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The Corrective Efficacy of the *n*-Hexane Fraction of the Hydro-Methanol Extract of the *Swietenia mahagoni* Seeds on Testicular Dysfunctions in Streptozotocin-Induced Diabetic Male Rats

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Abstract

Diabetes mellitus (DM) is one of the most common chronic metabolic diseases worldwide affecting male reproductive dysfunctions (RD). Male diabetics are more prone to having varieties of sexual disorders such as impotence, retrograde ejaculation, sexual drive, decreased libido, delayed sexual maturation, and erectile dysfunction (ED). The present study is designed to investigate the diabetes-induced testicular dysfunction and its treatment by the n-hexane fraction of the hydro-methanolic (2:3) seed extract of *Swietenia mahagoni* in Wistar male albino rats. Diabetes was induced by the intramuscular injection of streptozotocin (STZ) at a dose of 4 mg/mL of citrate buffer/100 gm body weight. Diabetes-induced testicular oxidative stress injuries were assessed by the determination of antioxidant enzyme-activities including catalase (CAT), superoxide dismutase (SOD), and glutathione-s-transferase (GST), along with thiobarbituric acid-reactive substances (TBARS), and conjugated dienes (CD) levels. The numbers of the different generations of germ cells [i.e. spermatogonia-A (SgA), preleptotine spermatocytes (pLSc), mid-pachytene spermatocyte (mPSc), step 7 spermatids (7Sd) and 19 spermatids (19Sd)], at stage VII of spermatogenic cycle were assessed. Testicular CAT, SOD and GST activities were decreased along with elevation in the levels of TBARS and CD in diabetic animals. Numbers of different generations of germ cells i.e. SgA, pLSc, mPSc, 7Sd and 19Sd, at stage VII of the spermatogenic cycle were decreased in diabetic animals. Significant improvement in the levels of blood glucose, serum insulin, testosterone, and testicular oxidative stress parameters were noted towards control after the treatment of diabetic rats with the fraction at a dose of 10 mg/100 gm body weight/day for twenty-eight days. The above-mentioned different generations of germ cell numbers along with the diameter and number of pancreatic islets were recovered towards control after the treatment with the fraction. The results support the validity of this n-hexane fraction for the management of testicular disorders noted in diabetic rats.

Keywords: *Swietenia mahagoni* (L.) Jacq., Streptozotocin, Antioxidant enzymes, Pancreas, Testis

1. Introduction

Diabetes mellitus (DM) represents one of the greatest threats to modern global health. The estimated number of adults with diabetes in 2007 was 246 million; of these, 80% live in developing countries, with the largest numbers being from the Indian subcontinent and China. India has forty-one million diabetics, and this number it is expected to increase to seventy million by 2025 (Sicree *et al.,* 2006). According to the World Health Organization (WHO) estimates, the urban population in developing regions will increase from 1.9 billion in 2000 to 3.9 billion in 2030. It is estimated that, by 2030, nearly 46% of India's population will be living in urban areas (WHO, 2002). The Diabetes' incidence is rising rapidly. It is a heterogeneous group of diseases, known as a syndrome, and has become one of the greatest threats to modern global health and the third leading cause of death (after heart disease and cancer) in many countries (Can *et al.,* 2004).

DM may affect the male reproductive function at multiple levels as a result of its effects on the endocrine control of spermatogenesis, or by impairing penile erection and ejaculation (Soudamani *et al.,* 2005). There are a number of reports in the related literature examining the effects of diabetes on the endocrine control of spermatogenesis (Baccetti *et al.,* 2002). Diabetes is, however, a well-recognized cause of male sexual dysfunction, which in itself may contribute to subfertility. Data from animal models strongly suggest that DM impairs male fertility. Furthermore, numerous studies have demonstrated a marked reduction in fecundity when male

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animals are diabetic, as well as an impairment of sperm quality (Gnoth *et al.*, 2005). If similar effects exist in the context of human male reproduction, the rising rates of diabetes may well pose a significant problem to human fertility. Despite this, the potential impact of the increase in diabetes in young men and its effect on their reproductive health have received comparatively little attention to date.

However, there are several reports about the dysfunctions of the male reproductive activity in diabetic conditions, but the data are confusing, and the exact role that insulin plays in the regulation of male reproductive function is still unclear. Insulin is known to influence the hypothalamic-pituitary axis (Bucholtz *et al*., 2000), which can alter serum hormone levels significant in spermatogenesis. It is also known that insulin may be able to interact with receptors on the Leydig cells directly to mediate the process of steroidogenesis; thus, we cannot exclude the possibility that plasma insulin also directly interacts with Leydig cells, in addition to the indirect signaling through the pituitary. Leydig cells contain insulin receptors (Brüning *et al*., 2000), and the administration of insulin to Leydig primary cultures causes an increase in testosterone formation (Pakarainen *et al.*, 2005). In the practices of traditional medicine, medicinal plants are used in many countries to control diabetes mellitus. The National Centre for Complementary and Alternative Medicine was established in 1998 by the United States Government for the development of herbal medicine as an important subject of study (Sing *et al.* 2005; Mallick *et al.*, 2010). Recently, there is a greater global interest in non-synthetic, natural drugs derived from plants and herbal sources due to their better tolerance and minimum potential adverse drug reactions (Pari and Umamaheswari, 2000). Plants play a significant role in human health, and have been considered valuable sources of natural products for maintaining a good health for many years. The WHO suggested that medicinal plants would be the best source from which to develop a variety of medications (WHO, 2001).

The plant, *Swietenia mahagoni* (L.) Jacq. from the (Family- Meliaceae), is a lofty, evergreen large tree, native to tropical America, Mexico, and South America, as well as India (Kirk, 2009). The seed extracts of *S. mahagoni* are widely used in Indonesia as folk medicine to cure diabetes (Li *et al.*, 2005). In an earlier study, the authors have reported the antidiabetic activity of n-hexane fraction of the hydro-methanol (2:3) extract of *S. mahagoni* seeds on an animal model (Bera *et al.*, 2015). Thus, this study has been undertaken to investigate the diabetes-related testicular disorders, and to evaluate the remedial effects of n-hexane of the hydro-methanol (2:3) extract of the *S. mahagoni* seeds on testicular dysfunctions in diabetic conditions.

2. Materials and Methods

2.1. Plant Materials

The seeds of *Swietenia mahagoni* (L.) Jacq. of the family- Meliaceae were collected from Medinipur in the District of Paschim Medinipur in West Bengal, India during December 2016. The materials were taxonomically identified by Prof. R. K. Bhakat of the Department of Botany and Forestry at Vidyasagar University in Medinipur. The voucher specimen was deposited in the Department of Botany at Vidyasagar University (Ref. No. *S. mahagoni* (L.) Jacq. / VU / 01 / 09).

2.2. Chemicals

Streptozotocin (STZ) was purchased from Sigma–Aldrich Diagnostic Ltd. USA. The insulin- enzyme linked immunosorbent assay (ELISA) kit was purchased from Boehringer Mannheim Diagnostic, Mannheim (Germany). The testosterone kit was purchased from IBL–Germany. The biochemical kits were purchased from Span Diagnostic Ltd. Surat, India. The blood- glucose levels were measured using a one touch electronic glucometer of Ascensia Entrust, Bayer Diagnostics Ltd., Borada, India.

2.3. The Selection of Animal and Animal Care

The current study was conducted on matured Wistar strain male albino rats of three months of age weighing approximately 150 ± 10 g. The rats were acclimated for a period of fifteen days in this study's laboratory conditions prior to the experiment. They were housed in Tarson cages (Tarson Products Pvt. Ltd., Kolkata, India) at an ambient temperature of 25 ± 2 ^{0}C with a twelve-hour light: twelve-hour dark cycle. The rats were allowed free access to standard food and water *ad libitum.* Normoglycemic animals selected for this experiment had a fasting blood glucose level of 75±5 mg/dL.

2.4. Preparation of the n-Hexane Fraction of the Hydro-Methanol (2:3) Extract from the Seeds of Swietenia mahagoni (L.) Jacq.

Fresh seeds of *S. mahagoni* were dried in an incubator for two days at 40 ^{0}C, crushed separately in an electric grinder, and then pulverized. Out of this powder, 500 g were suspended in 400 mL of water and 600 mL methanol (2:3) and were kept in an incubator at 37 ^{0}C for thirty-six hours. The slurry was stirred intermittently for two hours and was left overnight. About 96.8 g of the light brown colored lyophilized extract was collected. In a one-litre separate flask, the extract (96.8 g) was dissolved with 500 mL of a hydro-methanolic (2:3) solution, and the solvent fractionation was carried out using solvents (*n*-hexane, chloroform, ethyl acetate and *n*-butanol) with increasing polarity. Finally, from the 96.8 g- lyophilized extract of *S. mahagoni*, an amount of 27.6 g of *n*-hexane fraction was obtained.

2.5. Induction of Diabetes Mellitus

The Streptozotocin-induced diabetes was done by the standard method as mentioned earlier (Panda *et al.*, 2009). In brief, twenty-five rats fasting for twenty-four hours were subjected to a single intramuscular injection of streptozotocin (STZ) at a dose of 4 mg/ 0.1 mL of citrate buffer (pH 4.5) / 100 g body weight / rat. On the seventh day of the STZ injection, fasting blood sugar (FBG) levels were measured, and the eighteen diabetic rats were placed in group II to group IV having six rats in each group. The remaining seven rats having FBG less than 250 mg/ dL were not selected for this experiment. Six other normoglycemic rats were subjected to a single injection of citrate buffer at a dose of 0.1 mL/ 100 g body weight/ rat at

the time of the STZ injection in other rats, and these are kept under the vehicle control group.

2.6. Animal Treatment

Twenty-four rats were divided into four groups equally as follows; the duration of the experiment was thirty-five days.

Vehicle control: Normoglycemic healthy rats of this group were treated with 0.5 mL of 3.0% DMSO solution / 100 gm body weight / day for twenty-eight days at the time of the n-hexane fraction treatment of the diabetic rats.

Vehicle diabetic: Diabetic rats of this group were treated with 0.5 mL of 3.0% DMSO solution/ 100 gm body weight/ day for twenty-eight days at the time of the *n*-hexane fraction treatment of the diabetic rats.

Diabetic + *n*-hexane fraction: Diabetic rats of this group were treated with n-hexane fraction of the hydro-methanol (2:3) extract of the *S. mahagoni* seeds at a dose of 10 mg / 0.5 mL of 3.0% DMSO solution/ 100 gm body weight / day for twenty-eight days.

Diabetic + metformin: Diabetic animals of this group were treated with metformin (standard antidiabetic drug) at a dose 1 mg / 0.5 mL of 3.0 % DMSO solution / 100 gm body weight / day for twenty-eight days.

The fraction was administered orally using a feeding cannula daily for twenty-eight days starting from the eighth day of the STZ injection considering the first day of the fraction/ metformin treatment. From the first day of the fraction / metformin treatment of the diabetic rats, a fasting blood glucose level was measured every seventh day using a glucometer (Bera *et al*., 2010). On the twenty-ninth day of the experiment (the thirty-sixth day following the day of the STZ injection), all the animals were sacrificed by decapitation after recording the final body weight. Blood was collected from the dorsal aorta using a syringe. Serum was separated from part of the collected blood by centrifugation at 3000 g for five minutes to evaluate the levels of the serum insulin, and testosterone, in addition to the activities of serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT. Epididymis and testes were dissected out, and one testis was stored at –20 ^{0}C for the biochemical analysis, and other testes were used for the histological study. Cauda epididymis of each animal was stored at 37^{o}C in buffer, and was used for epididymal sperm count, motility and viability.

2.7. Measurement of the Fasting Blood Glucose (FBG) Level

At the time of grouping the animals, the FBG level was measured. On every seventh day of treatment, FBG was further recorded from all the animals in all groups. Blood was collected from the tip of the tail vein, or by an orbital puncture alternatively, and the FBG levels were measured by the one-touch glucometer (Bera *et al*., 2010).

2.8. Serum Insulin Level

Serum insulin was measured by an enzyme-linked immunosorbent assay (ELISA) using the kit (Boehringer Mannheim Diagnostic, Mannheim, Germany) (Briner *et al*., 1988). The insulin level in the serum was expressed in µIU/mL.

2.9. Serum Testosterone Level

Serum levels of testosterone were measured using the testosterone kit from IBL- Germany according to the standard protocols supplied by that company (Srivastava, 2001). In this solid phase-conjugated assay, an alkaline phosphatase conjugated hormone was used. There is no inter-assay variation as all the samples were assayed at a time.

2.10. Sperm Viability

The sperm viability was performed by the eosin nigrosin staining (WHO, 1999). One drop of semen was mixed with two drops of 1% eosin Y. After thirty seconds, three drops of 10% nigrosin were added and mixed well. A smear was made by placing a drop of the mixture on a clean glass slide, and was allowed to air-dry. Pink-stained dead sperms were differentiated from unstained live sperms, and their numbers were recorded.

2.11. Estimation of Testicular Δ5, 3β- Hydroxysteroid Dehydrogenase (Δ5, 3β- HSD) and 17 β- Hydroxysteroid Dehydrogenase (17 β- HSD) Activities

The testicular Δ^5, 3β- HSD activity was measured spectrophotometrically (Talalay, 1962). One testis from each animal was homogenized carefully at 4^{o}C in 20% spectroscopic grade glycerol containing 5 mM potassium phosphate and 1 mM EDTA, at a tissue concentration of 100 mg/mL homogenizing mixture. This mixture was centrifuged at 10,000 X g for thirty minutes at 4^o C. The supernatant (1ml) was mixed with 100 µM sodium pyrophosphate buffer (pH 8.9), 40 µL ethanol, containing 30 µg dehydro-epiandrosterone, and 960 µL of 25 mg% BSA, bringing the incubation mixture to a total of 3 mL. One unit of the enzyme activity was the amount causing a change in absorbance of 0.001 / minute at 340 nm.

For the measurement of the testicular 17 β- HSD activity, another 1mL supernatant from the same homogenizing mixture was added to 440 µM sodium pyrophosphate buffer (pH 10.2), 40 µL ethanol containing 0.3 µM testosterone, and 960 µL of 25 mg% BSA, bringing the incubation mixture to a total of 3 mL. The enzyme activity was measured according to the method of Jarabak *et al.* (1962). One unit of the enzyme activity was equivalent to a change in absorbency of 0.001 / min at 340 nm.

2.12. Biochemical Assay of Testicular Catalase (CAT) Activity

The activity of testicular catalase was measured biochemically (Beers and Sizer, 1952). 0.5 mL of 0.00035 M H_2O_2 and 2.5 mL of distilled water were mixed in a spectrophotometric cuvette, and the reading of absorbance was noted at 240 nm. The supernatants of the testicular samples were added at a volume of 40 µL, and the subsequent six readings were noted at 30 sec interval.

2.13. Assessment of Testicular Superoxide Dismutase (SOD) Activity

The testis was homogenized in a chilled 100 mM/L tris HCl buffer containing 0.16 M/L potassium chloride (pH 7.4) to give a tissue concentration of 10% (weight/volume), and was centrifuged at 10,000 rpm for twenty minutes at 4^{0}C. The SOD activity of the sample was estimated by measuring the percentage inhibition of

the pyrogallol auto oxidation by SOD according to the standard method (Marklund and Marklund, 1974).

2.14. Determination of Testicular Glutathione-s-Transferase (GST) Activity

The activity of GST in the testis tissue was measured spectrophotometrically using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate (Hobig *et al*., 1974). The assay mixture of 3 mL contained 0.1 mL of 1 mM CDNB in ethanol, 0.1mM of 1 mL GSH, 2.7 mL of 100 mM potassium phosphate buffer (pH -6.5) and 0.1 mL of the supernatant of the tissue homogenate. The formation of the product of CDNB, S-2, 4-dinitrophenylglutathione, was monitored by measuring the net increase in absorbance at 340 nm against the blank.

2.15. Estimation of Testicular Lipid Peroxidation from the Concentration of Thiobarbituric Acid Reactive Substance (TBARS) and Conjugated Dienes (CD)

The testis was homogenized separately with a tissue concentration of 50 mg / mL in 0.1 M of ice-cold phosphate buffer (pH-7.4), and the homogenates were centrifuged at 10,000 g at 4^0C for five minutes individually. Each supernatant was used for the estimation of TBARS and CD (Okhawa *et al.,* 1979; Slater, 1984). For the measurement of TBARS, the homogenate mixture of 0.5 mL was mixed with 0.5 mL of normal saline (0.9 g% NaCl) and 2 mL of TBA-TCA mixture (0.392 g thiobarbituric acid in 75 mL of 0.25 N HCl with 15 g trichloroacetic acid (TCA). The volume of the mixture was made up to 100 mL by 95% ethanol) and boiled at 100 ^{0}C for 10 min. This mixture was then cooled at room temperature and was centrifuged at 4000 g for ten minutes. The whole supernatant was taken in a spectrophotometer cuvette, the reading was at 535 nm (Okhawa *et al*., 1979).

The quantification of the CD was performed by a standard method (Slater, 1984). The lipids were extracted with chloroform-methanol (2:1) followed by centrifugation at 1000 g for five minutes. The chloroform layer was evaporated to dryness under a stream of nitrogen. The lipid residue was dissolved in 1.5 mL of cyclohexane and the absorbance was noted at 233 nm to measure the amount of the hydro-peroxide formed.

2.16. Biochemical Assay of Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT)

The activities of GOT and GPT in serum were measured by specific kits supplied by Span Diagnostic Ltd., Surat, India. The activities of these enzymes were expressed as IU/L of serum (Henry *et al*., 1960).

2.17. Histopathology of Testis and Pancreas

The tissues were subjected to paraffin embedding followed by section cutting (5 µm thick) with Leica microtome (Leica Biosystem). Deparaffinized sections were stained with hematoxylin and eosin. Histological examinations were carried out on stained sections with a computer-aided microphotography system using Avar Cap (Avatar Capture, Version-2.5; Aver Media Technologies Inc.), and Dewinter Caliper Pro 3.0 software (Dewinter Optical Inc.). The quantification of different generations of germ cells at stage VII was performed according to the method of Leblond and Clermont (1952). The cells present in this stage are spermatogonia-A (SgA), preleptotine spermatocytes (pLSc), mid-pachytene spermatocyte (mPSc), step seven spermatids (7Sd) and nineteen spermatids (19Sd). The different nuclei of the germ cells (except step ninteen spermatids, which cannot be precisely counted) were counted at twenty round tubular cross-sections in each rat. All the nuclear count of germ cells was corrected for differences in nuclear diameter by the formulae of Abercrombie (1947). True count= (Crude count × section thickness) / (section thickness + diameter of germ cell), and tubular shrinkage by the Sertoli cell correction factor (Clermont and Morgentaler, 1955).

The diameters of the pancreatic islets were measured by the Avar Cap (Avatar Capture; (Version-2.5); Aver Media Technologies Inc.) and Dewinter Caliper Pro 3.0 software (Dewinter Optical Inc.). Islet cells were counted per islet under 1000X magnifications. Islet count per specific parts of the pancreas was performed by a total scanning of the section under a microscope (Mallick *et al*., 2009).

2.18. Phytochemical Analysis of n-hexane Fraction of Hydro-methanol (2:3) Extract of the Seeds of Swietenia mahagoni (L.) Jacq.

2.18.1. Test for Alkaloids

The 0.25 g *n*-hexane fraction was defatted with 5 % ethyl ether for fifteen minutes. The defatted sample was extracted for twenty minutes with 5 mL of aqueous HCl using a boiling water bath. The resulting mixture was centrifuged for ten minutes at 3000 rpm (Trease and Evans, 2002).

2.18.2. Test for Flavonoids

A portion of the crude powder was heated with 10 mL of ethyl acetate over a steam bath for three minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 mL of dilute ammonia solution, and a yellow coloration was observed (Edeoga *et al.,* 2005).

2.18.3. Test for Tannins

A crude powder of 0.25 g in amount was stirred with 10 mL of distilled water. This was filtered and the ferric chloride reagent was added to the filtrate. A blue-black precipitate was taken as evidence for the presence of tannin (Kolawole *et al*., 2006).

2.18.4. Test for Terpenoids

0.25 g of crude powder was dissolved in 5 mL of methanol. Two mL of the extract was treated with 1 mL of 2, 4-dinitrophenyl hydrazine dissolved in 100 mL of 2M HCl. A yellow-orange coloration was observed as an indication of terpenoids (Kolawole *et al.,* 2006).

2.18.5. Test for Steroids

0.25 g of crude powder was dissolved in 5 mL of methanol. One mL of the extract was treated with 0.5 mL of acetic acid anhydride, and was cooled in ice. This was mixed with 0.5 mL of chloroform, and 1 mL of concentrated sulphuric acid was then added carefully using a pipette (Kolawole *et al.,* 2006).

2.19. Statistical Analysis

The analysis of variance followed by multiple comparisons using the two-tailed Student's T-Test for the

statistical analysis of the collected data by Origin 6.1 software (Origin Lab Corporation) (Sokal and Rohle, 1997). All of the values were indicated in the tables and figures as mean ± standard deviation (mean ± SD). Differences were considered significant at the level of $p < 0.05$.

3. Results

3.1. Body Weight and Organo-Somatic Indices

Body weight and organo-somatic indices were decreased significantly ($p<0.001$) in STZ-induced vehicle diabetic rats in comparison to the vehicle control group. The treatment of the diabetic animals with the n-hexane fraction (10 mg / 100 gm body weight / day for twenty-eight days) resulted in significant ($p<0.05$) recovery in the level of these parameters towards the control. The metformin treatment of diabetic animals resulted in resettlement of the above-mentioned parameters to the control level with the exception of the epididymis-somatic index (Table 1).

Table 1. Effect of *n*-hexane fraction of hydro-methanol (2:3) extract of *S. mahagoni* seeds on body weight and organo-somatic indices in streptozotocin-induced diabetic male albino rats.

Groups	Body weight (gm)		Testiculo-somatic index (gm%)	Epididymis-somatic index (gm%)	Seminal vesiculo-somatic index (gm%)
	Initial	Final			
Vehicle control	153.62± 4.51	161.03 ± 4.54	2.63 ± 0.17	0.65 ± 0.03	0.42 ± 0.03
Vehicle diabetic	154.21± 5.30	140.52± 3.76*	1.24 ± 0.14*	0.40 ± 0.04 *	0.20 ± 0.04 *
Diabetic +n-hexane fraction	151.94± 4.42	155.01± 4.12**	2.12 ± 0.12**	0.58 ± 0.02 **	0.36 ± 0.02 **
Diabetic + metformin	152.75± 4.36	160.22± 4.23	2.58 ± 0.16	0.59 ± 0.03**	0.41 ± 0.02

All values are expressed as mean ± SD, n = 6. Analysis of variance followed by multiple comparisons using two-tailed Student- t test, where *indicates $P< 0.001$ and **indicates $P <0.05$ compared with vehicle control group.

3.2. Fasting Blood Glucose (FBG) Level

Streptozotocin-induced diabetic animals resulted in a significant ($p<0.001$) elevation in the fasting blood glucose (FBG) level in comparison to the vehicle control animals. The level of this parameter was significantly ($p<0.05$) recovered towards the control level after the treatment of the STZ-induced diabetic animals by the n-hexane fraction. Treatment of the diabetic animals with metformin (1 mg / 100 gm body weight / day for twenty-eight days) resulted in resettlement in the level of FBG compared to the control level on the thirty-fifth day. Moreover, there was no significant difference in the level of FBG between the vehicle control and the metformin- treated diabetic groups on the thirty-fifth day (Table 2).

Table 2. Remedial effect of *n*-hexane fraction of hydro-methanol (2:3) extract of *S. mahagoni* seeds on fasting blood glucose level in streptozotocin-induced diabetic male albino rats.

Groups	Fasting blood glucose (FBG) level (mg / dL)					
	1st day	7th day	14th day	21st day	28th day	35th day
Vehicle control	75.3± 3.3	74.5± 3.4	76.6± 3.2	73.4± 3.1	75.3± 3.3	74.6± 3.2
Vehicle diabetic	73.6± 3.6	302.7± 4.3*	321.4± 4.7*	338.5± 4.8*	356.5± 4.9*	371.8± 4.8*
Diabetic +n-hexane fraction	74.1± 4.1	296.3± 3.8*	228.5± 3.8*	176.7± 3.7*	118.9±3.5*	95.2± 3.6**
Diabetic + metformin	76.4± 3.5	298.5± 4.1*	201.7± 3.5*	138.3± 3.6*	94.6±3.4**	76.4± 3.1

All values are expressed as mean ± SD, n = 6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P< 0.001$ and **indicates $P <0.05$ compared with vehicle control group.

3.3. Levels of Serum Insulin and Testosterone

The levels of serum insulin and testosterone were decreased significantly in the vehicle diabetic animals in comparison to the vehicle control animals. The oral administration of n-hexane fraction at the above-mentioned dose to the diabetic animals resulted in substantial correction of the levels of these parameters towards the control level. No significant variations were noted in the levels of serum insulin and testosterone between the n-hexane fraction treated groups and the metformin treated diabetic groups (Figure 1).

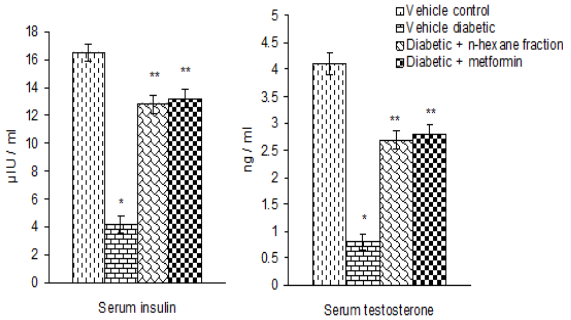

Figure 1. Effect of n-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on serum insulin and testosterone levels of STZ-induced diabetic male albino rats. All values are expressed as mean ± SD, n =6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P< 0.001$ and **indicates $P <0.05$ compared with vehicle control group.

3.4. Sperm Count, Viability and Motility

The epididymal sperm count and viability and motility were decreased significantly in the STZ-induced diabetic conditions when compared to the match vehicle control animals. Treatment of the diabetic animals with the n-hexane fraction resulted in significant ($p<0.05$) recovery of the above-mentioned parameters towards the control. The sperm count was recovered to the control level after the treatment of the diabetic animals with metformin though the sperm viability and motility were not recovered in this stage. No significant deviations were noted in sperm viability and motility between the n-hexane fraction-

treated group and the metformin treated diabetic groups (Table 3).

Table 3. Corrective role of *n*-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on sperm count, viability and motility in streptozotocin-induced diabetic male albino rats.

Groups	Sperm count (million/ml of epididymal fluid)	Sperm viability		Sperm motility (%)
		Alive sperm (%)	Dead sperm (%)	
Vehicle control	18.3±0.81	78.66±2.06	21.34±1.32	72.08±2.27
Vehicle diabetic	5.72±0.58*	33.51±1.87*	66.59±2.31*	41.71±1.92*
Diabetic +*n*-hexane fraction	13.2±0.69**	67.17±2.51**	30.83±1.7**	62.96±2.48**
Diabetic + metformin	17.8±0.76	68.47±2.63**	32.03±1.57**	64.17±2.61**

All values are expressed as mean ± SD, n = 6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P < 0.001$ and **indicates $P < 0.05$ compared with vehicle control group.

3.5. Activities of Testicular Δ5, 3β- Hydroxysteroid Dehydrogenase (Δ5, 3β- HSD) and 17 β- Hydroxysteroid Dehydrogenase (17 β- HSD) Enzymes

The testicular Δ^5, 3β- HSD and 17 β- HSD enzyme activities showed significant diminution in the vehicle diabetic rats when compared to the vehicle control animals. Activities of the above-mentioned enzymes in the testis were significantly recovered towards control after the treatment of the diabetic animals with n-hexane fraction. The 17 β- HSD enzyme activity was recovered to the control level after the metformin-treatment of the diabetic animals though the Δ^5, 3β- HSD enzyme activity was not recovered at this stage. There was no significant difference in the testicular Δ^5, 3β- HSD enzyme activity between the n-hexane fraction -treated groups and the metformin- treated diabetic groups (Figure 2).

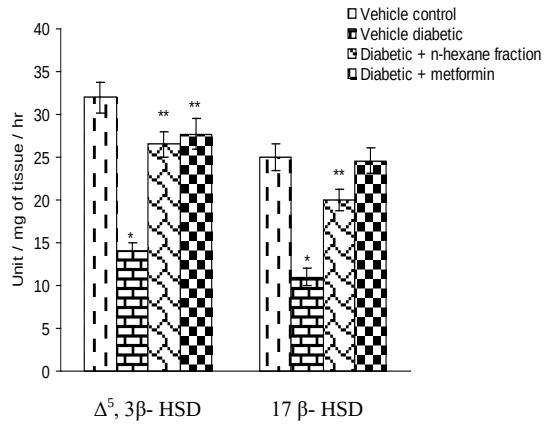

Figure 2. Corrective role of n-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on testicular Δ^5, 3β- HSD and 17β- HSD activities of STZ-induced diabetic male albino rats. All values are expressed as mean ± SD, n =6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P < 0.001$ and **indicates $P < 0.05$ compared with vehicle control group.

3.6. Testicular CAT, SOD and GST Enzymes Activities

Activities of CAT, SOD and GST in the testis were decreased significantly in vehicle diabetic animals in comparison to the vehicle control animals. After the n-hexane fraction treatment of the diabetic animals, the activities of the above-mentioned parameters were significantly recovered towards the control when comparison was made with the vehicle diabetic group. Metformin treatment of animals with diabetes resulted in resettlement of SOD activity to the control level whereas the CAT and GST activities were not recovered in this concern. Insignificant differences were noted in the activities of CAT and GST when a comparison was made between the n-hexane fraction treated groups, and the metformin treated diabetic groups (Figure 3).

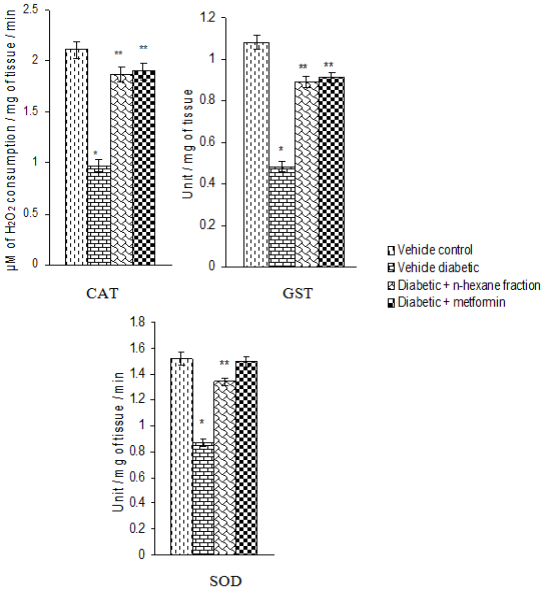

Figure 3. Remedial effect of *n*-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on testicular CAT, GST and SOD activities of STZ-induced diabetic male albino rats. All values are expressed as mean ± SD, n =6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P < 0.001$ and **indicates $P < 0.05$ compared with vehicle control group.

3.6. Levels of Conjugated Diene and Thiobarbituric Acid-Reactive Substances in Testis

Levels of testicular conjugated diene (CD) and thiobarbituric acid-reactive substances (TBARS) were significantly increased in the vehicle diabetic animals compared to the vehicle control animals. The treatment of the diabetic animals with n-hexane fraction or metformin at the above-mentioned dose resulted in significant recovery in the levels of these parameters towards the control. No significant deviations were noted in the levels of testicular CD and TBARS between the n-hexane fraction-treated groups and the metformin-treated diabetic groups (Figure 4).

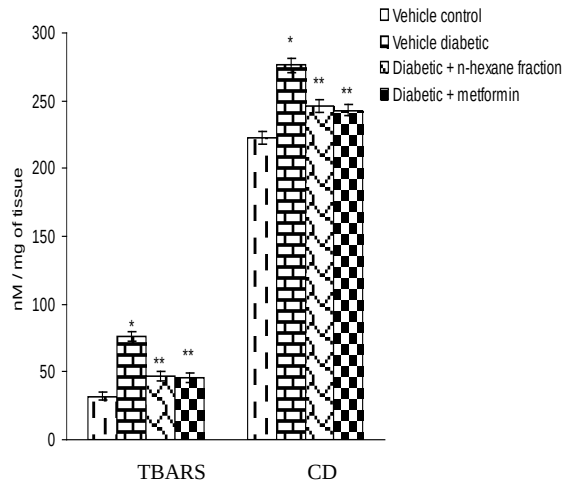

Figure 4. Effect of *n*-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on testicular TBARS and CD levels of STZ-induced diabetic male albino rats. All values are expressed as mean ± SD, n =6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P < 0.001$ and **indicates $P < 0.05$ compared with the vehicle control group.

3.7. Activities of SGOT and SGPT

Activities of transaminase enzymes, i.e., SGOT and SGPT, were increased significantly in vehicle diabetic animals in comparison with the vehicle control animals. After the treatment of the diabetic animals with n-hexane fraction or metformin at the above-mentioned dose in a fasting state, there was a substantial decrease in the activities of SGOT and SGPT towards the control level (Figure 5).

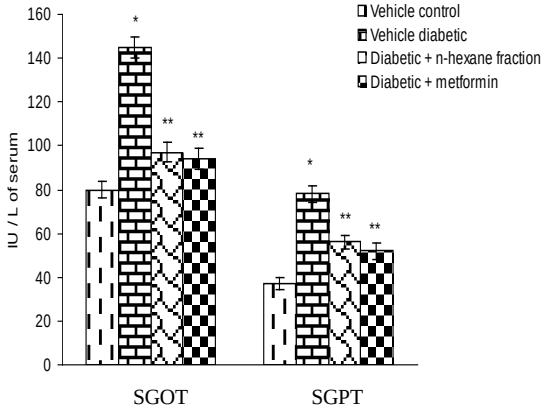

Figure 5. Corrective effect of *n*-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on serum GOT and GPT activities of STZ-induced diabetic male albino rats. All values are expressed as mean ± SD, n =6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P < 0.001$ and **indicates $P < 0.05$ compared with vehicle control group.

3.8. Histopathology of Pancreas

The diameters of pancreatic islets, count of islets, and islet cells per islet were substantially decreased. In addition, degeneration of pancreatic acini was also noted in the STZ-induced diabetic group in comparison with the vehicle control animals. The treatment of the diabetic animals with n-hexane fraction or metformin resulted in marked recovery of these parameters toward the control level. No significant deviations were noted in the above-mentioned parameters between the *n*-hexane fraction treated groups and the metformin-treated diabetic groups (Table 4 and Figure 6).

Table 4. Corrective effect of *n*-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on islet number, islet cell number, diameter of islets in streptozotocin-induced diabetic male albino rats.

Groups	Islet number (count per field in 1000X magnification)	No. Islet cells / Islet	Islet diameter (μ m)
Vehicle control	7.41±0.17	126.32±4.77	234.07±4.62
Vehicle diabetic	1.65±0.11*	71.74±3.38*	162.41±3.48*
Diabetic +*n*-hexane fraction	4.92±0.15**	106.59±4.32**	208.53±4.17**
Diabetic + metformin	5.06±0.16**	109.15±4.63**	210.76±3.95**

All values are expressed as mean ± SD, n = 6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P < 0.001$ and **indicates $P < 0.05$ compared with vehicle control group.

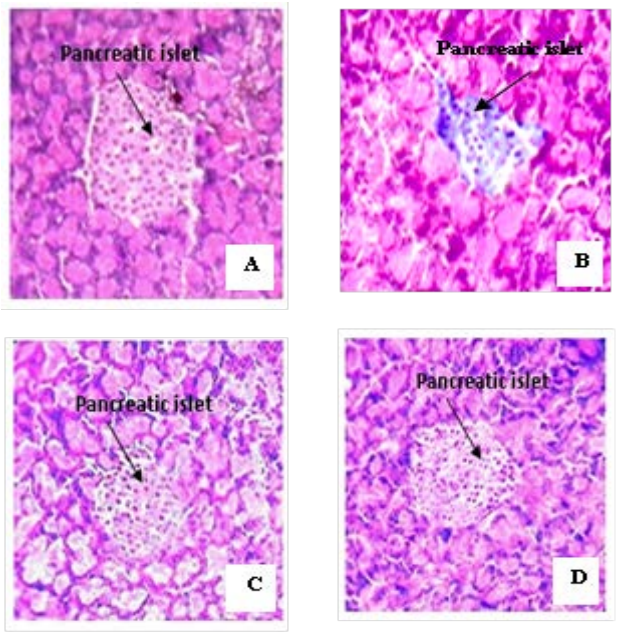

Figure 6. Histology of pancreas. **A:** Normal histoarchitecture of pancreatic islet, i.e. normal vehicle control group. **B:** Diminution in the diameter of the islet and islet cell population and degeneration of pancreatic acini cells in the vehicle diabetic group. **C and D:** Recovery of islet cell population and islet's diameter in n-hexane fraction-treated groups and metformin-treated diabetic groups (Haematoxylin-Eosin stain, X 400).

3.9. Histopathology of Testis

Vehicle treated control animals exhibited a normal histomorphological structure of testis, and dense lining of germ cells was noted in the periphery as well as in the middle part of the testis at stage-VII of the seminiferous epithelial cycle with normal diameter. Numbers of SgA, pLSc, mPSc, and 7Sd were all decreased significantly at

stage VII of the seminiferous epithelial cycle in the STZ-induced diabetic conditions in comparison to the vehicle control animals. The numbers of germ cells at stage VII of the seminiferous epithelial cycle were significantly recovered towards the control level after the n-hexane fraction treatment of the diabetic animals. Insignificant differences were noted in the numbers of SgA, pLSc, mPSc, and 7Sd germ cells at stage-VII of the spermatogenic cycle when comparisons were made between the *n*-hexane fraction treated groups and the metformin-treated diabetic groups (Table 5 and Figure 7).

Table 5. Effect of n-hexane fraction of hydro-methanol (2:3) extract of the *S. mahagoni* seeds on different generations of germ at stage VII in streptozotocin-induced diabetic male albino rats.

Groups	SgA	pLSc	mPSc	7Sd
Vehicle control	0.60±0.04	20.47±0.42	24.68±0.47	67.42±3.7
Vehicle diabetic	0.33±0.02*	9.75±0.32*	11.52±0.38*	34.05±2.8*
Diabetic +*n*-hexane fraction	0.53±0.03**	15.63±0.37**	19.27±0.40**	52.96±3.3**
Diabetic + metformin	0.54±0.04**	16.05±0.40**	19.86±0.45**	54.02±3.8**

All values are expressed as mean ± SD, n = 6. Analysis of variance followed by multiple comparisons using the two-tailed Student-t test, where *indicates $P < 0.001$ and **indicates $P < 0.05$ compared with vehicle control group.

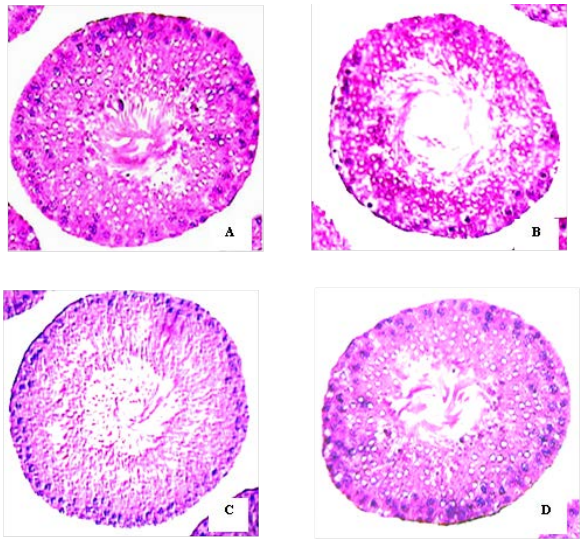

Figure 7. Histology of Testis. **A:** Normal density of different generations of germ cells in vehicle control group; **B:** Diminution in the number of different generations of germ cells at stage VII of vehicle diabetic group; **C and D:** Recovery in the number of different generations of germ cells at stage VII of n-hexane fraction and metformin-treated diabetic groups (Haematoxylin-Eosin stain, X 400).

3.10. Phytoingredients of n-Hexane Fraction

The *n*-hexane fraction of the hydro-methanol (2:3) extract from the seeds of *Swietenia mahagoni* (L.) Jacq. revealed the presence of medicinally-active antidiabetic and antioxidative constituents. The alkaloid, flavonoid and steroid constituents were present within the investigated fraction while terpenoid, and tannin were absent (Table 6).

Table 6. Phyto-constituents of *n*-hexane fraction of aqueous-methanol (2:3) extract of the sseds of *S. mahagoni* (L.) Jacq.

Phytoingredient(s)	*Swietenia mahagoni* (L.) Jacq.
Alkaloid	+
Flavonoid	+
Steroid	+
Terpenoid	-
Tannin	-

'+' indicate the presence of constituent and '–' indicate the bsence of constituent

4. Discussion

Diabetes mellitus (DM) is a leading disease of public health significance, which causes cardiovascular, psychological, and sexual dysfunctions. It is a well-known cause of male sexual dysfunction (MSD), with prevalence rates approaching 50% of both type 1 and type 2 diabetes mellitus (Johannes *et al.*, 2000). Day by day, the prevalence of MSD in diabetic patients is increasing. The incidences of impotence in diabetics are 2-5 times higher than non-diabetics (Zimmet, 2001). Impaired glucose metabolism leads to oxidative stress and protein glycation that in turn leads to a free radical generation. Diabetes suppresses the reproductive function resulting in male and female infertility (Rehman *et al.*, 2001; Enzlin *et al.*, 2002). Our data showed abnormal spermatogenesis following the STZ injection in male rats, which is concordant with data by other authors (Rabbani *et al.*, 2010). Our observations illustrate depressed sperm count and motility along with an increase in the percentage of dead sperm in diabetic animals. The number of different generations of germ cells at stage VII of the spermatogenic cycle was decreased significantly in diabetic rats in comparison to the control rats. This finding suggests that the conversion of spermatogonia into primary spermatocytes is reduced in diabetic conditions. These alterations in cellular conversion and/or activity lead to a reduction in the spermatozoid production. The current study was undertaken to investigate the remedial effect of the n-hexane fraction of the hydro-methanol (2:3) extract of *S. mahagoni* seeds on the management of diabetes-induced testicular dysfunctions noted in diabetic conditions. The effect was compared with a standard antidiabetic drug, namely, metformin.

The n-hexane fraction of the plant used here exhibited the most effective antidiabetic potentiality than other solvent fractions (i.e. chloroform, ethyl acetate, and n-butanol), supported by the study's report (Bera *et al.*, 2015). After the streptozotocin injection, the fasting blood glucose level increased and serum insulin levels were decreased; these results are in accordance with the authors' previous work (Bera *et al.*, 2015). After the treatment with the n-hexane fraction, there was a significant recovery in the levels of fasting blood glucose and serum insulin which can be attributed to the regeneration of the pancreatic β cells as confirmed by the present study and an earlier report by the same authors (Mallick *et al.*, 2007). Streptozotocin-induced diabetes resulted in significant diminution in testiculo-somatic, epididymal-somatic and

seminal vesiculo-somatic indices which may be attributed to the low- serum level of the testosterone because testosterone is the key regulator of the normal growth of these organs (Shrilatha and Muralidhara, 2007). After the treatment with this n-hexane fraction, the above-mentioned organo-somatic indices were recovered towards the control level which can be attributed to the elevation in serum testosterone. This elevation in serum testosterone after the administration of this fraction may be attributed to the elevation in the activities of androgenic key-enzymes i.e., Δ5, 3β-HSD and 17β-HSD, as well as by the recovery of serum insulin because insulin has a positive role in the testicular testosterone synthesis (Baccetti *et al*., 2002). This fraction may operate the proper tuning system of the pituitary-testicular axis by the insulin-glucose axis as proposed by others (Glenn *et al.*, 2003). Moreover, this fraction has the property to regenerate pancreatic β-cells; thus insulin levels will also increase and stimulate the testicular androgenesis. The n-hexane fraction has no general toxic effects reflected here by the body weight recovery and the measurement of the activities of SGOT and SGPT (Ghosh and Suryawanshi, 2001).

Another explanation for the corrective role of this *n*-hexane fraction on testis may be the correction in testicular oxidative stress noted here in the diabetic conditions by the measurement of testicular CAT, SOD and GST activities, and the important scavenging enzymes of reactive oxygen species (ROS) (Pillai and Gupta, 2005), in addition to the quantification of testicular TBARS and CD, which are the free radicals and end product (De-Young *et al*., 2004). The sperm count, which was decreased in diabetic states, may be due to the inhibition in spermatogenesis reflected here by the quantification of different generations of germ cells at stage VII of the spermatogenic cycle, an important reflector of holistic approach of spermatogenesis (Holstein *et al.*, 2003). Another possible reason behind the low sperm count is the effect of testicular oxidative stress on diabetic conditions reported by (Vincent *et al.*, 2002). Sperm motility and viability are also affected by the oxidative damaging effect of free radicals (De-Young *et al*., 2004). Increased cellular oxidative stress and altered antioxidant pool have been implicated in the pathogenesis of the chronic complications of diabetes (Vincent *et al*., 2002).

To comprehend the exact effect of oxidative stress on germ cells maturation and function, it is important to emphasize that some studies indicated the presence of high inducible cytochrome P-4502E1 isoform (CYP2E1) in male gonads (Healy *et al.*, 1999; Oropeza-Hernandez *et al.*, 2003; Quintans *et al.*, 2005). It is known that the CYP2E1 expression is affected by a variety of patho-physiological situations including diabetes (Knockaert *et al.*, 2011). Elevated oxidative stress and ROS production in diabetes often parallel an increased expression of CYP2E1 (Raza *et al.*, 2004). CYP2E1 generates reactive oxygen intermediates, such as superoxide radicals, which in turn could rapidly react with organic molecules generating secondary free radicals and ROS (Lieber, 1997). Such cascades may alter the antioxidant milieu of testis and epididymis; producing conditions for the spermatogenic cycle to interfere. After the administration of *n*-hexane fraction as protection from the testicular oxidative stress imposed in STZ-induced diabetic conditions, the sperm count, and viability, as well as a number of different generations of germ cells at stage VII were corrected towards the control level. This antioxidant activity of the n-hexane fraction may be due to the presence of phytoingredients such as alkaloid, flavonoid, and steroid which are traditional natural antioxidants.

5. Conclusion

From the preceding discussion, it may be concluded that this *n*-hexane fraction has a significant protective effect on testicular dysfunctions noted in STZ-induced diabetic conditions. The exact chemical compound(s) of n-hexane fraction responsible for such protection remains speculative, needing further studies to isolate, identify, and characterize the active ingredients and their molecular action in this regard.

Conflict of Interest Statement

We declare that we have no conflict of interest

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