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LETTER TO THE EDITOR

EFFECT OF *OCIMUM SANCTUM* (OS) LEAF EXTRACT ON HEPATOTOXICITY INDUCED BY ANTITUBERCULAR DRUGS IN RATS

Sir,

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Drug induced hepatotoxicity is a major form of iatrogenic disease. The combination of isoniazid, rifampicin and pyrazinamide is commonly used for chemotherapy of pulmonary tuberculosis and all the three are known to be potentially hepatotoxic (1). Treatment of drug induced hepatotoxicity is largely directed towards discontinuing the offending drug (2), which is mostly undesirable in chemotherapy of tuberculosis. Hence the need of an easily available hepatoprotective agent.

Ocimum sanctum (OS), popularly known as Tulsi is a holy plant common to most Indian households. Ancient Hindu literature is abundant with the medicinal actions of OS. Indian people can easily comply with the daily dose of this holy plant. *Ocimum sanctum* is known to possess anti-inflammatory, antimicrobial and antidiabetic activity (3, 4, 5). *Ocimum sanctum* leaves have been shown to exert hepatoprotective effect in the models of predictable hepatotoxicity like paracetamol and carbon tetrachloride induced liver damage in rats (6, 7). However the effect of OS on the model of unpredictable hepatotoxicity has not yet been studied. Hence the present study has been undertaken to study the effect of OS leaf extract on hepatotoxicity induced by antitubercular drugs in rats. At the same time the therapeutic effect of OS leaf extract on reversal of hepatotoxicity was also studied.

Healthy adult albino rats of either sex of Wistar strain weighing 150 to 250 g were used after approval of the Institutional Ethics Committee. They were kept on standard pellet diet and were allowed free access to food and water. The doses of anti-tubercular drugs (isoniazid (I)-27 mg/kg/day, rifampicin (R)-54 mg/kg/day, pyrazinamide (Z)-135 mg/kg/day) were extrapolated from daily human dose using the conversion table based on body surface area (8). All the three drugs were obtained from Plethico Pharmaceuticals Ltd. Indore. *Ocimum sanctum* leaves were collected after authentication by pharmacognosist. Air dried powder of *Ocimum sanctum* leaves was extracted by percolation at room temperature with 70% ethyl alcohol. The extract was concentrated under reduced pressure (bath temperature 50°C) and finally dried in vaccum desiccator. The residue of *Ocimum sanctum* leaf extract was dissolved in propylene glycol at a concentration of 100 mg/ml and was used in the experiment (7). OS leaf extract was administered in the dose of 200 mg/kg/day

orally as this dose has been shown to be hepatoprotective in other models of hepatotoxicity (6, 7). The animals were divided into six groups. Each group contained twelve animals. The groups were treated as follows :

Group I : control i.e. propylene glycol orally for 30 days.

Group II : control i.e. gum acacia orally for 30 days.

Group III : (I + R + Z) suspension orally for 30 days.

Group IV : (I + R + Z) suspension + OS leaf extract 200 mg/kg orally for 30 days.

Group V : (I + R + Z) suspension orally for 30 days + no treatment from day 31 to day 50.

Group VI : (I + R + Z) suspension orally for 30 days + OS leaf extract from day 31 to day 50.

Oral feeding was done by 18 gauze blunted needle of 10 cm length with a small solder ball applied around the tip and a gentle bend just proximal to the solder. Blood samples of animals from groups I, II, III and IV were taken for liver function tests by cardiac puncture under ether anaesthesia and the livers removed for histopathological examination on 30th day. Blood samples of groups V and VI were taken on 40th day from tail vein and on 50th day by cardiac puncture for estimation of liver function tests. On 50th day animals of these two groups were sacrificed by stunning and livers removed for histopathological examination.

Assessment of liver damage was done by biochemical investigations of Serum alanine aminotransferase (serum ALT) and Serum aspartate aminotransferase (serum AST) by Reitman and Frankel Method (9) and Serum protein, Serum bilirubin and serum alkaline phosphatase (serum ALP) by Biuret method (10), Modified Jendrassik and Grofs method (11) and Kind and King method (12) respectively. Histopathological assessment of liver damage was done by using a method of scoring of the structural changes described by National health services Maryland USA (13). Statistical analysis was done in consultation with biostatistician from department of preventive and social medicine S.R.T.R. Medical College, Ambajogai. All the groups were first subjected to analysis of variance by using oneway ANOVA; and unpaired 't' test was then applied for comparison between two groups i.e. group III was compared with group I, group IV, group V and group VI. $P<0.05$ was taken as significant. As there was no statistical difference between the two control groups I and II, only one control group i.e. group I of propylene glycol was used for comparison.

Group III receiving antitubercular drugs for 30 days showed significant fall in serum protein level and rise in the levels of serum bilirubin, serum ALT, serum AST and serum ALP as compared to the control group. Co-administration of OS leaf extract along with the antitubercular drugs significantly prevented all these changes. Mere

TABLE I: Effect of *Ocimum sanctum* leaf extract on liver function tests in anti tubercular drugs treated rats.

Group (n = 12)	*Serum total protein (gm/dl)*	*Serum bilirubin (mg/dl)*	*Sr. AST (units/ml)*	*Sr. ALT (units/ml)*	*Sr. ALP (KA units)*
Group I	7.6±0.50	2.1±0.32	31.0±2.30	35.16±0.80	11.0±0.89
Group II	7.7±0.40	2.0±0.22	32.1±3.20	34.26±0.50	11.5±0.66
Group III	5.0±0.036##	4.05±0.50###	75±2.91###	144±2.02###	42.6±0.99###
Group IV	7.5±0.56*	2.5±0.5*	33±1.53***	30±0.58***	11.5±0.77***
Group V 40th day	4.8±0.34	3.86±0.45	74.6±3.22	140±0.96	41±0.84
Group VI 40th day	5.08±0.27	2±0.28*	72±2.19	92±0.97***	40±1.54
Group V 50th day	5.3±0.5	3.2±0.52	69.5±0.88	80.66±2.11***	37.6±1.21**
Group VI 50 day	6.66±0.17**	1±1.24***	28±2.79***	29.8±2.15***	11±1.99***

(All values are Mean ± SEM for each group.
One-way ANOVA revealed significant difference between the groups.
Unpaired 't' test :–
= Vs group I * = Vs group III
#,* = P<0.05 ##,** = P<0.01 ###,*** = P<0.001

withdrawal of antitubercular drugs failed to produce significant reversal of any of the biochemical parameters within 10 days (group V, 40th day). Treatment with OS leaf extract for 10 days after withdrawal of antitubercular therapy (group VI, 40th day) significantly reversed only two biochemical parameters i.e. serum ALT and serum bilirubin as compared to the group III. Mere withdrawal of antitubercular drugs significantly reversed only two parameters i.e. of serum ALT and serum ALP within 20 days (group V, 50th day). On the other hand treatment with OS leaf extract for 20 days (group VI, 50th day) after withdrawal of antitubercular therapy significantly reversed all the biochemical changes as compared to group III (Table I). Administration of antitubercular drugs for 30 days to group III produced changes of degeneration, necrosis and fibrosis on histological examination of rat livers (Fig. 1). Concurrent administration of OS leaf extract to group IV preserved the histological structure of liver (Fig. 2) by significantly reducing the scores of degeneration, necrosis and fibrosis with evidence of significant regeneration. In group V for which the 30 days antitubercular therapy was merely withdrawn for 20 days, only the scores of fibrosis and regeneration changed significantly as compared to group III; the other scores reversed slightly but the change was not significant. Treatment with OS leaf extract for 20 days after withdrawal of antitubercular therapy to group VI 50th day significantly reversed all the histopathological scores as compared to group III (Table II).

Co-administration of OS leaf extract along with the anti – tubercular drugs significantly prevented all the biochemical and histological alterations caused by the antitubercular drugs. Administration of OS leaf extract was found to be more effective than mere withdrawal of antitubercular

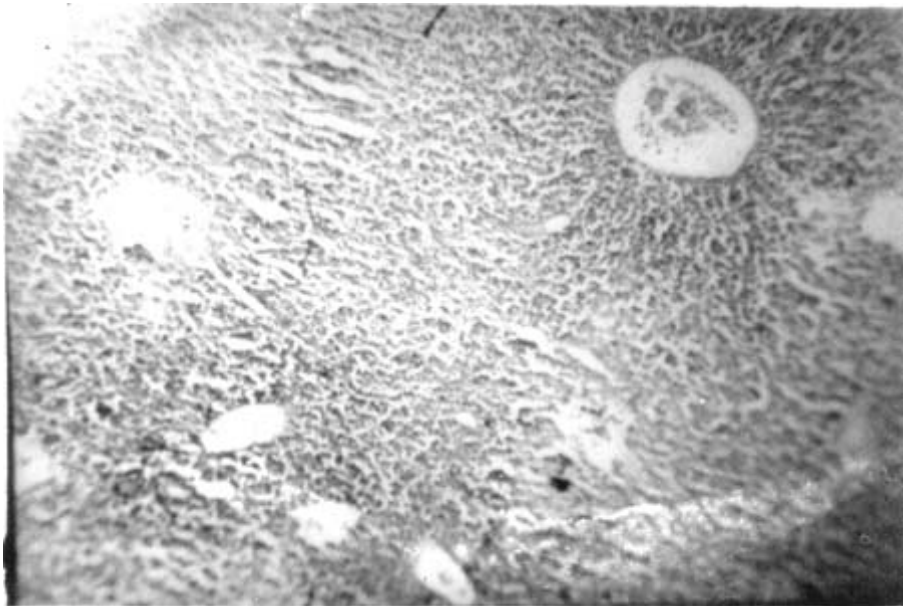

Fig. 1 : Photograph showing changes of degeneration, necrosis and fibrosis in livers of rats fed on antitubercular drugs.

TABLE II : Effect of *Ocimum sanctum* leaf extract on histopathology score in antitubercular drugs treated rats.

Group (n = 12)	*Degeneration*	*Necrosis*	*Fibrosis*	*Regeneration*
Group I	0	0	0	0
Group II	0	0	0	0
Group III	$2.1 \pm 0.48^{\#\#\#}$	$2.8 \pm 0.48^{\#\#}$	$2.5 \pm 0.43^{\#\#\#}$	0
Group IV	$0.5 \pm 0.22^{**}$	$1.66 \pm 0.33^{*}$	$0.83 \pm 0.4^{*}$	$1.66 \pm 0.33^{***}$
Group V 50th day	1.5 ± 0.22	1.0 ± 0.26	$0.66 \pm 0.33^{**}$	$0.83 \pm 0.30^{*}$
Group VI 50th day	$0.33 \pm 0.49^{*}$	$0.66 \pm 0.30^{*}$	$0.50 \pm 0.22^{**}$	$1.0 \pm 0.33^{*}$

(All values are Mean ± SEM for each group.
One-way ANOVA revealed significant difference between the groups.
Unpaired 't' test :–
$^{\#}$ = Vs group I * = Vs group III
$^{\#,*}$ = P<0.05 $^{\#\#,**}$ = P<0.01 $^{\#\#\#,***}$ = P<0.001

drugs in reversing the biochemical and histological changes. Oxidative stress has been found to be the most important mechanism in hepatotoxicity of antitubercular

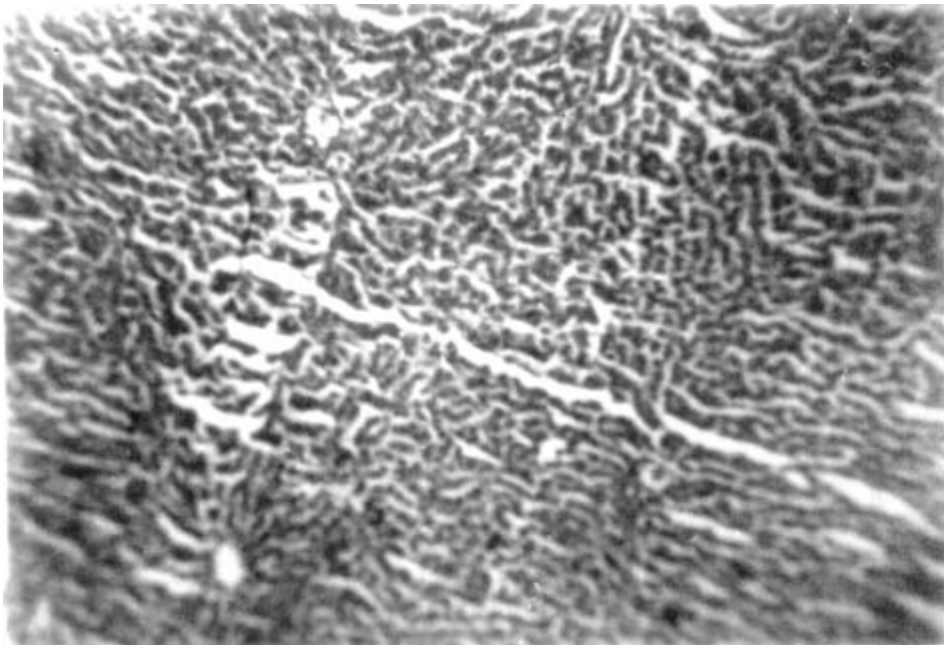

Fig. 2 : Photograph showing preservation of histological structure of livers in rats treated with *Ocimum sanctum* leaf extract.

drugs (14, 15). OS leaf extract has been found to significantly decrease the levels of hepatic lipid peroxidase and increase the levels of superoxide dismutase and catalase (16). Pretreatment with OS leaf extract has been found to prevent the radiation induced depletion of glutathione, glutathione peroxidase and superoxide dismutase and to prevent increase in lipid peroxidation rate (17). Antioxidant mechanism of OS leaf extract has been found to play a role in its anticataract activity (18).

Similarly, it may be presumed that ursolic acid, which is one of the key constituents of OS leaf extract, is responsible for inhibition of lipid peroxidation (19, 20). Membrane stabilizing property of OS has been shown to be responsible for its hepatoprotective action (21).

Thus though the exact mechanism of hepatoprotective action of OS leaf extract is not yet known, its antioxidant activity as revealed in some earlier studies seems to be the most important mode of its hepatoprotective action. Further extensive

studies and trials may find its role as a hepatoprotective agent or as an adjuvant in the management of antitubercular drugs induced hepatotoxicity.

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REFERENCES

1. Parthasarathy R, G. Raghupati Sarma, Janardhanan B, Padma Ramchandran, Santha T, Sivasubramanian S. et al. Hepatic toxicity in South Indian patients during treatment of tuberculosis with short-course regimens containing isoniazid, rifampicin, and pyrazinamide. *Tubercle* 1986; 76.
2. Speeg KV and Bay MK. Prevention and treatment of drug induced liver disease. *Gastroenterol Clin North Am* 1995; 24: 1047–1064.
3. Singh S and Majumdar DK. Evaluation of anti-inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. *Indian J Exp Biol* 1997; 35(4): 380–383.
4. Gupta SK, Jaiprakash and Sushma S. Validation of traditional claim of tulsi *Ocimum sanctum Linn.* as a medicinal plant. *Indian J Exp Biol* 2002; 40: 765–773.
5. Agarwal P, Rai V and Singh RB. Randomized placebo controlled, single blind trial of holy basil leaves in-patients with non insulin-dependent diabetes mellitus. *Int J Clin Pharmacol Ther* 1996; 34(9): 406–409.
6. Chattopadhyay RR, Sarkar SK, Ganguly S, Medda C and Basu TK. Hepatoprotective activity of *ocimum sanctum* leaf extract against paracetamol induced hepatic damage in rats. *Indian J Pharmacol* 1992; 24: 163–165.
7. Bhargava KP and Singh N. Antistress activity of *Ocimum sanctum linn. Indian J Med Res* 1981; 73: 443–451.
8. Ghosh MN, Fundamentals of experimental pharmacology. Scientific book agency, Calcutta, 1984; 155.
9. Reitman S and Frankel S. In vitro determination of transaminase activity in serum. *Am J Clin Pathol* 1957; 28: 56–60.
10. Doumas BT. Estimation of total protein in serum/plasma by biuret method. *Clin Chem Acta* 1978; 23: 663.
11. Jendrassik L and Grof P. Quantitative estimation of bilirubin in serum/plasma by Jendrassik and Grof's method. *Biochem Z* 1938; 29: 81.
12. Kind PRN and King EJ. Estimation of plasma phosphatases by determination of hydrolysed phenol with aminoantipyrine. *J Clin Path* 1954; 7: 322–326.
13. Davidson CS. Guidelines for detection of hepatotoxicity due to drugs and chemicals. NIH publication, U.S. Department of Health and Education and Welfare NIH U.S.A. 1979.
14. Sodhi CP, Rana S, Mehta S, Vaiphei K, Goel RC, Mehta SK. Study of oxidative stress in rifampicin induced hepatic injury in growing rats with and without protein energy malnutrition. *Hum Exp Toxicol* 1997; 16: 315–321.
15. Sodhi CP, Rana SV, Mehta SK, Vaiphei K, Attri S, Thakur S et al. Study of oxidative stress in isoniazid induced hepatic injury in young rats with and without protein energy malnutrition. *J Biochem Toxicol* 1996; 11: 139–146.
16. Panda S and Kar A. *Ocimum sanctum* leaf extract in regulation of thyroid function in male mouse. *Pharmacol Res* 1998; 38(2): 107–110.
17. Gana soundari A, Devi PV and Rao MN. Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury. *Indian J Exp Biol* 1999; 37(3): 262–268.
18. Sharma P, Kulshreshtha S and Sharma AL. Anti cataract activity of *Ocimum sanctum* on experimental cataract. *Indian J Pharmacol* 1998; 30: 16–20.
19. Balanehru S and Nagarajan B. Protective effect of oleandolic acid and ursolic acid against lipid Peroxidation. *Biochem Int* 1991; 24: 981–990.
20. Liu. J. Pharmacology of oleandolic acid and ursolic acid. *J Ethnophamacol* 1995; 49: 57–68.
21. Sen P, Dewan V, Bhattacharya SK, Gupta VS, Maiti PC and Mediratta PK. In brain and Psychophyioslogy of stress. New Delhi, ICMR Publication 1988; 245.

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