63 µm	Summer	$\bar{X}$ ±S.D	0.5±0.06	3459±11.40	1857±16.30	454±7.50	1402±14.50
		Winter	$\bar{X}$ ±S.D	5.1±0.07	8247±15	2095±7.10	597±10	1498±16
	Bulk	Summer	$\bar{X}$ ±S.D	0.53±0.06	4480±11	2020±14.20	543±5.70	1477±14.80
		Winter	$\bar{X}$ ±S.D	5.7±0.05	7567±6.30	2048±30.10	569±12.70	1479±9.20

$\bar{X}$: Mean. S.D: Standard Deviation.

Table (5): P-Values of nutrients and other chemical species in KTD sediments.

	Between sites	Between Fractions		Between seasons
		Summer	Winter	
OM %	0.72 (N.S)	<0.0001 (S)	0.002 (S)	<0.0001 (S)
Total Phosphors	<0.0001 (S)	0.04 (S)	0.6 (N.S)	0.0032 (S)
Inorganic Phosphorus	0.78 (N.S)	0.002 (S)	0.1 (N.S)	<0.0001 (S)
Total Nitrogen	0.0009 (S)	0.04 (S)	0.01 (S)	0.0002 (S)

(S): Significant (N.S): Not Significant

3.5. Comparison With Previous Studies:

Table (6) shows that the results of dissolved oxygen, nitrate, nitrite, ammonia and total nitrogen are either in agreement with or within the range of the results of the RSS (2005). The pH and water temperature results are within the range reported by the RSS (2005) and Mahasneh and Soub (1983). Transparency values are in agreement with the lower values reported by Mahasneh and Soub (1983). Table 6 shows that the concentrations ranges of total nitrogen and total phosphorus in the sediments of the present study are larger than the ranges reported by the RSS (1992). The high concentrations of nutrients in the present study are attributed to the increased industrial and wastewater effluents and increased load of nutrients in Amman-Zarqa area as well as to increased agricultural activities in the area.

Table (6): Comparing of water (mg/l) and sediments (µg/g) results with previous studies on KTD.

Phase	Year / Element	Mahasneh and Soub (1983)	RSS 2005 (Dam Center)	This Study		
				Site A	Site B	Site C
Water	pH (SU)	6.5-90	7.51-8.33	7.77	7.91	7.91
	Temperature ºC	12-32	12.1-27.30	17.60	15.90	16.80
	DO (mg/l)	-------	0.5-7.30	5	4.70	4.66
	Transparency (m)	0.6-60	------	0.72	0.67	0.59
	Nitrate (mg/l)	-------	<0.05-16.20	17.20	14	16.60
	Nitrite (mg/l)	-------	0.01-2.45	1.50	1.39	1.45
	Ammonia (mg/l)	-------	13-27	11.80	16.90	14
	Total Nitrogen (mg/l)	-------	17-38	31.80	35.50	34.90
	Total Phosphorus (mg/l)	-------	4.3-7.80	5.60	5.50	5
Sediments			**RSS 1992**			
		--------		Site A	Site B	Site C
	Total Phosphorus (µg/g)	--------	234-1981	2740	2509	2062
	Total Nitrogen (µg/g)	--------	78-4810	9464	7422	6506

Table (7) shows the correlation coefficient between nutrients in water and sediments. TN in surface water shows weak correlation with TN and TP in sediments, while TP in water shows high correlation with TN and TP in the same sediment samples. In contrast, TN in the near-bottom water shows negative correlation with TN and TP in sediments, while TP in the near-bottom water shows weak correlation with TN and TP in the same sediments samples.

Table (7): Correlation coefficient between nutrients in water and sediments of KTD.

Phase		Nutrients in sediments	
		TN	TP
Nutrients in surface water	TN	0.08	0.31
	TP	0.60	0.82
Nutrients in near-bottom water	TN	-0.54	-0.70
	TP	-0.24	0.005

4. Conclusions:

Temperature, pH, Do, and nitrate values in the surface water were higher than those of the near-bottom water. Temperature of the surface water ranged between 13.30 ˚C during winter and 28.90 ˚C during summer. By comparison, near-bottom water temperature ranged between 8.30 ˚C during winter and 17.10 ˚C during summer. Dissolved oxygen is low in the near-bottom water (1.10 mg/l) when compared with that in the surface water 13.90 mg/l. The high values of DO in surface water are mainly attributed to the direct contact of the surface water with air and to the effect of photosynthetic activity. The high DO values in winter is attributable to relatively low temperature of water and high input of floodwater that contains high oxygen due to its possible mixing with atmospheric oxygen. The levels of pH, temperature, DO, in KTD water vary significantly during different seasons. The generally higher concentration of nitrate in the surface water can be attributed to the higher nitrification rate that usually occur at this level, while the generally higher ammonia concentration in the near-bottom water is due to the higher denitrification rate that prevails at this level. The concentrations of nitrogen and phosphate species entering KTD reservoir are affected mainly by the input of nitrogen and phosphorus from Zarqa River and Wadi Rmemeen both of which receives effluents from major wastewater treatment plants. High concentrations of phosphorus and total phosphorus in KTD water were recorded in winter, due to rainwater that carries soil erosion products and agricultural waste.

The concentrations of nutrients and organic matter are higher in the clay–silt size fraction (<63µm) of the sediments. They showed more or less similar general trend of spatial distribution, where maximum values occurred during winter. Most pollutants decrease towards the far end of the dam (outlet point). The statistical examination of nutrients results in water and sediments showed that the spatial and depth differences are apparent and insignificant which is attributed to the fact that King Talal Dam is a

small body of water with no big depth difference between its surface and bottom. Most of the measured species showed significant temporal variation.

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In Vitro Multiplication of *Chrysanthemum morifolium* Ramat and it is Responses to NaCl Induced Salinity

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Abstract

A micropropgation method by multiple shoot formation of *Chrysanthemum morifolium* has been developed. Explants growing in greenhouse were used to establish cultures of *C. morifolium*. Shoot tips were surface sterilized and cultured on Murashige and Skoog (MS) media. Successful *in vitro* multiplication of chrysanthemum was achieved on MS medium supplemented with benzyl amino purine (BAP) at 0.3 mg l^{-1} . *In vitro* rooting was successfully achieved on MS media supplemented with different concentration of auxins. The *in vitro* response to salinity stress (0 to 300 mM NaCl) was also tested. Shoot proliferation was gradually reduced at higher NaCl concentrations. Shoot length, number of leaves, fresh and dry weight, chlorophyll, and carotenoid decreased with elevated salinity concentration. Proline and sodium contents increased with elevated salinity, whereas potassium, nitrogen and protein content decreased. Plantlets grown *in vitro* presented tolerance and their growth was negatively affected at high salt concentrations. Elevated salinity significantly reduced microshoot protein. It is concluded that in *C. morifolium* response to *in vitro* salinity stress may provide a system for production under field conditions.

الملخص

لقد تم تطوير طريقة للاكثار بواسطة تكوين الاغصان المضاعفة لنبات غريب (الكريزنثم). ان الاغصان النامية من النسيج قد تم زراعتها في البيت الزجاجي لاجل تاسيس مزرعة نسيجية من الغريب. لقد تم اجراء التعقيم السطحي الاغصان وثم زراعتها في وسط حار موراشيج وسكوج (MS) ان التضاعف الناجح داخل الانابيب لنبات غريب قد تم الحصول علية في وسط (MS) والمزود بمنظم النمو ينزل امينوبيورين (BAP) بتركيز 0.3 مغم/لتر. ان التجديد داخل الانابيب قد تم التوصل اليها في وسط (MS) وباستخدام تراكيز مختلفة من الاوكسجين. بالاضافة لذلك، فقد تم اختبار الاستجابة داخل الانابيب الى الاجهاد الملحي (بتراكيز من ملح الطعام من صفر الى 300 ملمول) . ان توالد الاغصان قد نقص تدريجياً في التراكيز العالية من ملح الطعام، ان التركيز العالي من ملح الطعام قد نتج عنه نقصاً في طول الغصن ، عدد الاوراق الورق الطري والجاف المحتوى من الكلورفيل والكاروتين . ان المحتوي من البلورين والصوديوم قد ازداد مع زيادة تركيز ملح الطعام في حيث قل مثل المحتوى من البوتاسيوم والنتروجين. ان النبيتات النامية داخل الانابيب قد اظهرت مقاومة وان نموها قد تأثر سلبياً في تراكيز الملح العالية. ان التركيز العالي من الملح قد خفضً معنوياً نمو الاغصان الصغيرة من البروتين. نستنتج من ذلك بان نبات الغريب الذي يستجيب للاجهاد الملحي يجب ان يزود بنظام للانتاج تحت الظروف الحقلية. اخيراً فقد تم الحصول على النبيتات الكاملة النمو المنتجة داخل الانابيب وتحت مستويات مختلفة من المزراع الملحية .

Keywords: *Chrysanthemum morifolium*, salinity, shoot multiplication, root formation.

1. Introduction

Simple screening of plantlets by tissue culture provides a unique opportunity for studying many aspects of plant growth and development under well defined conditions (Shatnawi, 2006). Moreover, tissue culture provides an important tool for studying the physiological effects of salt at the cellular level under controlled environment (Olmos *et al*., 1994). Cell and tissue culture systems have been considered for selection of plant tolerance to salinity, drought, and other stresses (Luttus *et al*., 1999). Salinity affects crop production and agricultural sustainability as it reduces productivity of the effected land (Al-Karaki, 2000). Plants frequently respond to water deficit, salt, or osmotic stress with identical or similar physiological and growth adaptations (McCue and Hanson, 1990).

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Chrysanthemum morifolium belongs to the family *Composaetae* (*Asteraceae*) (Arora, 1990). Chrysanthemum is one of the largest cut-flower among the ornamental plants traded in the global flower market. Chrysanthemums are important not only for their outstanding aesthetic beauty and long lasting as marketing cut flowers. They can be propagated vegetatively either through root suckers or terminal cuttings; this conventional process of shoot cutting is very slow (Nhut *et al*., 2005). However, clonal propagation through *in vitro* culture can enhance the multiplication rates (Sauvaire and Galgy, 1978).

In vitro culture allows monitoring plant responses to salinity at biochemical and physiological level (Shibli *et al*., 2000; 2007). Thus, it is crucial to establish a relationship between *in vitro* and *in vivo* responses (Lutus *et al*., 1999). Response of cultures to *in vitro* and *in vivo* induced stress was similar (Sawwan *et al*., 2000). Artificial salt stress on plant tissue grown *in vitro* is imposed by adding sodium chloride to the media (Shibli *et al*., 2007) because NaCl is the most predominant salt in saline condition.

Salinity is one of the most significant abiotic stresses for plant agriculture. It causes a serious problem as it reduces the value and productivity of the effected land (Al-Karaki, 2000). Salinity disrupts physiological processes in plants, leading to reduction in growth and yield (Cordovilla *et al*., 1995). Salt destroys the vital physiological process in plants by slowing cell division, cell enlargement, or both in the growing region (Ali *et al*., 1994), and increasing salt concentration and ions composition cause growth restriction or even lead to plant death (Cavagnaro *et al*., 2006). The mechanisms to determine plant responses to salinity involve complex interactions, since plants grow under saline conditions subjected to different types of stress including water stress caused by osmoticum (Schwarz and Kuchenbuch, 1998), mineral toxicity (Shannon 1985), and disturbance in mineral nutrition of the plants (Franco *et al*., 1999).

In vitro culture constitutes a useful tool for rapidly and economically evaluating tolerance in plants, especially for species that have long reproductive cycles. Shoots are easy to propagate *in vitro* and plant material selected from drought or salt stressed cultures can be used to establish plantations in dry or saline soils. Therefore, the aim of this study was, to initially establish an effective way for *in vitro* proliferation method for *C. morifolium* Ramat and furthermore to investigate the *in vitro* response of *C. morifolium* when being subject to NaCl stress.

2. Materials and methods

2.1. Establishment of in vitro culture

Microshoots of *C. morifolium* Ramat, (Balady) shoot tips were washed under running tap water and then sterilized with 70% ethanol for 30 seconds, then dipped in 3.5% sodium hypochlorite for 15 minutes. Finally, excess detergent was removed by rinsing in sterile distilled water four times each for five minute, under the laminar air-flow cabinet.

Medium was solidified using 8.0 g l^{-1} agar agar, then dispensed in test tubes (8 ml each), and autoclaved at 121 ºC. Shoot tips were grown on solid half strength MS medium (Murashige and Skoog, 1962). Cultures were maintained in the growth chamber at 24 ± 2 ºC and 16 h light/8 h dark. Microshoots were then subcultured on full strength MS medium for six times to have enough mother stock prior to experiments initiation. The pH of MS medium was adjusted to 5.8. Medium was solidifying 8.0 g l^{-1} agar agar, dispensed in flasks (60 ml), and autoclaved at 121 ºC. Cultures were maintained in the growth chamber at 23 ± 2 ºC and 16 h lights (50 µmol $m^{-2}s^{-1}$) / 8 dark.

2.2. In vitro propagation

Microshoots were subcultured to hormone-free MS medium for two weeks to eliminate any carry-over effects of the basic cytokinin. For shoot proliferation, microshoots (15 mm in length) were subcultured to MS medium supplemented with either, benzyl amino purine (BAP) or kinetin, at 0.0, 0.3, 0.6, 0.9, 1.2 and 1.5 mg l^{-1}. The pH of MS medium was adjusted to 5.8. Medium was solidified using containing 8 g l^{-1} agar and 30 g l^{-1} sucrose supplemented to the medium. 60 ml / flask of the MS medium was dispensed into 250 ml Erlenmeyer flasks and autoclaved at 121 ºC. Explants were incubated in a growth room under 16 h light/ 8 h dark (50 µmol $m^{-2}s^{-1}$). After six weeks growth periods, data were collected on shoot length and number of shoot per explants.

2.3. In vitro root formation

Microshoots were subcultured to hormone-free MS medium for 2 weeks to eliminate any carry-over effects of the cytokinin. Microshoots (15 mm in length) were subcultured on MS medium supplemented with (0.0, 0.2, 0.4, 0.6, 0.8, or 1.0 mg l^{-1}) IBA (indole-3-butyric-acid), IAA (indole-3-acetic-acid) or NAA (1- naphthalene acetic acid containing 8 g l^{-1} agar and 30 g l^{-1} sucrose. Explants were incubated in a growth room under 16 h light/8 h dark (50 µmol $m^{-2}s^{-1}$). Data were collected after six weeks growth period on number of roots, roots length, and number of leaves.

2.4. Effect of salinity on microshoots physiological responses

2.4.1. Plant growth

Microshoots 15 mm in length with two leaves were subcultured on MS medium on proliferation medium containing 0.3 mg l^{-1} BAP, and supplemented with different concentration of NaCl (0, 20, 40, 60, 80, 100, 150, 300 mM). Data were collected after six weeks growth period on shoot length, number of shoot per explants, number of new leaves, fresh weight, and dry weights.

2.4.2. Chlorophyll and carotenoid content

A fresh weight of 0.1 g of leaves was sampled per replicate, with 5 replicates per treatment. Samples were extracted by acetone, following the method of De Filippis *et al*. (1981). Pigments assay was prepared after six weeks exposure to salts. A fresh weight of 0.1 g shoot material was homogenized in 2.0 ml of 80% acetone using a pestle and mortar. The extract was pipetted into 2.0 ml microtubes and centrifuged at 15000 g for 2 min Helitch Microfuge. The clear green supernatant was collected using a Pasteur pipette, and made up to 3.0 ml in a 10 ml measuring cylinder with extra 80 % acetone. The

absorption spectra were measured by a spectrophotometer (Pye Unicam Sp6) at 480, 510, 626, 645, 649, 663 and 665 nm absorbance, 80 % acetone was used as a reference. Chlorophyll *a*, and Chlorophyll *b* contents were calculated according to Anderson and Boardman (1964), whereas carotenoids content was calculated according to Duxbury and Yentsch (1956).

2.4.3. Proline content

After six weeks growth period, free proline was extracted from leaves and measured colorimetrically. About 0.5 g of fresh weight plant was homogenized in 10 ml of 3% aqueous 5- sulfosalicylic acid and the extract was filtered through Whatman # 2 filter paper. Two ml of the filtrate and 2 ml of both acidic ninhydrin and glacial acetic acid were mixed in a test tube and placed in a boiling water bath (100 ºC) for 1 h to allow color development. The reaction was stopped by placing the tubes in an ice bath. To extract the chromophore, 4 ml of toluene were added to the tubes and mixed vigorously for 15-20 sec. and left at room temperature until the aqueous phase separated from toluene. The extract was quantitatively transferred to a clean test tube. Absorbance of the tested extract was compared with toluene blank by Milton Roy spectrophotometer 1001 at 520 nm. Stock solution of 250 mg praline ml l^{-1}, in 3% aqueous 5-sulfosalicylic acid, was used and a linear standard curve was constructed over a rang of 30 to 250 mg l^{-1}.

2.4.4. Mineral composition

Plant samples were dried at 80 ºC for 24 h and grounded to analyzed nitrogen, sodium and potassium contents. Total nitrogen was determined using Micro-Kjeldahl digestion procedure. A 0.5 g of dry weight plant samples were placed in large test tubes. 10 g of digestion mixture (1M K_2SO_4 + 1M $CuSO_4.5H_2O$) and 20 ml of concentrated H_2SO_4 was added to each test tube. Test tubes were placed in the digester (Buchi Digest Automat K-438) for 135 min at 400 ºC. After cooling down, samples were placed in the distilater (Buchi Autokjeldahl Unit K-370) to determine nitrogen concentration (Bremner *et al*., 1992). Crude protein content in the microshoots was determined by multiplying the total nitrogen by a factor of 6.25 (Balman and Smith, 1993). Na and K concentration were determined by using flame photometer. Plant samples were ashed at 500-550 ºC for about 20-24 h using Thermolyne muffle furnace (6000 Furnace). 10 ml of 2N HCl was added to the samples and gently heated 7-10 min on 75-80 ºC. The solution was filtered using Whatman # 42 filter paper, the solution was filtered and diluted to 50 ml with distilled water then mixed gently. Using Flame photometer 410, Na and K concentration were determined after calibration with different concentration of either Na or K solutions (Chapman and Pratt, 1961).

2.4.5. Experimental design and statistical analysis

Experimental design was performed as a completely randomized design. Each experiment consisted of 5 treatments and was replicated 4 times; each experiment was repeated at least twice. Data were subjected to ANOVA; differences between individual means were determined by Fishers' Least significant difference (LSD) at the 0.05 probability level. Data were analyzed using STATISTICA (StatSoft, Inc 1995).

3. Results

3.1. In vitro shoot formation

Increasing BAP from 0.0 to 0.3 mg l^{-1} increased the number of proliferated shoots from 1.98 to 4.35 (P=0.05). Number of proliferated shoots at 1.5 mg l^{-1} BAP was lower compared to those at 0.3 mg l^{-1} BAP (Table 1). Maximum shoot production was obtained on a medium containing 0.3 mg l^{-1} BAP (Table 1). Similarly, kinetin increased average number of shoots/explants. Increasing concentration of BAP or kinetin decreased shoot length.

3.2. In vitro root formation

Microshoots were successfully rooted *in vitro* on MS medium supplemented with 0.0, 0.2, 0.4, 0.6, 0.8 or 1.0 mg l^{-1} of IBA, IAA or NAA. Root formation start after 14 days growing period. Rooting occurred in bases of shoots growth on solid media supplemented with IBA, IAA, or NAA (Table 2). No callus formation appeared at the bases of the cuttings. Increasing IBA, IAA or NAA concentrations resulted in a significant effect on root length (Table 2). Maximum root number was obtained with the addition of IBA at 0.2 mg l^{-1}, with an average of 18.75 roots per microshoot. Root length was significantly decreased with the use of IBA, IAA and NAA at 1.0 mg l^{-1} (Table 2). Increasing IBA concentrations significantly increased number new leaves formation (Table 2). Maximum number (17.07) of leaves was obtained at 0.4 mg l^{-1} IAA, followed by 14.75 at 0.4 mg l^{-1} IBA (Table 2).

Table 1; Influence of BAP or kinetin on shoot length, number of shoots after six weeks growth period of *in vitro* grown *C. morifolium*

Growth regulators (mg l^{-1})	Shoot length (cm)	Number of new shoot
	BAP	
0.00	2.51	1.98
0.30	3.79	4.35
0.60	2.12	4.15
0.90	2.04	4.13
1.20	1.98	3.90
1.50	1.90	3.45
	Kinetin	
0.30	3.11	1.98
0.60	3.26	1.65
0.90	3.00	2.10
1.20	2.97	3.00
1.50	2.44	1.95
Means	2.88	1.45
LSD at 0.05	0.287	2.02

Values represented means; each experiment consists of five treatments, each treatment consisted of 4 replicates and each experiment was repeated twice. Differences between individual treatments were determined with the least significant difference (LSD) at 0.05 level of probability.

3.3. Physiological responses of microshoots to salinity levels

3.3.1. Growth

Effect of NaCl on shoot length, number of new shoots, number of new leaves, fresh weight, and dry weight after six weeks growth periods on MS medium supplemented with 0.3 mg l^{-1} BAP were reported (Table 3). After six weeks growth periods on MS medium supplemented with 0.3 mg l^{-1} BAP, shoot length and number were decreased with the increased salt concentration in the medium (P=0.05). Number of shoots, number of new leaves, fresh weight, and dry weight decreased with increasing salt in the medium. The highest number of shoots was 2.80 at 20 mM NaCl whereas the lowest number of shoots was 1.00 at 300 mM NaCl (P=0.05). Whereas the highest number of new leaves was produced (26.15) at 40 mM NaCl and then it was decreased with increasing NaCl and it was ceased at 300 mM NaCl. Control treatment produced 13.79 new leaves. Fresh weight was increased with the increase in NaCl concentration (P=0.05). Maximum fresh weight (1.68 g) was obtained at 60 mM NaCl, whereas the minimum fresh weight (0.24 g) was obtained at 300 mM NaCl (Table 3). Similar to fresh weight, dry weight was decreased with increasing salt in the medium, and the highest dry weight was obtained at 60 mM NaCl (Table 3).

Table 2: Effect of IBA, IAA or NAA on roots number, root length, number of new leaves after six weeks growth periods of *in vitro* grown *C. morifolium*

Growth regulator (mg l^{-1})	Root number	Root length (cm)	Number of new leaves
0.0	5.65	3.23	9.33
IBA			
0.2	18.75	4.94	13.32
0.4	16.68	4.63	14.75
0.6	15.52	3.82	13.35
0.8	15.35	3.61	11.95
1.0	14.18	3.20	9.41
IAA			
0.2	10.68	4.59	14.36
0.4	10.26	3.89	17.07
0.6	9.62	3.48	16.99
0.8	8.32	3.01	16.25
1.0	7.20	2.44	13.60
NAA			
0.2	14.82	4.94	11.20
0.4	14.58	4.70	13.31
0.6	13.72	4.20	13.05
0.8	13.52	3.93	12.32
1.0	12.45	1.94	9.70
LSD at 0.05	0.950	0.484	0.958

Values represented means; each experiment consists of five treatments, each treatment consisted of 4 replicates and each experiment was repeated twice. Differences between individual treatments were determined with the least significant difference (LSD) at 0.05 level of probability.

3.3.2. Chlorophyll and carotenoid content

Chlorophyll content decreased as NaCl concentration increased in the media (Table 4). Chlorophyll a and chlorophyll b contents were declined with elevated salinity. Maximum value for chlorophyll a was 122.56 (µg g^{-1} Fw) at 0.0 NaCl (Table 4). Chlorophyll b content decreased with increasing salt in the medium (P=0.05). However, the chlorophyll a/b ratio was not affected by NaCl concentration. Carotenoid content decreased with increasing salts in the medium. Carotenoid content was the highest (39.61 µg g^{-1} Fw) at 0.0 mM NaCl concentration, whereas the lowest content (13.306 µg g^{-1} Fw) was obtained at 300 mM. Maximum carotenoid-chlorophyl ratio (0.2005 µg g^{-1}) was obtained at 300 mM NaCl which was lower than the ratio (0.2001 µg g^{-1}) obtained at the control (Table 4).

Table 3: Effect of NaCl on shoot length, number of new shoots, number of new leaves, fresh weight, and dry weight of *in vitro* grown *C. morifolium* after six weeks growth period on MS medium supplemented with 0.3 mg l^{-1} BAP.

NaCl (mM)	Shoot length	Shoot number	Leaves number	Fresh weight (g)	Dry weight (g)
0	2.70	1.94	13.79	0.31	0.01
20	2.98	2.80	21.90	0.57	0.04
40.0	2.08	2.20	26.15	0.75	0.07
60.0	1.95	2.15	25.30	1.68	0.17
80.0	1.94	2.14	24.90	1.17	0.15
100.0	1.88	1.89	21.16	0.97	0.13
150.0	1.76	1.40	16.88	0.67	0.10
300.0	1.59	1.00	0.00	0.24	0.07
LSD at 0.05	0.146	0.153	0.613	0.055	0.003

Values represented means; each experiment consists of five treatments, each treatment consisted of 4 replicates and each experiment was repeated twice. Differences between individual treatments were determined with the least significant difference (LSD) at 0.05 level of probability.

3.3.3. Mineral composition

Table 5 represents the Na, K, N contents in leaf tissue of *C. morifolium* after six weeks growth periods. As NaCl increased, nitrogen content decreased. Maximum value 7.33% was obtained at control, and then it started to decline dramatically until it reached its minimum value (3.61) at 300 mM NaCl (Table 5). Na content in leaf tissue of *C. morifolium was* significantly increased as NaCl increased.

As NaCl in the medium increased, sodium content increased in plant tissue after six weeks growth periods (Table 5). Na concentration reached maximum value 7.21% at 300 mM. Maximum value was significantly different as compared to minimum value (0.30) which was obtained at the control treatment. Potassium content in leaf tissue of *C. morifolium* was significantly decreased as NaCl level increased in the medium (Table 5). Maximum value (6.02) occurred at control (0.0 mM NaCl) and then it

decreased with salinity elevated salinity reaching a minimum value of 1.28 at 300 mM NaCl. There are significant differences among all values (Table 5).

Table 4: Effect of NaCl on chlorophyll and carotenoid content of *in vitro* grwon *C. morifolium* after six weeks growth periods on MS medium supplemented with 0.3 mg l^{-1} BAP.

Concentration (mM)	Chl a (µg g^{-1} Fw)	Chl b (µg g^{-1} Fw)	Chl a/b ratio	Total Chl (µg g^{-1} Fw)	Carotenoid (µg g^{-1} Fw)	Carot-Chl ratio (µg g^{-1})
0	122.56	75.41	1.63	197.96	39.61	0.2001
20	111.10	68.79	1.61	179.89	36.02	0.2002
40	100.83	62.51	1.61	163.34	32.71	0.2002
60	88.34	54.81	1.61	143.15	28.67	0.2002
80	78.93	49.05	1.61	127.97	25.62	0.2002
100	65.17	40.57	1.61	105.74	21.18	0.2003
150	52.87	32.95	1.60	85.83	17.19	0.2003
300	40.87	25.51	1.60	66.37	13.31	0.2005
LSD at 0.05	0.046	0.033	0.001	0.074	0.017	0.001

Values represented means; each experiment consists of five treatments, each treatment consisted of 4 replicates and each experiment was repeated twice. Differences between individual treatments were determined with the least significant difference (LSD) at 0.05 level of probability.

Table 5: Effect of different concentrations of NaCl on sodium, potassium and nitrogen contents of *in vitro* micoshoots of *C. morifolium* grown on medium supplemented with 0.3 mg l^{-1} BAP after six weeks growth periods

Concentreation mM	**Na%**	**K%**	**N%**
0	0.30	6.02	7.33
20	1.13	5.11	6.83
40	1.96	4.62	6.67
60	3.18	4.24	6.20
80	4.44	3.81	5.63
100	4.76	3.46	5.34
150	6.30	2.81	4.22
300	7.21	1.28	3.61
LSD at 0.05	0.130	0.504	0.277

Values represented means; each experiment consists of five treatments, each treatment consisted of 4 replicates and each experiment was repeated twice. Differences between individual treatments were determined with the least significant difference (LSD) at 0.05 level of probability**.**

As NaCl in the medium increased, sodium content increased in plant tissue after six weeks growth periods (Table 5). Na concentration reached maximum value 7.21% at 300 mM. Maximum value was significantly different as compared to minimum value (0.30) which was obtained at the control treatment. Potassium content in leaf tissue of *C. morifolium* was significantly decreased as NaCl level increased in the medium (Table 5). Maximum value (6.02) occurred at control (0.0 mM NaCl) and then it decreased with salinity elevated salinity reaching a minimum value of 1.28 at 300 mM NaCl. There ere significant differences among all values (Table 5).

3.3.4. Proline and protein content

Proline content increased with the increases in NaCl (Figure 1). The lowest proline value was obtained at 0.0

mM NaCl, whereas the highest proline content was obtained at 300 mM NaCl. Protein contents declined with the increases in NaCl concentration (Figuer. 2). Protein content was 40.41 and 22.58 mg g l^{-1} . FW when the medium was supplemented with 20 and 300 mM NaCl, respectively.

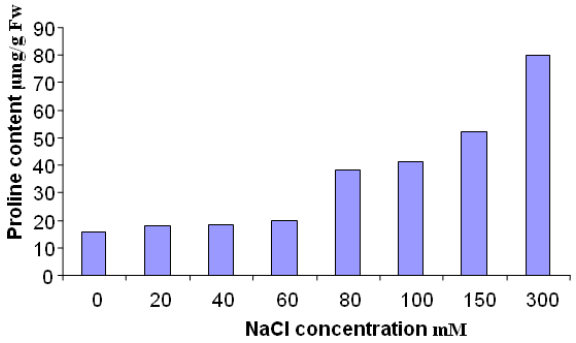

Figure 1: Effects of different level of NaCl on proline content of *in vitro* grwon *C. morifolium* after six weeks growth period on MS medium supplemented with 0.3 mg l^{-1} BAP. Values represented means; each experiment consists of five treatments, each treatment consisted of 4 replicates and each experiment was repeated twice. *LSD at 0.05=0.2325*

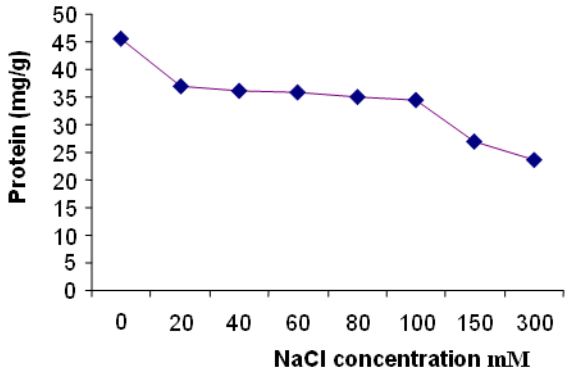

Figure 2: Effect of different level of NaCl on protein content of *in vitro* grwon *C. morifolium* after six weeks growth periods. Values represented means; each experiment consists of five treatments, each treatment consisted of 4 replicates and each experiment was repeated twice. ***LSD at 0.05= 3.199.***

4. Discussion

4.1. In vitro propagation

The study aimed at identifying the best media for shoot proliferation and studying the effect of NaCl on *in vitro grown C. morifolium*. The results of the investigations indicated that an efficient *in vitro* propagation method, with high levels of survival and reproducibility, could be achieved for *C. mirifolium.* BAP and Kinetin stimulate cell division (Table 1). Increased BAP to 0.3 mg l^{-1} produce maximum microshoot. Optimum shoot proliferation of juvenile and adult chrysanthemum plants was obtained on MS medium containing 2.22 µM BAP (Long *et al*., 2006). However, proliferation was inhibited at 4.4 µM BAP, which is similar to our finding in *C. morifolium* where proliferation rate decreases at higher BAP concentration (Table 1).

Kinetin has been used for shoot proliferation on cotton (Meloni *et al*., 2001), and chrysanthemum (Karim *et al*., 2002; Long *et al*., 2006); thus the effect of kinetin was varied with concentration (Table 1). The effectiveness of BAP was proved to be superior to kinetin. MS media supplemented with BAP have been satisfactory for many species and cultivars (Hutchinson, 1981). Superiority of BAP over other growth regulators in producing *in vitro* shoots has also been confirmed in other plants like *Prunus amygdalus* (Shatnawi, 2006), *Arachis hypogaea* (Mhatre *et al*., 1985) and *Atropa beladona* (Benjamin *et al*., 1987). On the other hand, Karim *et al.* (2002) approved the superiority of BAP over Kinetin in regeneration of shoots from chrysanthemum explants. Karim *et al*. (2003) reported that, among different concentrations used, best response towards shoot proliferation from nodal and shoot tip explants was obtained on MS supplements with 1.0 mg l^{-1} BAP.

Root induction was undertaken using three growth regulators IBA, IAA or NAA. Percentage of root induction and number of roots per shoots were highly influenced by concentration and types of auxin (Table 2). IBA confirmed its superiority over other auxins regarding number of new leaves, number of roots and root length (Table 2). Similar results on chrysanthemum were obtained by Long *et al*. (2006) and Karim *et al*. (2002). Similar to Hoque *et al*. (1995) on C. *morifolium*, maximum number of roots was obtained on MS media supplemented with 0.2 mg/L IBA (Table 2). Whereas Long *et al*. (2006) reported that highest number of roots where obtained when chrysanthemum microshoot cuttings where treated with IBA. In our study maximum root length (4.94) was obtained by using 0.2 mg l^{-1} IBA or NAA (Table 2). This result is similar to previous finding by Karim *et al.* (2002), who reported that 0.2 mg l^{-1} IBA produced the highest root length. IAA produces the highest number of leaves, whereas NAA produce the highest fresh weight and dry weight at 0.2 and 0.4 mg l^{-1} concentration (Table 2).

4.2. Physiological responses of microshoots to salinity

Increasing salinity reduced growth rate of chrysanthemum microshoots. Number and length of new shoots were declined with increasing salt in the growth media (Table 3). Similar results were obtained in microshoots of *Citrus aurantium* (Shiyab *et al*., 2003). Carvaja *et al*. (1998) found that muskmelon shoot growth decreased as salt concentration increased. Similar results were obtained on olive (Shibli and Al-Juboory, 2002). Number of new leaves was reduced at 60 mM NaCl, generating zero number of new leaves at 300 mM NaCl (Table 3). This reduction could be caused by toxicity associated with excessive uptake of Na (Yong *et al*., 2004) and nutrition imbalance (Cabanero *et al*., 2004).

Increased salt concentration caused an increase in fresh weight, up to 80m M NaCl) (Table 3). This agreed with the result obtained by Shibli *et al.* (2000) on apple microshoot. On the other hand, dry weight showed a similar pattern in which it increased until reaching the maximum at 80 mM NaCl (Table 3). Thus the reduction in fresh weight and dry weight could be due to salinity induced water deficit which reduced translocation of assimilates. Plant responds differently to alternation in the medium and this causes change in physiological responses

through it metabolic pathways (Harrak *et al*., 1999). Different stress conditions can cause changes in the physiological process of the plants. Chlorophyll and caretenoid content were decreased with progressive increasing in NaCl concentration (Table 4).

Mineral nutrients and uptack is adversely affected by high salinity levels (Al-Karaki *et al*., 1995). In this study nitrogen and potassium responded in a similar pattern. As salinity increased, N and K contents were decreased whereas Na contents were increased (Table 4). A similar result was obtained in hydroponic tomato (Al-Karaki, 2000; Alian *et al*., 2000; Flores *et al*., 2001.), cucumber microshoot (Abu-Romman and Suwwan, 2008) and lettuce (Irigoyen *et al*., 1992; Tarakcioglu and Inal, 2002), sour orange (Shiyab *et al*., 2003). Reduction of K content in plant leaves adversely affect metabolic function and eventually reduce plant growth (Greenway and Munns 1980).

Proline contents in chrysanthemum leaves increased significantly with increasing salt concentration (Figure 1). A similar result was obtained in tomato leaves (Bolarin *et al*., 1995). Proline, an osmotically active substance, is usually released from the cell due to salt and osmotic shocks (Gangopdhyay *et al*., 1997). Proline accumulation may be increased in plants tissue; this may be due to hyperosmotic stresses, primarily drought (Balibrea *et al*., 1997), and salt stress (Guerrier, 1998; Aziz *et al*., 1999). Proline accumulation could be used as a salt sensitive trait because it inhibits callus growth (Bolarin *et al.,* 1995; Cano *et al*., 1996).

Salinity reduced protein content of chrysanthemum microshoots (Figure 2). The reduction in the protein level in stressed tissues appeared to be due to more degradation of proteins as well as overall inhibition of protein synthesis under stress (Kumar and Singh, 1991).

The results of this study indicated that an efficient *in vitro* propagation method, with high levels of survival and reproducibility, could be achieved. BAP at 0.3 mg l^{-1} produced the highest number of new shoots, with the highest proliferation. Growth of *C. morfolium* microshoots was adversely affected by elevated NaCl levels. Plant growth at 20 mM NaCl was significantly improved. Furthermore, the production of adventitious microshoots was reduced by using higher concentrations of NaCl (Ben Amor *et al*., 2005). Chlorophyll and caretenoid content were decreased with progressive increase in medium NaCl supplements.

Mineral content in *C. morifolium* tissue were significantly affected by increasing NaCl levels. The effectiveness of this system, however, should be further tested on a greater number of genotypes with known performance for root characteristics related to drought tolerance under field conditions. In such comparisons, data from field evaluations must be based on well-conducted trials repeated over years. Unfortunately, such evaluations for root characteristics are available for only a few genotypes. Nevertheless, the results of the present study clearly showed that screening of *C. morifolium* simulate the *in vivo* conditions which might provide a high efficacy *in vitro* screening method for abiotic stresses. This might identify promising cultivars recommended for growers in salt-affected areas of the world. Further physiological and molecular studies are still needed to understand many physiological issues of *C. morifolium*, to salinity tolerance**.**

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Cypermethrin Induced Biochemical Alterations in the Blood of Albino Rats

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Abstract

This study was carried out to investigate blood biochemical alteration in albino rats after cypermethrin treatment. Nitrogen contents of blood in the albino rats have been influenced by the amount of cypermethrin used for toxicity in the acute (1d) and sub chronic (7,14and21days) treatment in the present investigation. The free amino acid, total protein, urea, urea nitrogen, uric acid and creatinine of blood were increased while blood albumin was decreased after both the treatments.

Keywords: Nitrogen contents, blood, cypermethrin.

1. Introduction

Pesticides are widely used throughout the world in agriculture to protect crops and their residues have affected the environment adversely. Their poisoning is an important cause of morbidity and mortality in developing countries. The uses of such biologically active compounds possess potential problems of toxicity among those who manufacture, formulate or use these compounds. The pyrethroid represents a relatively new group of synthetic insecticides, although members of the group have been commercialized since mid 1950s. Their popularity has been increasing substantially in recent years, and new members are constantly being developed and commercialized. The pyrethroid has been proved as effective insecticides, and thus was applied at low doses.

The toxicity of pyrethroid insecticides to mammalian animals has received much attention in resent years because animals exposed to these insecticides exhibited changes in their physiological activities beside other pathological features (Sakr 2002). Although extensive research work is under way in different laboratories on various aspect of synthetic pyrethroid, including metabolism, pharmacological characteristics, ecotoxicity and detection of residues (Cremer &Seville1982, Ray 1982,Casida *et al.*1983), little attention has been paid to their biochemical effects in non target species. Cypermethrin is a synthetic pyrethroid. It is widely used as insecticide in developing countries controlling pests (Jayakumar *et al*. 2008). The present report aims at studying the effects of sub lethal doses of cypermethrin on some blood biochemical changes in albino rats after 1day and 21 days continuous feedings.

2. Materials and methods

Adult albino rats (*Rattus norvegicus)* weighing 125-135gm from an inbred colony representing both the sexes were selected for the experimentation. Rats were housed in polypropylene cage and were allowed standard pellet diet and water *ad libitum*. Cypermethrin 25%EC was procured from Syngenta group company, India .Cypermethrin suspended in coconut oil was administered orally by gavage. The acute oral LD_{50} value was calculated as 620 mg/kg body weights .The doses for acute (1 day) and sub chronic (7, 14, and 21 days) studies were 310mg/kg.bwt. and 15mg/ kg.b.wt., respectively. Doses were selected on the basis of LD50. The control animals received the same volume of coconut oil alone. Rats used for experimentation were selected randomly either of sex and anaesthetized with chloroform. The blood samples, collected from the rats with the help of sterilized disposable syringe, fitted with a hypodermic needle and stored in plain vials. Serum was assayed for free amino acid, total proteins and albumin, urea, urea nitrogen, uric acid, and creatinine.

The free amino acid contents obtained from serum were analyzed according to the Ninhydrin method of Moore and Stein (1957). Total serum proteins and albumin were estimated by Biuret method of Gornall *et al.* (1949) modified by Dumas *et al.* (1971). The estimation was done by using diagnostic reagent kit for *in vitro* determination of total proteins in serum, manufactured by Span diagnostics Ltd. India. The estimation of urea was performed according to the DAM method (Evans, 1968) using the diagnostic reagent kit for *in vitro* determination of urea in serum, manufactured by Span diagnostics Ltd. India. Urea nitrogen was estimated by mathematical

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calculation taking the serum urea value in consideration. The uric acid was determined by uricase/PAP method (Fossati&Prencipe, 1980) using the diagnostic reagent kit manufactured by Crest bio systems, a division of coral clinical systems Goa, India. Concentration of serum creatinine was determined by alkaline picrate method (Henry *et al.* 1974) using the diagnostic reagent kit same above. Statistical significance difference between experimental and controls values were calculated according to Fisher's student't' test (Fisher and Yates, 1963).

3. Results

Results (Table-1) indicate that treatment with cypermethrin caused a significant increase in serum, free amino acid, total proteins, urea, urea nitrogen, and uric acid, while serum albumin decreased significantly after all the treatments. Serum creatinine declined after acute treatment but increased after 14 & 21days treatment.

Table-1 Effect of Cypermethrin on Serum Biochemical Parameters in Albino Rats

Serum Biochemical Parameters	Control[o]	Post Treatments Days			
		1st Day(acute)	7th Day (sub chronic)	14th Day (sub chronic)	21stDay (sub chronic)
Free amino acid (mg/dl)	11.13±.46	15.27±.65**	11.47±.26	13.67±.33*	16.00±.52**
Total Protein (gm/dl)	6.06±.21	6.83±.12*	6.71±.06*	6.69±.06*	6.94±.05*
Albumin (gm/dl)	4.14±.14	3.48±.05*	3.78±.09	3.62±.14	3.94±.02
Urea (mg/dl)	23.40±.79	32.21±.32***	28.02±.23**	29.41±.21**	32.82±.08***
Urea Nitrogen (mg/dl)	10.93±.37	15.04±.15***	13.09±.11**	13.73±.10**	15.33±.40**
Uric Acid (mg/dl)	4.20±.44	6.70±.01***	4.41±.25	5.84±.21***	6.56±.14***
Creatinine (mg/dl)	0.64±.41	0.13±.08*	0.82±.08	1.41±.01***	2.85±.01***

O=controls were given the same quantity of diluent (coconut oil), *=Significant p<0.05, **=Highly Significant <0.01, ***=Very Highly Significant<0.001

4. Discussion

Table 1 shows the overall means of serum free amino acid, protein, albumin, urea, urea nitrogen, and creatinin. Present observation suggests that the level of free amino acid, protein, and urea content in treated animals were influenced by the amount of cypermethrin used for poisoning, i.e., it shows dose dependent toxicity and duration of toxic effects.

Alteration in serum free amino acid (FAA) content reflects either an increase or a decrease in protein break down or synthesis. Alteration in deamination and transamination of amino acid are associated with changes in nitrogen metabolism, which can be observed in terms of serum urea level. FAA is also involved in the formation of excretory product by the process of conjugation. Increase in FAA content associated with increase in urea level probably reflects an increase in transamination and production of biogenic nitrogenous compound in the form of urea. This is well supported by the increase in urea level in present investigation after cypermethrin toxicity. Lower doses for long period may exert hypertrophy of hepatocytes and thus increase the total protein and FAA in serum of rats (Shakoori *et al.*1988). The increased level of protein in human blood is due to flumethrin (Box and Lee 1996) and in albino rat after cybil, a synthetic pyrethroid (Saxena and Saxena 1997). This once again supports the finding in the present study. The changing levels of serum albumin, thus, provide valuable indices of severity, progress, and prognosis in hepatic disease. Decreased albumin in serum indicates hepatocellular origin of liver disease (Sood 2006).

The increased blood urea concentration in rats treated with cypermethrin are in agreement with the results obtained by AI-Qarawi *et al.*, (1999) and Yousef *et al.,* (2003). Elevated serum urea is also correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea because of increased synthesis of enzyme involved in urea production (Murray *et al.*, 1990). Pesticides induced increase in urea level observed in the present study may be due to the effect of pesticides on liver function, as urea is the end product of protein catabolism (Coles 1986). An increase in serum creatinine was recorded in cypermethrin-treated rats. Abu-El-Zahab *et al.*, (1993) and Sakr *et al.,* (2001) obtained the same results in rats treated with pyrethroid. In the light of these observations, it is recommended that cypermethrin should be used with caution, at it could be hazardous to domestic animals and human beings as well.

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Seasonal variation in physico-chemical properties and zooplankton biomass in Greater Zab River -Iraq

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Abstract

Seasonal variation of zooplankton biomass has been carried out at two selected sites on Greater Zab River. Monthly samples of water were collected during period from January to December 2008. Some physico-chemical properties of water were studied including water temperature (from 8 to 30.5 °C), hydrogen ion concentration (from 6.3 to 8), EC (from 255 to 821 $\mu s.cm^{-1}$), turbidity (10- 85 NTU), dissolved oxygen (from 4.2 to 12.35 $mg.l^{-1}$), BOD_5 (from 0.6 to 24 $mg.l^{-1}$), COD (from 15 to 294 $mg.l^{-1}$), ammonium level (from 0.51 to 1.66 µg NH_4-$N.l^{-1}$), nitrite (from 29.1 to 71.3 µg $NO_2^-N.l^{-1}$), nitrate (from 112.9 to 327.4 µg $NO_3^-N.l^{-1}$), reactive phosphate (from 195.7 to 558 µg PO_4-$P.l^{-1}$) and calcium concentration (from 24.7 to 72 $mg.l^{-1}$). Concerning planktonic communities, the results showed that the total zooplankton number was ranged from 100 $individuals.l^{-1}$ to 6650 $individuals.l^{-1}$ and the total phytoplankton population was 18773 $cell.l^{-1}$ to 269448 $cell.l^{-1}$. Statistical analysis showed that there was a positive correlation between total count of zooplankton and total count of phytoplankton during studied period with r = 0.38 in site (1) and r = 0.31 in site (2). However, the results of water quality of Greater Zab River showed that the values of WQI were 66.15% and 64.61% respectively in both studied sites which can be considered as fair according CCME (2001) and medium according EU (1975).

الملخص

تم دراسة التغيرات الفصلية لمجتمع الهائمات النباتية و الحيوانية في موقعين على نهر زاب الكبير. جمعت العينات شهرياً خلال فترة كانون الثاني الى كانون الاول 2008. وقد تم دراسة بعض الصفات الكيمياوية والفيزياوية للماء وكانت النتائج على النحو التالي: درجة حرارة الماء (تراوحت من 8 الى 30.5)، تركيز ايون الهايدروجيني (تراوحت من 6.3 الى 8)، التوصيل الكهربائي (تراوحت من 255 الى 821 ميكروسمنز/سم)، عكورة المياه (10 الى 85 وحدة عكورة)، الاوكسجين المذاب (4.2 الى 12.35 ملغم/لتر)، المتطلب الحيوي للاوكسجين (0.6 الى 24 ملغم/لتر)، المتطلب الكيمياوي للاوكسجين (15 الى 294 ملغم/لتر)، امونيا (0.51 الى 1.66 ميكرو غرام NH4-N/لتر)، نتريت (29.1 الى 71.3 ميكرو غرام NO2- N/لتر)، نترات (112.9 الى 327.4 ميكرو غرام NO3- N/لتر)، الفوسفات (195.7 الى 558 ميكرو غرام PO4- P/لتر) وتركيز ايون الكالسيوم (24.7 الى 72 ملغم/لتر). اما بالنسبة الى دراسة كتلة الهائمات، فان النتائج اوضحت بان مجموع الهائمات الحيوانية كانت تتراوح بين 100 فرد/لتر الى 6650 فرد/لتر ومجموع الهائمات النباتية كانت تتراوح بين 18773 خلية/لتر الى 269448 خلية/لتر، كما ظهرت احصائيا بان هناك علاقة موجبة بين اعداد الهائمات الحيوانية والنباتية حيث كانت قيمة r= 0.38 للموقع الاول و r= 0.31 للموقع الثاني. كما ان نتائج اختبار نوعية المياه اعتماداً على (EU, 1975) و (CCUM, 2001) اوضحت بان قيمة تقيم نوعية المياه كانت 66.15% و 64.61% على التوالى في كلا من محطتي الدراسة كوركوسك و جمة دبس.

Keywords: Zooplankton, Phytoplankton, Physico-chemical properties, Water quality, Greater Zab River.

1. Introduction

Zooplankton is tiny animals found in all aquatic ecosystems, particularly the pelagic and littoral zones in the ocean, also in ponds, lakes, and rivers. They are classified by size and/or by developmental stage. Size categories include: **picoplankton** that measure less than 2 micrometers, **nanoplankton** measure between 2 - 20 micrometers, **microplankton** measure between 20 - 200 micrometers, **mesoplankton** measure between 0.2 - 20 millimeters, **macroplankton** measure between 20 -200 millimeters, and **megaplankton**, which measure over 200 millimeters (Lynn, 2007).

The zooplankton community is composed of both primary consumers (which eat phytoplankton) and secondary consumers (which feed on the other zooplankton). They provide a direct link between primary producers and higher trophic levels such as fish. Nearly all fish depend on zooplankton for food during their larval phases, and some fish continue to eat zooplankton in their entire lives (Madin *et al.*, 2001).

Many studies on zooplankton community and grazing with phytoplankton were conducted in different parts of the world. Merrick and Ganf (1988) made a study on effects of zooplankton grazing on phytoplankton communities in Mt Bold Reservoir in Australia. Zooplankton grazing on bacteria and phytoplankton in

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Nakdong River was carried out by Kim *et al.* (2000) in Korea. However, Tan *et al.* (2004) made an investigation on seasonal variation in zooplankton composition and grazing impact on phytoplankton in Pearl River estuary in China. The present investigation aimed to study the seasonal variations of zooplankton and its relations to phytoplankton, in addition to study of some physio-chemical properties and water quality of Greater Zab River.

2. Material and Methods

2.1. Study area

Greater Zab River is a large river (392 km) in Iraq. This river is one of the main tributary of the Tigris. It is originated mainly from mountainous area of Iran and Turkey. It is situated between 36°-37° north latitudes and 43°-44° east longitude (Susa, 1960). During this study samples were collected in two sites; the first was located near Kaurgosk village and second was located at Chamadbz village about 20 Km from site one (Fig. 1).

2.2. Sampling

Samples for physical, chemical and biological variables were performed from two sites during period extended from the January to the December 2008. Water samples were collected for chemical and biological analysis using pre-washed polyethylene bottle by water sample twice before filling.

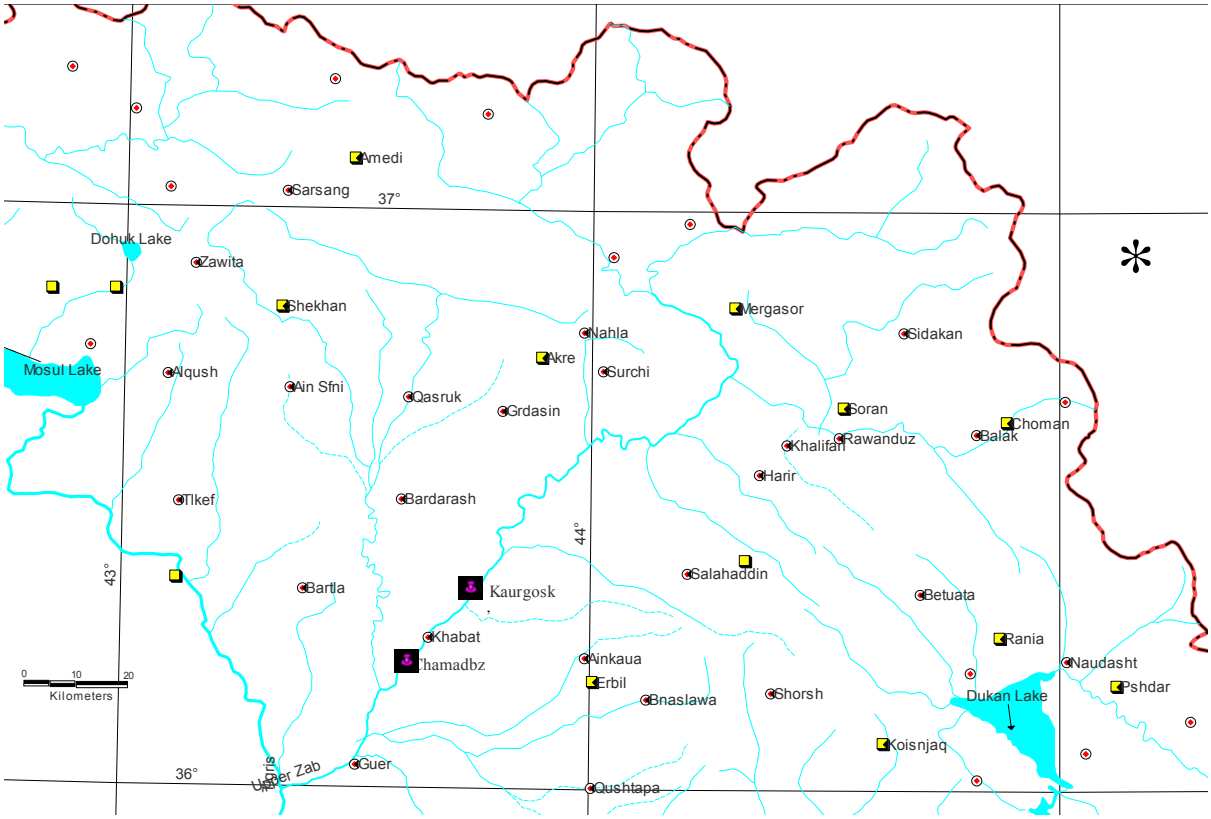

Fig. (1) Map of Iraq showing the studying sites on Greater Zab River

The studied physico-chemical parameters:- water temperature (by using precise mercury thermometer), hydrogen ion concentration (by using pH-meter), electrical conductivity (by using EC-meter), turbidity level (by using turbidity-meter), dissolved oxygen (titrimetric methods), biological and chemical oxygen demand (titrimetric methods), ammonium, nitrite and nitrate-nitrogen, reactive phosphate (by using spectrophotometric methods) and calcium ion content(by using titrimetric methods) were measured according to A.P.H.A. (1998).

Empirical equation was described by (Sanchez *et al.*, 2007) were used for water quality index evaluation:-

$$WQI = k\frac{\sum_i CiPi}{\sum_i Pi} \quad (1)$$

Where:

K= is a subjective constant

Ci= is the normalized value of the parameter

Pi= is the relative weight assigned to each parameter.

The obtained results of the equation were compared with the Canadian and European standard for surface water quality.

Enumeration of phytoplankton was conducted based on a modification of the membrane filtration technique of McNabb (1960), Hinton and Maulood (1979). The phytoplankton was counted by using Olympus compound

microscope, the number of algal cell per liter was calculated by the following formula:

Total No. of phytoplankton (cell/l) =
(D * area of filter)/(area of 30 fields * liter of sample filtered)
D = total count of 30 fields

Zooplankton sample were collected by filtering 30 liter of the river water using plankton net (55 µm mesh size), concentrated sample were fixed with 5% formalin.

Counting of zooplankton samples was undertaken in the laboratory using a compound microscope and the following references were used:-Edmondson (1959), Scourfield and Harding (1966) and Smith (2001). The results expressed as individual.l^{-1}.

2.3. Statistical analysis:

Statistical analysis of physico-chemical data was done using Completely Randomized Design (CRD) to study the effect of different site and date of sampling, and LSD values were calculated to compare between each of ecological data. Also, simple correlation analysis was done between total count of zooplankton and total count of phytoplankton (Snedecor and Cochran, 1980).

3. Results:-

In this study, a number of phisco-chemical parameters were studied (Table 1). Water temperature of studied sites during studied period was ranged between 8 to 30 ˚C, and statistically the regional and monthly variation showed non significant differences ($p< 0.05$).

Hydrogen ion concentration of studied river in studied sites was ranged from 6.3 to 8. Statistical analysis showed that pH value was significantly different ($P< 0.05$) between studied sites and date of sampling.

Electrical conductivity of studied sites ranged between 255 to 821 µs.cm^{-1}, and the results showed that there was a significant difference ($P< 0.05$) between studied sites and time of sampling.

Turbidity levels revealed that they were obviously significant differences ($p<0.05$) during studied period it was ranged between 10 to 85 NTU.

Dissolved oxygen concentrations were ranged from 4 to 12.35 mg.l^{-1} . The statistical analysis showed that there was no significant differences ($p< 0.05$) were observed between studied sites.

The results were revealed that the BOD_5 in both studied sites was ranged between 0.6 to 24 mg.l^{-1}. While, COD value was ranged from 15 to 294 mg.l^{-1}, and the statistical analysis showed that there was a significant differences ($p<0.05$) between studied site and sampling date.

Data of ammonia, nitrite and nitrate nitrogen indicated significant differences ($p< 0.05$) between studied sites and sampling date. Ammonia level was ranged from 0.51 to 1.66 µg NH_4-N.l^{-1}. While, nitrite was ranged between 29.1 to 71.3 µg NO_2-N.l^{-1} and nitrate ranged between 112.9 to 327.4 µg NO_3-N.l^{-1}.

Phosphate content of studied river was ranged from 195.7 to 558 µg PO_4-P.l^{-1}, and statistically the results showed significant differences ($p< 0.05$) between the studied sites.

Table (1): Physico-chemical properties of Greater Zab River, data represented as mean ± S.E., with minimum and maximum value from January to December 2008.

Studied parameters	Kaurgosk	Chamadbz
Water Temperature (C°)	19.54 ± 0.71 10-26.5	19.06 ± 0.905 8-30.5
Hydrogen ion concentration	7.31 ± 0.007 6.3-8	7.16 ± 0.004 6.74-7.89
Electrical conductivity	1127.08 ± 65.9 283-821	442.5 ± 35.7 255-711
Turbidity level (NTU)	40.08 ± 13.21 10-85	48.16 ± 7.40 16-80
Dissolved oxygen (mg/l)	9.54 ± 0.25 6.5-11.34	9.36 ±0.34 4-12.34
BOD_5 (mg/l)	3.71 ± 1.98 0.6-23	3.80 ± 1.96 0.8-24
COD (mg/l)	91 ± 28.1 15-280	98 ± 26.7 15-294
NH_4 (µg NH_4-N/l)	0.92 ± 0.09 0.51-1.06	0.98 ± 0.09 0.7-1.66
NO_2 (µg NO_2-N/l)	50.2 ± 3.60 29.1-65	50.8 ± 3.58 29.1-71.3
NO_3 (µg NO_3-N/l)	208.4 ± 25.1 112.9-327.4	233.7 ± 26.2 135.4-293.5
PO_4 (µg PO_4-P/l)	424.21 ± 26.58 212.1- 515.5	393.1 ± 25.37 195.7- 558
Calcium concentrations (mg/l)	38.45 ± 3.78 27.4-72	42.4 ± 4.27 24.7-67

Calcium concentration of studied Zab was ranged from 24.7 to 72 mg.l^{-1}, and it is variations showed significant differences ($p< 0.05$) among studied sites and sampling date.

Concerning to biological study, the results showed that the total count of phytoplankton ranged between 18773 to 269446 cell.l^{-1}. Higher number of phytoplankton was observed in site (2) during September 2008, while lower number was recorded in the same site during June 2008. However, the total count of zooplankton was ranged from 100 to 6650 individual.l^{-1}. Higher number of zooplankton was recorded in site (1) during November 2008, whereas lower number was observed in site (2) during January 2008. From the statistical analysis observed that there is a positive correlation between total count of zooplankton and total count of phytoplankton with $r =0.38$ and $r = 0.31$ in both site respectively.

The result of water quality assessment showed that the value WQI were 66.15% in site (1) and 64.61% in site (2).

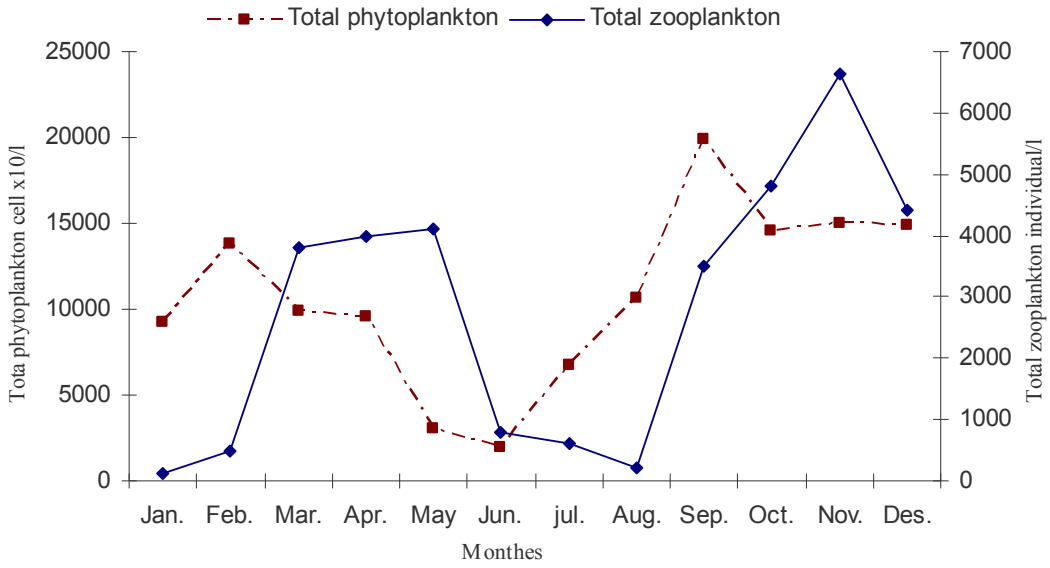

Fig. (2)Relationship between total zooplankton and total phytoplankton in site (1) during study period

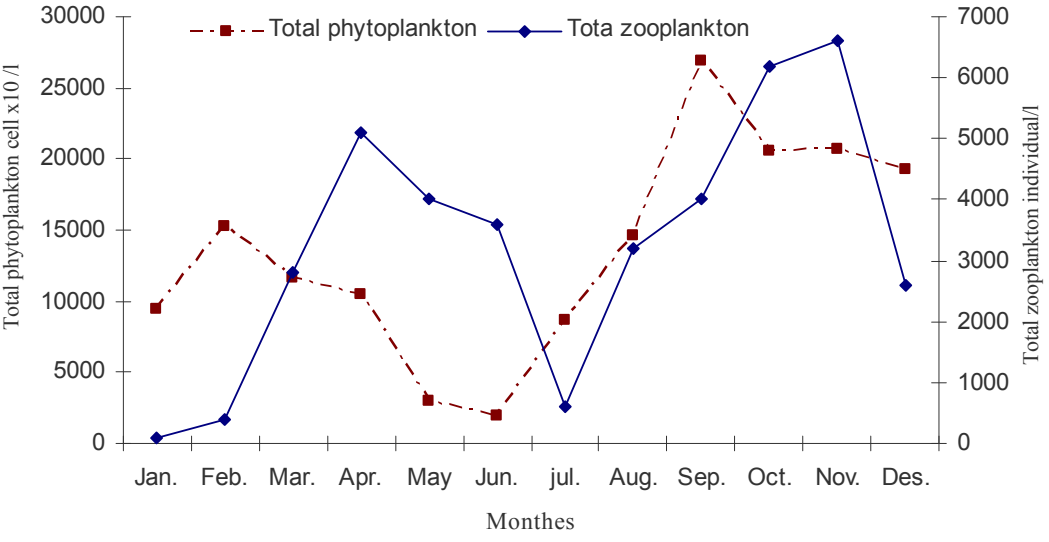

Fig. (3)Relationship between total zooplankton and total phytoplankton in Chamadbz during study period

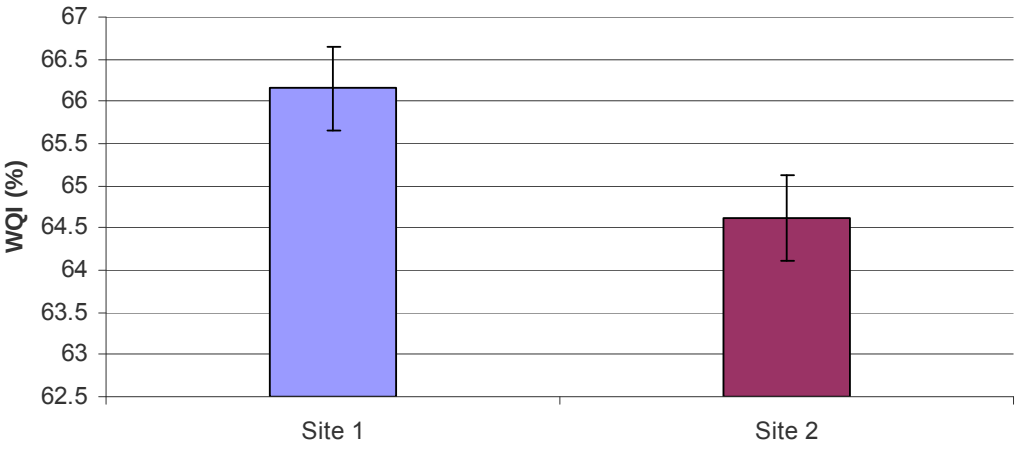

Fig. (4) Water quality index in Greater Zab River (value as mean)

4. Discussion:-

Water temperature is an important factor in any aquatic environments affecting on biological processes, in this study it was ranged between 8 to 30.5 ˚C. This variation may be due to changes in air temperature, and this result was similar to previous studies done by Ali (2007) and Shekha (2008). The pH value of Greater Zab River in study sites during of most studied period was alkaline side above 7, and this result agreed with Maulood *et al.* (1980) they reported that Iraqi inland water is regarded to be on the alkaline side of neutrality, reflecting geological formations of the area and the results are agree with the finding that recorded by Ali (2007) and Shekha (2008) in the same river. Electrical conductivity used as an indicator of water quality based on total dissolved salts (Rasheed, 1994). Generally, EC value was less than 500 µs.cm^{-1} in most of studied period in both sites and the results came in accordance with the known EC value for Iraqi inland water (Al-Naqshbandi, 2002). On the other hand, lower level of turbidity was 10 NTU recorded in June, while the higher level was observed during september 2008 which may be due to the increase of planktonic algal growth (Antoine and Alsaadi, 1982).

Oxygen content of water is one of the important factors, and it is very necessary for all living organisms (WHO, 2006). The DO content of studied river was ranged between 4 to4- 12.35 mg.l^{-1}. However, BOD_5 and COD values were ranged between 0.6 – 24 mg.l^{-1} and 15 – 294 mg.l^{-1}, respectively. Generally, high value of BOD_5 and COD were observed during the warm sommer months (July and August) which coincided with a high water temperature and low DO. These results were slightly lower than that reported by Shekha (2008) at the same river.

Ammonium nitrogen is commonly used as an indicator for organic matter content, while nitrite is another source of inorganic nitrogen and concerning as an indicator of water pollution. The high level of nitrite may be due to domestic sewage input to the river from the surrounding village. Nitrate is the stable form of combined nitrogen and it is an important factor which might limit growth of phytoplankton (ref.…). The results of ammonia, nitrite and nitrate nitrogen are agreed with those of Ali (2007) and Shekha (2008). Phosphorus is essential to the growth of algae and other biological organisms. The reactive phosphate concentration in studied river was ranged between 195.7 to 558 µg PO_4-P.l^{-1}. The high concentration of phosphate may be due to sewage water effluent and fertilizer application in surrounding agricultural area. This result was close to that reported by Shekha (2008). Calcium concentration in the studied Zab was ranged from 24.7 72 mg.l^{-1}. The present result was similar to that reported by Ali (2007), and it was lower than that reported by Al-Kubasi (1996).

Concerning to phytoplankton community, the results showed that the range of total phytoplankton population was 18773 cell.l^{-1} to 269448 cell.l^{-1}. Diatoms were the most dominant group of phytoplankton in the studied river, with dominancy of ***Cyclotella*** *sp.* in both sites. The total zooplankton population was from 100 individual.l^{-1} to 6650 individual.l^{-1} with mean value of 3266.7 ± 95.24x10^3 individuals.l^{-1} in site (1) and 2782.1 ± 102.01x10^3 individual.l^{-1} in site (2), which was lower to the value reported by Ahmed *et al.* (2004) and Chowdhury *et al.* (2007). Crustacea was dominant group among zooplankton especially Copepods with the dominancy of ***Eucyclops*** *sp.* in both sites during studied period. Similarly Patra and Azadi (1987) reported same result in Hulda River and Ali (2007) and Shekha (2008) also reported similar results in the greater Zab River. In site (1) zooplankton showed two main peaks, one extended from March to May and another one in November. In site (2) the first peak was in April to June the other one from October to November. The lower value of zooplankton was 100 individual.l^{-1} observed in site (2) during January 2008, and this may be due to decrease of phytoplankton number in which zooplankton grazing on it, in addition to low temperature during cold winter months. These results agreed with those results reported by Das and Srivastava (1956) in a pond in India and Chowdhury *et al.* (2007) in Borobila beel. Razzaque *et al.* (1995) reported that the zooplankton showed two peaks, one in the May and another in the October in Halti Beel. Both zooplankton and phytoplankton showed direct

grazing relationship with r = 0.38 in Kaurgosk and r = 0.31 in Chamadbz (Fig. 2, 3). Similar relationship was also reported by Ali *et al.* (1985) in a Lucknow pond in India, Patra and Azadi (1987) in Halda River, Chowdhury *et al.* (2007) in Borobila Beel.

The results of the seasonal variation in zooplankton population suggest that the most favourable period for growth is from the August to the November, and this may be due to increase of phytoplankton population. The same phenomenon was reported by Razzaque *et al.* (1995) and Ehshan *et al.* (2000).

Regarding to the evaluation of the water quality with using of WQI, the results showed that the values of WQI were 66.15% in site (1) and decreased to 64.61% in Chamadbz. Taking in to account both the points sampled, the water from Greater Zab River may be classified as fair (Grade D) depending on the classification of CCME (2001), meanwhile according to EU (1975) classification of water of both sites can be regarded as medium (Fig. 4).

Further study should be conducted on seasonal variation of zooplankton and phytoplankton in relation to some other parameters as water temperature, nutrients and pollution should be taken in Greater Zab River.

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Screening of *Terminalia bellirica* Fruits Extracts for its Analgesic and Antipyretic Activities

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Abstract

The study was designed to investigate the analgesic and antipyretic activities of ethanolic and aqueous extracts of ***Terminalia bellirica*** (family: Combrataceae) fruits (200 mg/kg, p.o.) in acetic acid-induced writhing, Eddy's hot plate method and brewer's yeast-induced fever models in mice and rats. Both extracts showed a significant decrease in the number of the writhes in acetic acid-induced writhing and increase in paw licking time to heat stimuli in the hot plate method. Both extracts showed a significant inhibition of elevated body temperature when compared to corresponding control. The results suggested that the ethanolic and aqueous extracts possessed significant analgesic and antipyretic activities.

Keywords: ***Terminalia bellirica***, analgesic activity, acetic acid-induced writhing, hot plate method, antipyretic activity, brewer's yeast-induced fever model.

1. Introduction

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states (Chattopadhyay *et al*., 2005 a). It is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive (Chattopadhyay *et al*., 2005 b). Normal body temperature is regulated by a center in the hypothalamus that ensures a balance between heat loss and production. Fever occurs when there is a disturbance of this hypothalamic 'thermostat', which leads to the set-point of body temperature being raised. Once there has been a return to the normal set point, the temperature regulating mechanisms (dilatation of superficial blood vessels, sweating etc.) then operate to reduce temperature (Anonymous, 1976). Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PgE2 biosynthesis (Cheng *et al*., 2005). Analgesia is the inability to feel pain while still conscious. From the Greek an-, without + algesis, sense of pain.

In view of this, different therapeutic agents are employed like NSAIDS, Opiods etc. However, on chronic usage most of these agents produced several side effect including gastrointestinal, renal, hepatic, central nervous system and dermatological effects (Chaudhary, 2001).

Therefore, today a large section of world population relies on traditional remedies to treat plethora of disease due to their low cost, easy access and reduced side effects (Marino-Betlolo, 1980).

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Terminalia bellirica Roxb, belonging to Combrataceae family, commonly known as Belliric Myrobalan (Bahera in Hindi, one of the important constituents of Indian herbal preparation Triphala), is a deciduous tree found throughout Indian forests and plains. The Fruit is bitter, analgesic, astringent, brain tonic, expectorant and laxative (Chaudhary, 2008). It is chiefly used for fever, leprosy, diarrhea, and piles. The fruits exhibited bronchodilatory, antispasmodic, antiasthematic, hypoglycemic, wound healing, spermicidal activities; they contain tannin, β-sitosterol, gallic acid, ellagic acid, ethyl gallate, and chebulic acid in various proportions (Dhingra and Valecha, 2007). A survey of literature revealed that no scientific study on the analgesic and antipyretic activities has been reported on the fruits of the plant. Therefore, the present study was designed to evaluate the said activities of the fruit extracts of the ***T. bellirica***.

2. Materials and Methods

2.1. Collection of plant materials and preparation of extracts

The fruits of ***Terminalia bellirica*** Roxb were collected in the month of November from the local market of Etawah, Uttar Pradesh state, India, and were authenticated by Dr. Harish .K. Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen was submitted at the Institute's Herbarium Department for future reference (AA 103). Shade dried fruits were ground to coarse powder. Powder was first defatted with pet. ether and then extracted with ethanol (80 %) which is further evaporated to dryness to obtain alcoholic extract. Aqueous extract were obtained by maceration for 24 hrs.

2.2. Drugs and chemicals

Ibuprofen was purchased from Cipla Pvt.Ltd, Pentazocine was purchased from Ranbaxy Pvt.Ltd, and Paracetamol was obtained from Zydus Cadilla Ltd. The solvents and the other chemicals of analytical grade were used and obtained from the institute's central store. Brewer's yeast was procured from a local source.

2.3. Phytochemical screening

Qualitative assay of ethanolic and aqueous extracts of ***T. bellirica*** fruits**,** for the presence of plant phytoconstituents such as flavonoids, phytosterol, alkaloids, glycosides, tannins and saponins, was carried out following standard procedure(Trease and Evans,2003).

2.4. Experimental Animals

Albino rats (160-180 g) and mice (40-50g) of either sex were procured from the institute's Animal House for experimental study. They were acclimated to laboratory conditions for seven days before the commencement of the experiments, and were allowed free access to standard dry pellet diet and water *ad libitum*. The experimental protocol was approved by the IAEC, for using animals in present study.

Animals were fasted overnight with free access to water prior to each experiment.

2.5. Acute Toxicity Studies

The acute toxicity study was carried out in adult female albino rats by the 'up and down' method (425 OECD, 2001). The animals were fasted overnight and the next day extracts of the ***T. bellirica*** Roxb fruits dissolved in normal saline were administered orally at different dose levels. Then the animals were observed continuously for 3 hours for general behavioral, neurological, and autonomic profiles, and then every 30 minutes for the next three hours, and finally till death after 24 hours.

3. Pharmacological evaluation

3.1. Acetic acid induced writhing in mice

The mice were divided into four groups of six mice in each. To each group 0.1 ml of 1% acetic acid was injected intraperitonially (Purnima *et al*., 2009). The control group mice received saline solution (0.9% w/v, NaCl) 2 ml/kg and standard group of mice received 40 mg/kg Ibuprofen. The test groups of mice were treated orally with 200 mg/kg of the ethnolic and aqueous extracts respectively, 60 min before acetic acid injection. The Number of writhes (abdominal muscle contraction), stretching of the hind limbs and trunk twisting were counted for 10 min after acetic acid injection. Percent inhibition was determined for each experimental group as follows: Percent inhibition = $(N\text{-}N^{t}/N) \acute{}100$, where N is the average number of writhing of control per group, and N^{t} is the average number of writhing of test per group.

3.2. Thermal stimulus-induced pain (hot plate test) in rats

The rats were divided into four groups of six each mice in each. The test was carried out using Eddy's hot plate apparatus (Eddy and Leimbach, 1953). The temperature was set at $55\pm1^{0.}$ Rats were placed on a hot plate and recorded the reaction time in second for licking of hind paw or jumping with cut-off time of 15 s., following the administration of the test extracts (ethanolic and aqueous at 200 mg/kg), reference standard Pentazocine (5 mg/kg) and control saline vehicle at 0, 30, 90 and 180 min.

3.3. Brewer's yeast-induced pyrexia

Albino rats were divided into four groups of six rats in each group. Fever was induced by injecting 20 mg/kg (subcutaneous) of 20% suspension of Brewer's yeast in normal saline below the nape of the neck (Somezeet Panda *et al.,* 2009)**.** Initial rectal temperature was recorded. After 18h, animals that showed an increase of $0.3\text{-}0.5^{0}$ in rectal temperature were selected. The test extracts (200mg/kg) reference standard Paracetamol (150 mg/kg) and control saline vehicle were administered orally. The rectal temperature was measured with clinical thermometer at 0, 0.5,1, 2 and 3 hours post dosing.

3.4. Statistical analysis

The results are presented as Mean ± SEM. Statistical analysis of data was performed using Student's't' test to study the differences amongst the means.

4. Results

4.1. Preliminary phytochemical screening

Phytochemical studies revealed that fruits extracts of ***T.bellirica*** contains phytosterol, alkaloids, flavonoids, glycosides, saponins and tannin.

4.2. Acute toxicity studies

The result of acute toxicity study in rats indicated that the ethanolic and aqueous extracts did not produce any significant changes in the behavioural or neurological responses up-to 2000 g/kg b. wt.

4.3. Analgesic activityIn acetic acid-induced writhing test, T.bellirica fruits extracts (200 mg/kg, p.o.) reduced writhing counts significantly Table 1.

Table 1. Effect of ***Terminalia bellirica*** fruits extracts on acetic acid induced writhing in mice.

Treatment	Dose (mg/kg)	Mean No. of Writhing±S.E.M (10Mins.)	% Inhibition
Control	0.2 ml	61.60±4.26	…….
Ibuprofen	40	15.20±1.20***	75.33
Ethanolic	200	23.60±1.94***	61.89
Aqueous	200	27.60±2.14**	55.20

Values are Mean±S.E.M. (n=6) Significance vs. control group: **P<0.01, ***P<0.001

In the hot plate method, the ethanolic and aqueous extracts at a dose of 200mg/kg showed significant increase in reaction time i.e. 7.15 s and 6.81 s, respectively at 30 min. when compared to control (5.15 s) Table 2. These increases were found to be statistically significant (**P<0.01, ***P<0.001). Hence the ethanolic extract was found to be more effective compared to aqueous extract.

Table 2. Effect of ***Terminalia bellirica*** fruits extracts on Thermal stimulus induced pain (Hot Plate Test) in Rats.

Treatment	Dose (mg/kg)	Reaction time in second at (Mins)			
		0	30	90	180
Control	0.2ml	4.12± 0.85	5.15± 0.75	5.00±0.35	5.50±0.46
Pentazocine	5.00	5.60 ±0.92	7.25 ±0.80***	10.83 ± 0.56***	13.92 ±0.77***
Ethanolic	200	5.35±0.48	7.15 ±0.37***	9.10 ± 0.50***	12.27±0.59***
Aqueous	200	5.10±0.24	6.81± 0.20**	8.72±0.31***	10.14 ±0.37***

Values are Mean ±S.E.M. (n=6) Significance vs. control group: **P<0.01, ***P<0.001

The present investigations suggested that both extracts showed a significant analgesic effect in chemical and mechanical induced pain models.

4.4. Antipyretic activity

The results of the antipyretic effect of the test compounds (ethanolic and aqueous), standard (Paracetamol 150 mg/kg), and control are depicted in Table 3. The Paracetamol as well as ethanolic extract at dose of 200 mg/kg started showing effective antipyretic activity after 1h of post dosing; while aqueous extract 200 mg/kg reduced temperature after 2 h, when compared with control. Antipyretic activity was observed up to 3 h after paracetamol and test extracts administration.

Table 3. Effect of ***Terminalia bellirica*** fruits extracts on Brewer's yeast-induced pyrexia in Rats.

Treatment	Dose (mg/kg)	Rectal temperature in °C at time (h)				
		0	0.5	1	2	3
Control	0.2ml	37.13±0.50	37.19 ±0.10	37.21 ± 0.11	37.50 ± 0.11	37.42 ± 0.13
Paracetamol	150	37.11±0.15	37.01 ±0.34	36.68 ±0.44***	36.10±0.17***	35.81±0.11***
Ethanolic	200	37.10 ± 1.0	37.05 ±0.23	36.78 ±0.05***	36.90 ± 0.67**	36.61±0.23***
Aqueous	200	37.41 ±0.22	37.21 ±0.31	37.20 ± 0.06	36.89 ± 0.72**	37.01 ±0.63*

Values are Mean±S.E.M. (n=6) Significance vs. control group: *P<0.05, **P<0.01, ***P<0.001

5. Discussion

Acetic acid-induced writhing and Eddy's hot plate induced thermal stimulation are models of pain that mainly involve peripheral and central mechanisms, respectively. Analgesic effect observed in these two models with 200 mg/kg ethanolic and aqueous extracts of ***T. bellirica*** indicates the involvement of both peripheral and central mechanisms. The acetic acid-induced writhing has been associated with an increased level of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products (Derardt *et al*., 1980). The present results revealed that a significant reduction in acetic acid-induced writhing, and increase reaction time to heat stimuli strongly suggests that the mechanism of both extracts may be linked partly to cyclooxygenase and/or lipoxygenase inhibition (Franzotti *et al*., 2002).

In addition, the flavonoids are known to inhibit prostaglandin synthetase (Ramaswamy *et al*., 1985 a). Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavonoids present in titled plant might be responsible for its analgesic effect.

It is well known that most of the anti-inflammatory and analgesic drugs possess antipyretic activity. Both extracts markedly decreased the rectal temperature of pyretic rats. This postulation is supported by the antipyretic effect of the extract, evidenced by its impact on the pathogenic fever induced by the administration of a yeast injection. Its etiology includes the production of prostaglandins in central nervous system which is the final common pathway responsible for fever induction (Howard, 1993). In general, NSAIDS produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus (Clark, 1975; Zeal, 1975). Therefore, it appears that the antipyretic action of the titled plant may also be related to the inhibition of prostaglandin synthesis in hypothalamus by present flavanoids in both extracts of ***T.bellirica*** (Ramaswamy *et al*., 1985 b).

Thus, the present study concludes that the ethanolic and aqueous extracts have analgesic and antipyretic activities in rats and mice at the doses 200 mg/kg, respectively. However, further study needs to be carried out for the isolation and identification of specific phytoconstituents present in titled plant.

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Regional Distribution of Superoxide Dismutase Activity in Human Placenta and its Correlation with Lipid Peroxidation

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Abstract

The objectives of the present study are to determine the activity of superoxide dismutase (SOD) in various regions of human placenta, and to correlate this activity with lipid peroxidation. To follow up changes in SOD activity and lipid peroxidation during gestation, a female rat model was used and pregnant females were sacrificed at 4, 7, 10, 14, and 18 days of gestation. Placentas were collected from 45 uncomplicated term pregnancies. Each placenta was sampled in 3 regions on the maternal and fetal side: central; mid placenta; and periphery. 5 gm of tissue were obtained for analysis and full thickness samples were obtained from the umbilical cord. In the rat, 2-3 placentas from each (female (n=4) were pooled and assayed while the cord for all placentas of the same female were pooled and assayed. The results show slight regional variations in the amount of lipid peroxidation as estimated by malondialdehyde (MDA) with the mid placental region of the fetal side being significantly higher than any of the other placental regions. MDA concentration in the placenta and umbilical cord of female rat progressively increased with progress of gestation (67% increase in the placenta and 90% increase in the umbilical cord). The results also show slight regional variations in SOD activity in the placenta being higher at the fetal site. The SOD activity in the umbilical cord was significantly higher than any of the placental regions. SOD activity in the placenta and umbilical cord of female rat progressively increased with the progress of gestation and after 10 days SOD activity in the umbilical cord was significantly higher than the placenta. The results clearly show that the increase of lipid peroxidation in the placenta and umbilical cord during gestation is coupled with an increase in SOD activity. The magnitude of SOD activity increase is higher to compensate for the increase in lipid peroxidation.

Keywords: placenta, umbilical cord, lipid peroxidation, SOD.

1. **Introduction**

Normal cells have a number of protective scavenger enzymes that act as antioxidants to detoxify the harmful reactive oxygen radical and prevent cell damage. One of these enzymes is superoxide dismutase (SOD: ECl.15.1.1) which catalyses the dismutation of superoxide radicals into oxygen and hydrogen peroxide and is widely distributed in mammalian tissues (McCord and Fridovich,1969; Bannister *et a1*., 1987) including the placenta (Beckman, *et al*., 1973; Sekiba and Yoshioka, 1979; and Takehara *et al*, 1990). There are three SOD isoenzymes (Beckman *et al*., 1973) in human beings: the souble cytosolic Cu/Zn SOD (Akbar *et al*., 1998); the manageese-contaning SOD (MnSOD) which is located in the matrix of the mitochondria (Bannsiter *et al*., 1987), and the extracellular form of SOD (Marklund, 1984; Chu *et al*., 2006).

Various studies dealt with the increase of lipid peroxidation and generation of free radicals during pregnancy (Ishihara 1978; Wickens et al., 1981; Wang , *et al*., 1991; Walsh 1994; Akihito *et al*., 2000; Warren *et al*., 2005), but there is little information about placental lipid peroxides. It has also been suggested that placenta could contribute to the increase of lipid peroxidation (Diamant *et al*., 1980; Hubel *et al*., 1989), but little is known about the placental regional variation of this possible contribution.

Although the presence of Cu/Zn SOD activity in human placenta has been known for more than 30 years (Beckman *et al*.,1973; Hien *et al*., 1974) there are only sporadic

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reports about the regional distribution of this enzyme in the placenta during gestation time. The objectives of this study are: (1) to measure the activity of the enzyme superoxide dismutase in various regions of human placenta immediately after delivery; (2) to determine the level of lipid peroxidation in the same placental regions; and (3) to correlate the level of lipid peroxidation with the activity of superoxide dismutase.

2. **Materials and methods**

2.1. Collection and processing of human placenta

Forty five placentas were collected from various maternity hospitals immediately after vaginal delivery and placed on ice. Each placenta was weighed and sampled in three main regions on both maternal and fetal sides as well as the umbilical cord as follows:

a- central region: within 3 cm of the cord insertion,
b- mid placenta: mid way between the cord insertion and margin,
c- peripheral region: within 3 cm of the placental edge,
umbilical cord: full thickness tissue samples were obtained from the umbilical cord (midway between the placenta and the umbilicus).

At each sampling site 5g of tissue was obtained, split into a liquotes and then kept in deep freeze (-80 C) until the time of processing.

2.2. Female rat model

Adult female rats weighing 250-300g were housed in groups of 4-5 in pvc cages (350x530 mm long x180 mm high) in an environment maintained at 19-22 C with 12/12 h light/ dark cycle. Food and water were always available. Female rats were allowed to mate with male rats and the males were removed after pregnancy which was confirmed with the observation of vaginal plug. Pregnant females were sacrificed at various gestational age (4, 7, 10, 14, and 18 days).

Embryos were carefully removed, placenta and umbilical cords were separated and processed similar to human placenta as mentioned in section 2.1. Each 2-3 placentas were pooled and ,assayed, but the cord of all placentas of the same female was pooled and assayed.

2.3. SOD assay

Placental tissues were homogenized in phosphate buffered saline (PBS, PH 7.4), sonicated and then centrifuged at 10.000 g for 25 min to obtain the cytosol fraction which was used to assay for Cu/Zn SOD. The assay was done according to the method of misra and Fridovich (1972) at 30 C . The amount of enzyme that causes 50% inhibition of epinephrine auto-oxidation is defined as 1 unit.

2.4. Measurement of lipid peroxidation

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) (Buege and Aust,1978). The MDA level was measured in the cytosol fraction of placental homogenate. Lipid peroxides were measured after addition of 2ml of TBA reagent (15% w/v trichloroacetic acid and 0.25 N HCL) to l ml of cytosol fraction. The mixture was treated in a boiling water bath for 15 minutes. After cooling, the suspension was centrifuged at 1000 g for 10 minutes. The supernate was then separated and absorbance was measured at 535 nm. The MDA concentration was determined by the specific absorbance coefficient (1.34x105mol/cm3).

2.5. Measurement of protein

Protein concentration in the cytosol suspension was determined by a modification of the Lowry procedure (1951) as reported by Markwell *et al.*, (1978) using bovine serum albumin as a standard.

2.6. Statistical methods

Statistical analysis was done using a t-test (SPSS for windows) by a personal computer. P values <0.05 were considered significant.

3. **Results**

3.1. Topographical data of maternal and placental records

The placental tissue was collected from 45 uncomplicated term pregnancies (38-40 weeks menestrual age). Mean weight of placenta was 720g (range 610- 945 g). Medical records for the women from whom placentas were recovered revealed that the mean maternal age was 28.4 years (range 19-38 years), mean gestational age was 38.6 weeks (range 37-42 weeks), mean parity was 3.7 (range 0-7), and mean birth weight of infants was 3028 g (range (2100-4030 g). Each placenta was sampled in 3 main regions on both the maternal and fetal sides. The various regions in the placenta which were dissected are indicated in Figure 1.

3.2. Regional distribution of SOD activity in the placenta

3.2.1. Regional distribution of SOD in human placenta

Distribution of SOD activity in various regions of the placenta and the umbilical cord are shown in Table 1. There are slight regional variations in the SOD activity being relatively higher at the fetal site.

There is no significant difference in SOD activity of various regions at the same site of the placenta. However, the central region of the fetal site has a significantly higher level of SOD ($P<0.05$) than any of the regions in the maternal site. Activity of SOD was significantly higher ($P < 0.01$) in the umbilical cord than any of the regions in either the maternal or fetal sites of the placenta.

3.2.2. SOD activity in placenta of female rat

Because the placenta of the rat embryo is much smaller than human placenta and the weight of each placenta was less than 0.5 g, regions of the placenta were not dissected. Two or three placentas were pooled and processed for SOD activity. Changes in the SOD activity, in the placenta

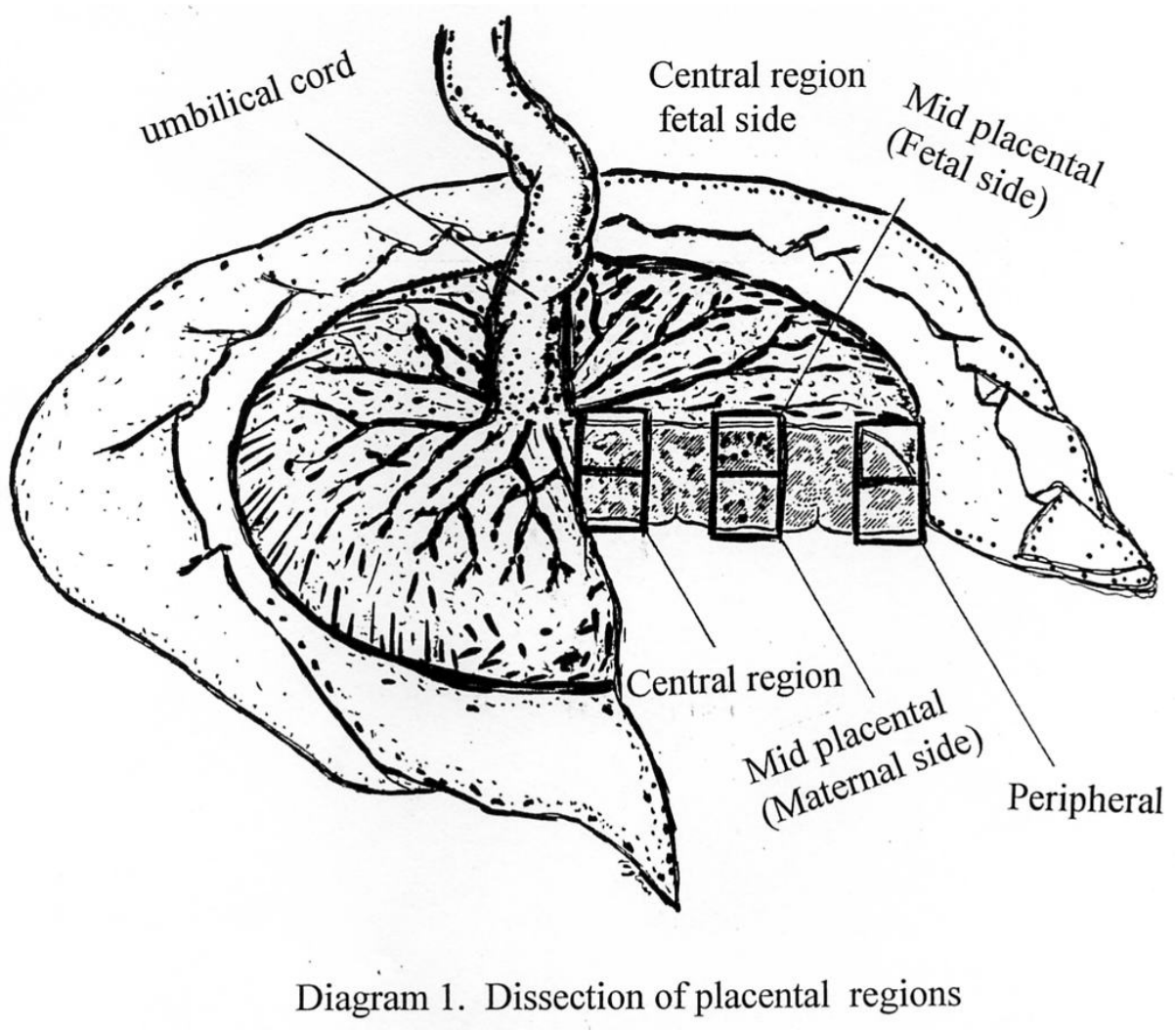

Figure 1. Dissection of placental regions showing maternal and fetal sides of the placenta and the umbilical cord. Site of obtaining samples are indicated by *. Modified after Jendryczko *et al.* 1991)

and the umbilical cord are shown in Table 2. SOD activity in either the placenta or the umbilical cord, progressively increased with the progression of gestational time. Until 7 days of gestation, there was no significant difference in SOD activity in the placenta compared to the umbilical cord. Although the activity of the enzyme in the placenta or the umbilical cord showed slight progressive increase, such increase was not significant. At 10 days of gestation, SOD activity in the placenta increased 58% while in the umbilical cord it increased about 2 folds. The increase in SOD activity in the placenta and the umbilical cord persisted throughout the rest of the gestational period. However, the increase was more evident in the umbilical cord. At 18 days, SOD activity in the placenta reached 2.1 folds, while in the umbilical cord it reached 3.1 folds.

3.3. Lipid peroxidation in the placenta

3.3.1. Lipid peroxidation in human placenta

Lipid peroxidation in various placental regions is shown in Table 3. The average MDA concentration in the maternal side was 1.42 nmol/mg (range 1.41 – 1.53). In spite of the slight regional variation, there was no significant difference ($P>0.05$). The average MDA concentration in the fetal side was 1.72 nmol/mg (range 1.62 – 1.83).

There are slight regional variations in the MDA concentration being relatively higher at the mid placental region. There is no significant difference in MDA concentration of various regions at the same site of the placenta.

However, the mid placental region of the fetal site was significantly higher ($P < 0.05$) than any of the regions at the maternal site. Also the average MDA concentration in the fetal site was significantly higher ($P < 0.05$) than the average maternal site. The MDA concentration in the umbilical cord was significantly higher than either the maternal or the fetal site of the placenta ($P<0.05$). It was 56% higher than the maternal site and 29% higher than the fetal site.

Table 1: Regional distribution of SOD activity in human placenta and umbilical cord

1. Placenta	
A. Maternal Side	
a. M1 (Central)	3.31 + 0.41
b. M2 (Mid placenta)	3.36 + 0.39
c. M3 (Periphery)	3.42 + 0.45
B. Fetal side	
a. F1 (central)	4.21 + 0.6*
b. F2 Mid placenta)	4,13 + 0.53
c. F3 (Periphery)	3.82 + 0.45
2. Umbilical cord	5.8 + 0.73**

N = 45, Values are mean + S.D , * P < 0.05 compared to maternal side , ** P < 0.01 Compared to placental region

Table 2 : Changes of SOD activity in placenta and umbiblical cord of female rat during gestation

Gestation Age (days)	Fetus weight (g)	Placenta weight (g)	SOD activity (unit/mg protein) Placenta	SOD activity (unit/mg protein) Umbilical cord
4	1.4 + 0.2	0.23 + .02	2.6 + 0.3	2.5 + 0.4
7	2.1 + 0.2	0.31 + .03	2.7 + 0.4	3.1 + 0.5
10	3.1 + 0.3	0.35 + .03	4.1 + 0.4*	5.2 + .07*
14	3.6 + 0.4	0.38 + .04	4.6 + 0.5*	5.7 + .08*
18	4.4 + 0.5	0.45 + .05	5.5 + 0.6 **	7.8 + .09 **

N = 4 female rats, 2-3 placentas were pooled from each female rat and umbilical cords of all placentas of the same female were pooled. values are mean + S.D, * P < 0 .05 , ** P < 0.01

Table 3. Lipid peroxidation in various regions of human placenta as estimated by MDA

Type of Tissue	MDA concentration nmol/ mg protein X 10 -1
1.Placenta	
A.Maternal side	
a. M1 (central	1.41 + 0.16
b. M2 (mid placental)	1.32 + 0.14
c. M3 (peripheral)	1.53 + 0.16
B. Fetal side	
a. F1 (central)	1.62 + 0.18
b. F2 (mid placenta)	1.83 + 0.21*
c. F3 (peripheral)	1.71 + 0.19
2.Umbilical cord	2.2 1 + 0.23**

N=45, values are mean + S.D , *P< 0.05 compared to maternal side. **P<0.01 compared to placental region

3.3.2. Lipid peroxidation in the placenta of female rat

Lipid peroxidation in the placenta of female rat is shown in Table 4. MDA concentration in the placenta and the umbilical cord progressively increased with the progression of gestational time. Until 10 days of gestation, there was no significant difference in lipid peroxidation between the placenta and umbilical cord. At 14 and 18 days the difference in MDA concentration between the placenta and the umbilical cord was significant ($P < 0.05$), with the umbilical cord being higher.

MDA concentration in the placenta increased 67% at 18 days while in the umbilical cord it increased 90%.

4. **Discussion**

The current study determined the level of lipid peroxidation in human and female rat placentas. We found that the level of peroxidation in both the human and the rat placenta are comparable. Concentration of peroxidation as estimated by MDA in the placenta of female rat at 18 days (2 days before delivery) was higher than that in full term delivered human placenta. The level was 2.45 nmol/mg in the rat versus 1.5 nmol/mg (average of the maternal and fetal regions) in humans (Table 3 and 4). The level is 63% higher in female rat.

The placenta is a heterogenous tissue with membraneous nature (Ali *et al*., 1996); so different tissue compartments probably contributed to the tissue level of lipid peroxides (Roijmakers *et al*., 2004). This could explain the slight variation in the level of peroxidation at various regions of the placenta being relatively higher at the fetal site. In both human and female rat, the level of lipid peroxidation in the umbilical cord was significantly higher than the placenta. In human placenta, the level of peroxidation in the umbilical cord was 47% higher than the placenta while in the female rat the level of peroxidation was about 12% higher. This indicates that the umbilical cord has a considerable contribution to the formation of lipid peroxides.

The level of peroxidation in the placenta or the umbilical cord, as shown in the present study, is high compared to other tissues like liver or lung as reported by Beckman *et al*., 1973; Romero *et al*., 1998; Janssen and Tazzero, 2002). This high level could be due to increased thromboxane production as reported by various investigators (Wang *et al*., 1992; Walsh and Wang, 1995; Cueto *et al*., 1997), which causes an increase in cyclooxygenase activity resulting in increased oxygen radical formation. Lipid peroxides are formed when oxygen radicals interact with polyunsaturated fatty acids. Since the placenta is a rich source of unsaturated fatty acids, the increased cyclooxygenase activity associated with increased thromboxane production could be coupled with increased placental peroxidation.

In this study, we also shown that lipid peroxidation progressively increases as gestation progress in both the placenta and the umbilical cord. The increase was 67% in

Table 4. Lipid peroxidation in the placenta and umbilical cord of female rat during gestation as estimated by MDA

Type of Tissue	MDA concentration nmol/ mg protein X 10 -1
1.Placenta	
A.Maternal side	
a. M1 (central	1.41 + 0.16
b. M2 (mid placental)	1.32 + 0.14
c. M3 (peripheral)	1.53 + 0.16
B. Fetal side	
a. F1 (central)	1.62 + 0.18
b. F2 (mid placenta)	1.83 + 0.21*
c. F3 (peripheral)	1.71 + 0.19
2.Umbilical cord	2.2 1 + 0.23**

N=45, values are mean + S.D , *P< 0.05 compared to maternal side. **P<0.01 compared to placental region

the placenta and almost doubled in the umbilical cord (Table 4). Although several investigators (Sekiba and

Yoshika, 1979; Wickens, 1981; Wang *et al*., 1992; Wang and Rogers, 1997) have also shown increased level of lipid peroxides during pregnancy (review Walch, 1994) this was in maternal blood and not in actual placental tissue as we have demonstrated. It is also worth mentioning that only scarce reports (Palan *et al*., 2001) are available about lipid peroxidaion in the umbilical cord compared to the placenta.

We have shown in a previous study that the walls of blood vessels do have considerable amount of lipid peroxidation and this is much higher in the varicose veins (Wali *et.al*., 2002).

In this study, we have also shown that SOD activity in the placenta exhibited slight regional variations being higher at the fetal site of the placenta, particularly the central region (Table 1). The SOD activity was significantly higher in the umbilical cord than any of the placental regions of either the maternal or the fetal site. The level of SOD in the placenta of female rat at 18 days of gestation was 48% higher than in the human placenta (5.5 units/mg in the female rat versus 3.71 units/mg in human).

The level of SOD activity in the umbilical cord of female rat was 35% higher than that in humans.

There was a progressive increase of SOD activity with progress gestation where the increase was 2 folds in the placenta and 3 folds in the umbilical cord (Table 2).

Results in this study clearly show that the increase of lipid peroxidation in the placenta and the umbilical cord during gestation is coupled with an increase in SOD activity. The magnitude of the increase in SOD activity is higher than the increase of lipid peroxidation in female rat. Correlation of the increase in lipid peroxidation and the increase in SOD activity is shown in Figure 2. This indicates that although lipid peroxidation increased with the progress of gestation, the antioxidant SOD activity has also increased to compensate for the increase in lipid peroxidation.

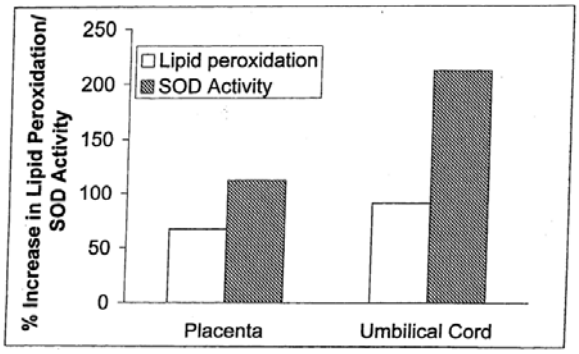

Figure 2. Correlation between the magnitude of increase of lipid peroxidation and SOD activity in the placenta and the umbilical cord of female rat with progress of gestation

Various investigators have reported the increase of lipid peroxidation during pregnancy (Diamant *et al*., 1980; Hubel *et al.*, 1989; Walsh, 1994) which seems to play an important factor in the pathogenesis of pre-eclampsia.

Recently, there has been a growing interest in using antioxidants to suppress lipid peroxidation in various tissues including the placenta (Chow, 2001; Raijmakers, *et al*., 2004). In a previous study, we have shown that treatment with vitamin E considerably reduced lipid peroxidation in spermatozoa and improved sperm motility. Therefore, supplementation with vitamin E could protect against the increase of lipid peroxidation in the placenta.

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