JJBS

Volume 1, Number 3, September. 2008
ISSN 1995-6673
Pages 105 -108

Jordan Journal of Biological Sciences

Hepatoprotective Activity of "*Orthosiphon stamineus*" on Liver Damage Caused by Paracetamol in Rats

C. Maheswari *, R.Maryammal and R. Venkatanarayanan

R.V.S College of Pharamaceutical sciences,Sulur, Coimbatore, Tamil Nadu,India.

Abstract

The objective of this study was to investigate the hepatoprotective activity of Methanol extract of leaves of Orthosiphon stamineus against paracetamol induced hepatotoxicity. The material was dried in shade, they were powdered and Extracted with methanol. Preliminary phytochemical tests were done. Methanol extract showed presence of phenolic compound and flavanoids. The hepatoprotective activity of the methanol extract was assessed in paracetamol induced hepatotoxic Rats.Alteration in the levels of biochemical markers of hepatic damage like SGOT, SGPT, ALP and lipid peroxides were tested in both Paracetamol treated and untreaed groups. Paracetamol (2g/kg) has enhanced the SGOT, SGPT, ALP and the Lipid peroxides in liver. Treatment of methanolic extract of O.Stamineus leaves(200mg/kg)has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner.Our findings suggested that O.Stamineus methanol leaf extract possessed hepatoprotective activity.

Keywords : Hepato protection, *Orthosiphon stamineus* ,Leaves, Methanol, paracetamol

1. Introduction

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc). Inspite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations (Handa SS et al.,1989; Hikino H, et al., 1988; Evans WC et al.,1996; Sharma A, et al., 1991).

Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels.In addition serum levels of many biochemical markers like SGOT, SGPT,triglycerides, cholesterol,bilirubin,alkaline phosphatase are elevated (Mascolo N et al 1998).

Orthosiphon stamineus benth (Lamiaceae) better known as poonai meesai by the locals is rich in flavanoids.Most flavanoids are bioactive compounds due to the presence of phenolic group in their molecule.Twenty phenolic compounds were isolated from this plant including nine lipophilic flavones,two flavonol glycosides, nine caffeic acid derivatives (Sumaryono W., et al 1991) and the new compound 5,6,7,8-tetra hydroxy-6-methoxy flavone was isolated from this plant (M Amzad Hossain et al.,2007). It is widely used in India for treatment of eruptivefever,urinarylithiasis, edema, hepatitis, jaundice,

Hypertension diabetes mellitus,Gout,Rheumatism, diuretic, anti-inflammatory and influenza. They exhibit excellentantibacterial Antifungal, antimicrobial, anti-tumer, and insect anti feed ant activities (Saravanan D., et al 2006; Hossain M.A et al 2001). OS have been reported to possess anti inflammatory (Masuda, T et al 1992) ,antihypertensive (Ohashi K et al 2000)Hypoglycemic activity (Mariam, A et al 1999) and Diuretic effect (Galyuteva, G.I., et al 1990; Dona DD et al 1992). In the present study we have evaluated the hepatoprotective activity of this plant against paracetamol overdose – induced hepatotoxicity in rats.

2. Materials and Methods

2.1. Drugs and Chemicals

Paracetamol (farmsons,Gujarat). All other chemicals were obtained from local sources and were of analytical grade.

2.2. Plant Materials

The leaves of *Orthosiphon stamineus* were collected from siddha research institute, Arumbakkam, Chennai. The plant was identified and voucher specimen was deposited in the herbarium of the department of biology (Specimen no;L-121),Annamalai University, chidambaram.The material was dried in shade and powdered leaves 1kg were extracted with methanol in a Soxhlet extractor for 36 hr. Extract was evaporated under low pressure by using Buchi type evaporator.

* Corresponding author. e-mail: mahi3kp@yahoo.co.in.

2.3. Animals

Adult male wistar rats weighing 200-250g were obtained from Raja Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu. They were maintained at standard housing conditions and fed with commercial diet and provided with water ad libitum during the experiment. The institutional animal ethical committee (Reg.no 160/1999/CPCSEA) permitted the study.

Table 1 Effect of *O.Stamineus* Extract on Biochemical Parameters in Rats Subjected to Paracetamol Induced Hepatotoxicity

Group		Lipid peroxides (nmole of MDA/mg protein)	ALP(IU/L)	SGOT (IU/L)	SGPT (IU/L)
I (Control)		46.5 ± 2.47	1.0817 ± 4.542	100.33 ±3.16	65.42 ± 2.16
II (Paracetamol)		128.17 ± 3.05	2.1067 ± 4.030	207± 4.05	180 ± 3.03
(Paracetamol & *O.Stamineus* extract)					
III	100 mg/kg	75.83 ± 2.04	1.6950 ± 2.918	174.5 ± 3.13	155.67 ± 3.84
IV	200 mg/kg	54.33 ± 3.16	1.2567± 2.171	115.17 ± 3.63	70.83 ± 3.32
One-way ANOVA	F	169.097	232.550	203.482	344.513
	d.f	23	23	23	23
	P	0.001	0.001	0.001	0.001

Values are mean ± SEM of 6 animals in each groups Group II compared with Group I (P<0.001), Group III and IV compared with Group II (P<0.001).

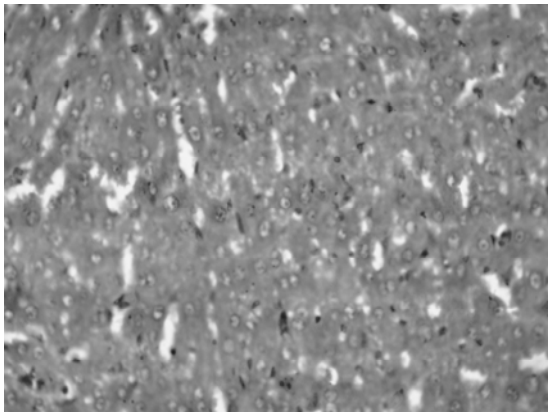

Figure 1. Liver tissue of control rats showing normal histology

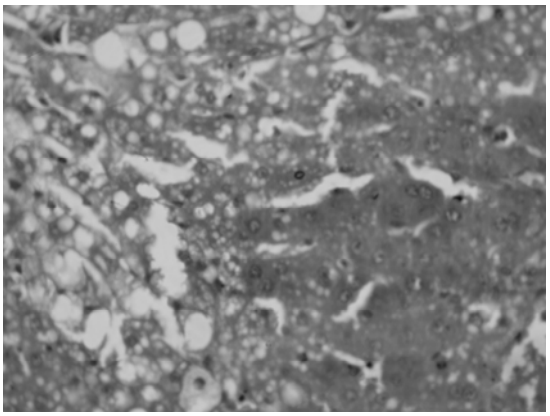

Figure 2. Liver tissue of paracetamol treated rats showing necrosis of the hepatic cells

2.4. Experimental Design

Four groups of six animals were used for the study.Control group received single daily dose of 5 % tween 80 (5 ml/Kg; po) for 4 days and a single dose of 40% sucrose solution (1 ml/rat; po) on day 3.

Paracetamol group received single daily dose of 5 % tween 80 (5ml/kg; po) for 4 days and a single dose of paracetamol suspension (2g/kg ,po)on day 3.

Test groups received daily doses (100mg/kg and 200mg/kg) of OS extract for 4 days and single dose of paracetamol suspension on day 3. Animals were sacrified under light ether anaesthesia, 48 hour after paracetamol administration.

2.5. Biochemical Study

Animals were sacrificed by cervical dislocation.The blood samples were cpllected by direct cardiac puncture . The blood samples were allowed to clot and serum were separated and the serum was used for the assay of maker enzymes viz.,Glutamate oxaloacetate transaminase(SGOT) ,Glutamate pyruvic transaminase,(SGPT) (Reitman S, et al1957) alkaline phosphatase (ALP)(Bessey OA et al 1964).

2.6. Estimation of Liver Lipid Peroxides

Estimation of liver lipid peroxides malondialdehyde (the product of lipid peroxidation) in the liver homogenate was measured as described(Ohkawa H et al 1979). Protein in the liver homogenate was measured according to the method of lowery et al (Lowry O et al 1951).

2.7. Histopathological Examination

Small pieces of liver tissue were collected in 10% formaldehyde solution for histopathological study. The pieces of liver were processed and embedded in paraffin wax sections were made about 4-6μm in thickness. They were stained with hematoxylin and eosin and photographed.

2.8. Statistical Analysis

The results were expressed as mean ± SEM of six animals from each group. The statistical analysis were carried out by one way analysis of variance (ANOVA)
P values < 0.05 were considered significant.

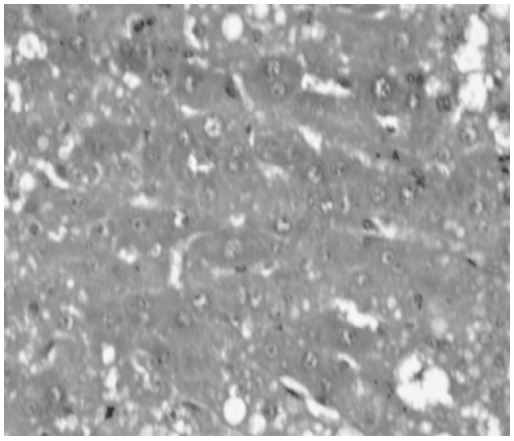

Figure 3. Liver tissue of paracetamol + OS extract (200 mg/kg) treated rats showing normal hepatic cells and central Vein

3. RESULTS

3.1. Paracetamol-Induced Hepatotoxicity

Preliminary phytochemical studies revealed the presence of phenolic compound and flavonoids were noticed in methanolic leaf extract. Table 1 shows that administration of paracetamol induced 48 hour after intoxication, a marked increased in serum SGOT, SGPT, alkaline phosphatase.

The toxic effect of paracetamol was controlled in the animals treated with methanol extracts (100mg/kg and 200 mg/kg) by way of restoration of the levels of the liver function.

At a dose of 100 mg/kg, the effect was only marginal whereas at higher dose (200mg/kg) the drug effectively prevented the paracetamol induced liver damage.

Paracetamol treatment group resulted in an increase in the lipid peroxide levels in liver homogenates. Administration of the methanol extract of O.stamineus leaves prevented the accumulation of lipid peroxides. At a lower dose (100mg/kg) there was a marginal effect in the lipid peroxide level where as at higher dose (200mg/kg) the drug effectively prevented paracetamol – induced elevation of lipid peroxides in liver (Table1)

3.2. Histopathology

Histological studies also confirmed the hepatoprotective effect of the methanol extract of O.stamineus. Paracetamol treated rat liver sections showed cloudy swelling and fatty degeneration of hepatocytes, necrosis of cells were also seen (Figure 1). The drug treatment (200mg/kg methanol extract) almost normalized these effects in the histoarchitecture of liver (Figure3).

4. DISCUSSION

Paracetamol is a known antipyretic and an analgesic which produces hepatic necrosis in high doses. Paracetamol is normally eliminated mainly as sulfate and glucuronide. Administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinemine by cytochrome-450 enzymes.Semiquinone radicals, obtained by one electron reduction of N-acetyl-p-benzoquineimine, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage.Higher doses of paracetamol and N-acetyl-p-benzoquineimine can alkylate and oxidise intracellular GSH, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage (Diadelis R et al 1995). In our experiments it is observed that the lipid peroxidation levels in the paracetamol group is increased.This clearly indicates that there is a significant hepatic damage due to paracetamol and this is further evident from the fact that there is elevation in the levels of various markers of hepati damage like SGOT, SGPT and ALP. Treatment with O.stamineus leaf extract has decreased the levels of lipid peroxidation and the elevated levels of above mentioned biochemical markers to the near normal levels. It may be concluded that the hepatoprotective effect of O.stamineus leaves is due to the prevention of the depletion in the tissue GSH levels. Literature review shows that the O.stamineus contains phenolic compound and flavanoids which are present in the methanol extract. Therefore there is a possibility that the O.stamineus leaf extract may possess hepatoprotective activity.

ACKNOWLEDGEMENT

The authors are grateful to Dr.R.Manavalan, Head of the department of pharmacy and Mr.R.SURESHKUMAR, Assistant professor in Horticulture , Annamalai University, AnnamalaiNagar, Chidambaram for providing all the necessary facilities to carry out this work.

References

Handa SS, Sharma A, Chakraborty KK. Natural products and plants as liver protecting drugs. Fitoterapia 1989; 57: 307-51.

Hikino H, Kiso Y. Natural products for liver diseases in Economic and medicinal plant research. Vol 2, Academic press, London. 1988:39-72.

Evans WC. An overview of drugs having anithepatotoxic and oral hypoglycaemic activities In: Trease and Evans pharmacognsoy, 14th ed. U.K., W.D. Sanders Company Ltd. 1996.

Sharma A, Shing RT, Sehgal V, Handa SS, Antihepatotoxic activity of some plants used in herbal formulations. Fitoterapia 1991; 62: 131-8.

Masuda, T., Masuda, et al. (1992). Orthosiphol A and B, Novel diterpenoid inhibitors of TPA (12-O-tetradecanoylphorbol – 13 – acetate) – induced inflammation, from *Orthosiphon stamineus*. Tetrahedron 48 (33) : 6787 – 6792.

Mariam, A., M.Z. Asmawi, et al. (1999) hypoglycaeic activity of the aqueous mextract of *Orthosiphon stamineus*. Fitoterapia 67 (5): 465 – 468.

Galyuteva, G.I., N.A. Benson, et al., (1990). Comparative evaluation of the diuretic activity of leaves and leaf tissue culture biomass of *orthosiphon stamineus* Benth. Rastite 'Nye Resursy 26(4) ; 559 – 565.

Dona DD, Nguyen NH, Doan HK, et al. studies on the Individual and combined Diuretic Effects of Four Vietnamese Traditional Herbal Remedied (Zea Mays, Imperate cylindrical, plantago major and *orthosiphon stamineus*). J.Ethnopharmacol. 1992; 36 (3) : 225 - 31.

Reitman S, Frankel S.A. Colorimetric method for the determination of serum glutamic Oxaloacetic and glutamic pyruvic transaminase. AM J Clin Pathol 1957; 28: 56 – 63.

Bessey OA, Lowery DH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic meters of serum, J. Biol chem. 1964; 164:321 –9.

Ohkawa H, Ohishin N & Yagi K, Anal Biochem, 95 (1979) 351.

Lowry O.Hm Rosebrough N.J Farr A L & Randall R.J, J Biol Chem, 193 (1951) 265.

Saravanan D., Hossain M.A., Salman Z.,Gam L.H., and Zhari I.,Chemometrics and Intelligent Laboratory Systems,2006,81,21

Hossain M.A.,Salehuddin S.M., and Tarafdar S.A Pakistan J.Sci.Ind. Res., 2001,44(4),191

Ohashi K, bohgak T, Shibuya H., Antihypertensive substances in the leaves Kumis Kucing(orthosiphon stamineus) in java island. *Yakugaku Zasshi* 2000;120(5):474-82

Sumaryono W., Proksch P.,Wray V., Witte L., L., Hartmaan T., Qulatative and Quantitative analysis of the phenolic constituents from *Orthosiphon aristatus*.Planta medica1991;57:176-180

M Amzad Hossain, S .M. Salehuddin and Zhari Ismail Isolation and Characterization of a new poly hydroxy Flavone from the leaves of *Orthosiphon Stamineu*s Indian J.Nat.prod.,2007,23(4).3-7

Diadelis R, Jan NM,Commandeur ED,Groot,Nico PE,Vermeulen.Eur J Pharmacol:Environ Toxicol Pharmacol Sect 1995;293:301

Mascolo N,Sharma R,Jain SC, Capasso F. J Ethnopharmacol 1998;22:211