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PREVENTION OF HEPATORENAL TOXICITY OF ACETAMINOPHEN WITH *OCIMUM SANCTUM* IN MICE

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ABSTRACT

Aim: To evaluate hepatorenal protective potential of leaves of *Ocimum sanctum* against lethal and sublethal doses of acetaminophen.

Materials and methods: Aqueous filtrate of dried leaf powder in distilled water is tested at three dose levels (100, 75 and 50 mg/kg./oral) against sub lethal dose (300mg/kg.bw/i.p.) of acetaminophen (paracetamol) towards liver and kidney of Swiss albino mice (Exp-I).In another study (Exp-II) test drug is tried at its maximum dose (1.5gm/kg/oral)against lethal dose (1gm/kg./i.p) of acetaminophen.

Result: Test drug could prevent acetaminophen induced mice in the serum levels of GOT, GPT, AP, Urea and Creatinine associated with histophysological changes in liver and kidney at two higher doses. Drug could not reduce lethality.

Conclusion: Leaves of *Ocimum sanctum* could reduce hepatorenal toxicity of acetaminophen in mice under laboratory conditions.

Key words: Hepatorenal toxicity, *ocimum sanctum*, paracetamol.

1. INTRODUCTION

During recent past several herbal compounds have been tested against paracetamol (acetaminophen) induced hepatic damage but kidney is badly ignored [1]. Common Indian medicinal plants *Osmium sanctum* (tulsi) is not an exception however,it is antihepatotoxic against acetaminophen (paracetamol) and antitubercular drugs and enen antinephrotoxic against mercuric chloride [2,3,4] Overdoses of acetaminophen damage both, liver and kidney hence hepatorenal protective role of some herbal compound have been tested in our lab[5,6,7.] and this paper deals with a study on the hepatorenal protection with the *ocimum sanctum* leaves in mice.

2. MATERIALS AND METHODS

2.1 Animal Model: Isogenic Swiss Albino male mice of 20-25 gm were obtained from Biological Production Division, Govt Veterinary College, Mhow, MP. They were maintained on standard food and tap water *ad-libitum*. They were housed in well ventilated animal house under natural day/night (light/dark) cycle.

2.2 Herbal Drugs: Dried leaves of *ocimum sanctum* were procured from local herbal drug shop. They were crushed to powder. The powder was thoroughly mixed in known amount of distilled water using mortar and pestle which was filtered by ordinary filter paper. This clear aqueous filtrate was orally administered to mice using blunt, bent, thick (no. 18) needle fitted on a syringe. The doses of drug were selected from described values in the literature on herbal drugs.

2.3 Experiment I: ***Protection at lethal dose****:* Preliminary experiments were performed on mice to estimate the protective effect of leaves against *lethal* dose of acetaminophen (1g/kg/ip). Mice were divided into two groups of 10 animals each. Mice of one group were treated orally with aqueous filtered of leaves at 1.5gm/kg/oral dose and mice of another group received equal volume of distilled water; mice of both groups also received an intraperitoneal injection of acetaminophen after 1 hour. The mortality was observed 24 hour after acetaminophen administration in both groups. Percentage protection against lethal effect of acetaminophen was calculated.

2.4 Experiment II: ***Hepatorenal protection at sublethal dose*** :Hepatic and renal injuries were induced in mice by administration of single *sublethal* dose (300mg/kg/i.p.) of acetaminophen injection (Intas Ltd.) following 07days prophylactic drug administration. Detail are shown in table -1.

Table-1: Experimental Design.

Group I (treated with vehicle)	*Control group* : Mice were treated orally with distilled water daily for 7 days followed by single ip injection of benzyl alcohol (vehicle) 2 hr after last treatment (vol. equal to that of injection of acetaminophen used in Gr-II) on 7th day.
Group II	*Acetaminophen treated group*: Mice were given distilled water orally for 7days and single injection of sub lethal dose of acetaminophen (300mg/kg/i.p.) on 7th day, 2 hr after last treatment.
Group III, IV, V	*Drug pre-treated and acetaminophen challenged Group:* Mice were treated with leave's filtrate in distilled water at *three doses* 100,75 & 50 mg/kg/oral daily for 7 days followed by single i.p. acetaminophen injection on 7th day 2 hr after last treatment (as in group II).

2.5 Biochemical Observations: On 9th day i.e. 48 hours after last treatment blood sample of each animal was taken directly from heart under mild chloroform anaesthesia and biochemical parameters GOT, GPT, billirubin & AP as liver function tests and creatinine and urea as kidney function tests were evaluated using ready to use available kits made by standard companies (i.e. BEACON diagnostics Pvt. Ltd., AGAPPE diagnostics,ACCUREX biomedical Pvt. Ltd., VITAL diagnostics (P) Ltd.) in a pathological clinic.

2.6 Histopathological Observation: Also, on 9th day pieces of liver and kidney from each animal were fixed in Bouin's fluid for routine histopathology. Haematoxylin eosin stained section were observed for histopathological study and were photographed for record.

(vii) Statistical Analysis: All experiments were done thrice. Data were subjected to statistical comparisons made by means of student's t-test.

3. RESULTS

3.1 Lethality test: (Experiment I).

Results are shown in Table 2. It is found that *ocimum sanctum* could afford very little protection against lethal dose of acetaminophen.

Table-2: Protection against lethal dose of acetaminophen by leaves of *ocimum sanctum*.

S.No.	Group	Total number of mice used	Mortality	Percentage protection
1.	Acetaminophen treated group	10	10 (100%)	0%
2.	Acetaminophen challenged to drug treated group	10	08 (80%)	20%

3.1 Histological Observations: (Figs 1-5 in both plates)

Among mice of control group liver and kidney sections revealed usual histology, free from any pathological sign (Fig-1).Single injection of sublethal dose of acetaminophen (300mg/kg) caused severe disorganization of liver and kidney tissue (Figure-2). Pretreatment with *ocimum sanctum* at highest dose (100 mg/kg) could afford protection against acetaminophen challenge as very mild toxic effect is seen in Figure-3. At lower dose (75mg/kg) drug could afford partial protection (Figure-4) but at lowest dose (50 mg/kg) it could not combat against hepatorenal toxicity of acetaminophen.

3.2 Physiological Observations(Table:03):

Acetaminophen injection caused sharp rise in the serum level of all parameters i.e. GOT, GPT, AP, Bilirubin, Creatinine and Urea confirming severe hepatorenal injury. All parameters remained unaffected among mice that were pretreated with highest dose (100 mg/kg) of *ocimum sanctum* before acetaminophen challange. Lower dose of *ocimum sanctum* could not keep narmal level of serum enzymes but significantly lower values are recorded than the values obtained in Group II (acetaminophen exposed) showing better situation but lowest dose of drug could not prevent rise in the serum level of enzymes indicating no protective effect.

Histological and physiological findings corroborate each other.

Table 3:

Influence of pretreatment with *ocimum sanctum* leaves on acetaminophen induced changes in the levels of serum enzymes in mice (Mean ± SEM; n=6).

S.NO.	GROUPS	LIVER FUNCTION TEST				KIDNEY FUNCTION TEST	
		AST (U/L)	ALT (U/L)	ALP (U/L)	BILIRUBIN (MG/DL)	CREATININE (MG/DL)	UREA (MG/DL)
1.	Group I (Controls)	66.56±0.83	54.56±0.77	125.23±1.10	0.35±0.02	0.46±0.05	54.28±0.29
2.	Group II (Acetaminophen challenged at 300mg/kg bw)% change vs control	135.28±1.68[a] 103.24%↑	157.75±107[a] 178.13%↑	177.81±1.38[a] 41.98% ↑	0.89±0.07[a] 154.28%↑	1.25±0.08[a] 171.73%↑	107.11±1.21[a] 97.32%↑
3.	Group III (Pretreated with higher dose 100gm/kgbw of *Ocimum sanctum* & challenged with acetaminophen at 300mg/kg bw) % change vs control % difference from group II	66.66±1.37[b] NIL	53.33±1.63[b] NIL	126.65±1.62[b] NIL	0.34±0.03[b] NIL	0.44±0.03[b] NIL	55.51±1.10[b] NIL
4.	Group IV (Pretreated with lower dose 75mg/kg bw of *Ocimum sanctum* & challenged with acetaminophen at 300mg/kg bw) % change vs control % difference from group II	98.28±1.38[a,b] 47.65%↑ 27.35%↓	94.63±2.04[a,b] 73.44%↑ 37.64%↓	150.69±2.09[a,b] 20.33% ↑ 15.25% ↓	0.62±0.05[a,b] 77.14% ↑ 30.33% ↓	0.73±0.04[a,b] 58.69% ↑ 41.6%↓	77.31±1.18[a,b] 83.21% ↑ 27.82%↓
5.	Group V(Pretreated with lowest dose 50mg/kg bw of *Ocimum sanctum* & challenged with acetaminophen at 300mg/kg bw) % change vs control % difference from group II	133.88±1.68[a,] NS	153.67±2.09[a,] NS	161.79±2.06[a,b] NS	0.88±0.04[a,b] NS	1.23±0.06[a] NS	105.81±1.13[a] NS

Stastically significant at 5% level when: 'a'= group I *vs* all groups compared and

'b'=group II *vs.* groups III, IV& V compared ,NS=Non Significant-; ↑ High ↓ Low

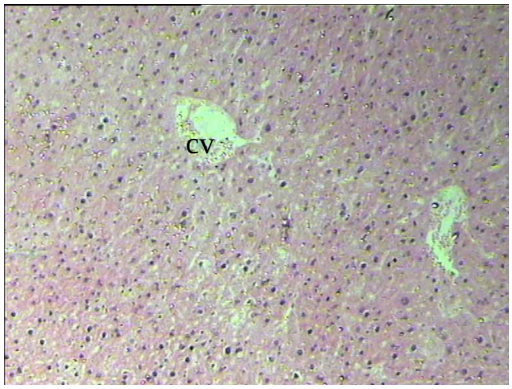

Fig- 1 Showing normal histology of liver of control group of mice. Radiating chords of hepatocytes arounds central vein (cv), indicate well organized histoarchitecture. No inclusion and no infiltration.

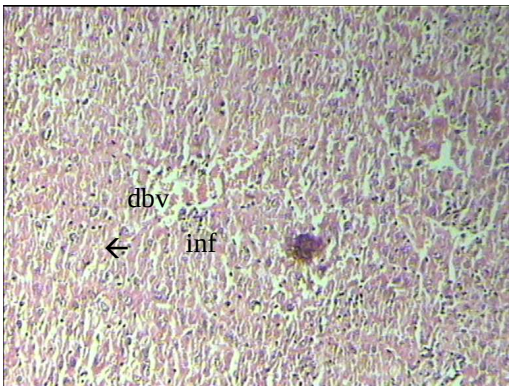

Fig- 2 Showing severe disorganizion of mice liver at 48 hr after single injection of acetaminophen (300mg/kg i.p.). Damaged hepatocytes are seen as eosinophilic spots (←). Damaged & collapsed blood vessels (dbv) are seen ,infittration seen.

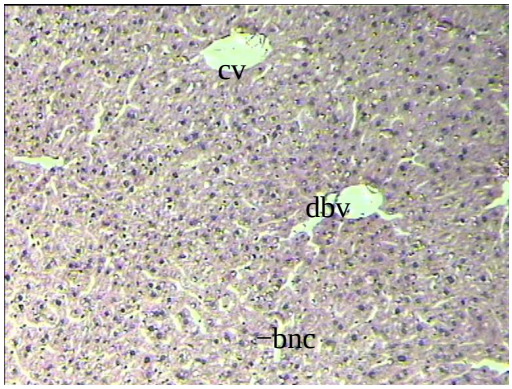

Fig- 3 Liver of mice at 48 hr after single dose of acetaminophen (300mg/kg i.p.) after 7 days pretreatment of *ocimum sanctum* at highest dose (100mg/kg) showing both normal hepatocytes as well as slightly affected ones. Mild damage to blood cells & infiltration are evident..Histoarchitecture is quite better than what is seen in Fig-2. Binucleatede cell (bnc)are seen.Drug could appreciably reduce acetaminophen toxicity.

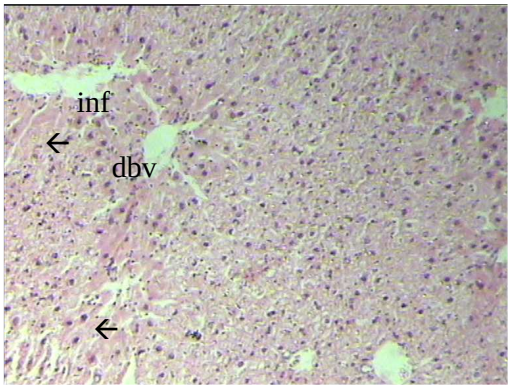

Fig- 4 Liver of mice at 48 hr after single dose of acetaminophen (300mg/kg i.p.)after 7 days pretreatment of *ocimum sanctum* at lower test dose (75mg/kg). Infiltration from damaged blood vessels is evident. Both damaged hepatocytes (←) and normal hepatocytes are seen. Damage is more pronounced than what is seen in earlier figure. Still drug could reduce acetaminophen toxicity as better histology is seen than Fig-2.

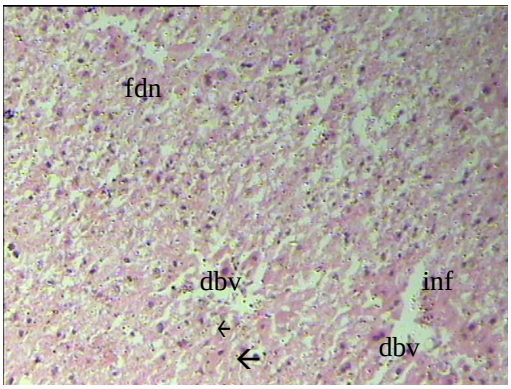

Fig - 5 Liver of mice at 48 hr after single dose of acetaminophen (300mg/kg i.p.)after 7 days pretreatment of *ocimum sanctum* at lowest test dose (50mg/kg). Showing infiltration from damaged blood vessels among fatty degenerated (fdn) damaged hepatocytes (←). Drug could not afford protection here.

Plate:1 Histology Of Mice Liver HE 150X

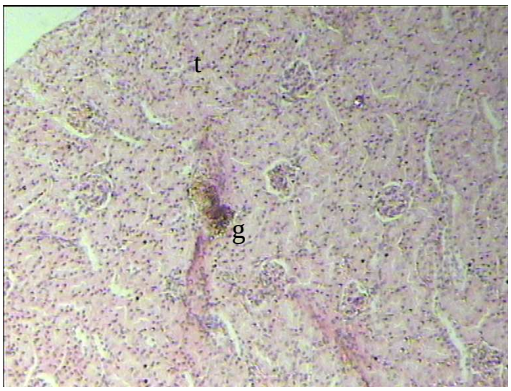

Fig-1: Showing normal histology of kidney of control group of mice, with well organized glomeruli(g) and tubules(t).

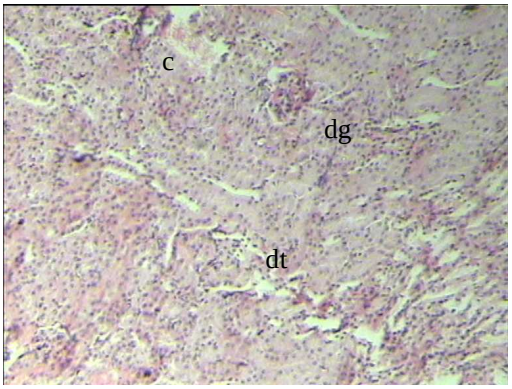

Fig-2:Showing severe disorganization of mice kidney 48hr after single injection of acetaminophen (300mg/kg i.p.). Damaged glomeruli (dg), dilated tubules (dt) are seen. Dead tubules are i.e. cast (c),evident.

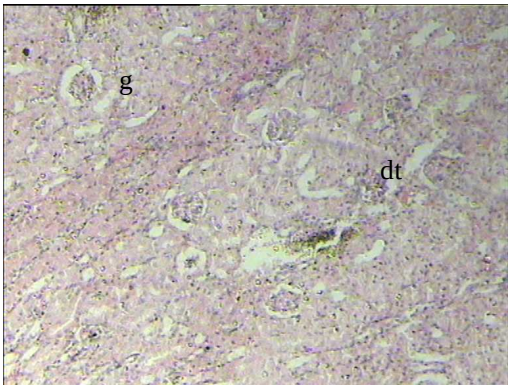

Fig-3:Showing mice kidney at 48 hr after single injection of acetaminophen (300mg/kg i.p.) after 7 days pretreatment of *ocimum sanctum* at highest test dose (100mg/kg). Showing control like histoarchitecture except mild tubular dilation. Drug could appreciably afford protection.

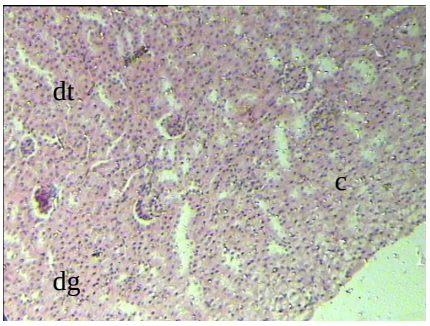

Fig-4:Showing mice kidney at 48 hr after single injection of acetaminophen (300mg/kg i.p.) after 7 days pretreatment of *ocimum sanctum* at lower test dose (75mg/kg). Tubular dilation with disorganized glomeruli are seen but better histology is seen than as in Fig-2. Drug could afford partial protection.

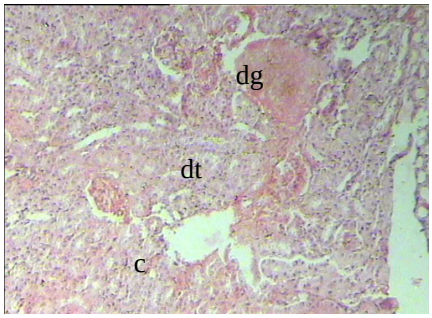

Fig-5:Showing mice kidney at 48 hr after single injection of acetaminophen (300mg/kg i.p.) after 7 days pretreatment of *ocimum sanctum* at lowest test dose (50mg/kg). Severe tubular dilation, disorganized glomeruli and few casts are seen like Fig-2. Drug could not protect.

Plate: 2 Histology of Mice Kidney HE 150X

4. DISCUSSION

Result show that acetaminophen-induced structure and functional i.e. histophysiologial impairment of liver and kidneys in mice.Peroxidative toxicity of acetaminophen in animals including mice and in human is an established fact [8,9, 10].Due to this reason acetaminophen is used to induce hepatorenal toxicity [1.] hence this observation does not deserve further discussion.

Prior administration of *ocimum sanctum* leaves filtrate did not enhance toxicity of acetaminophen, this observation suggests that simultaneous intake of both acetaminophen and *ocimum sanctum* leaves poses no threat even if consumed together intentionally or accidentally; interesting *ocimum sanctum* has been found as antiinflammlamatory, analgesic and antipyretic [11] and there are possibilities of their simultaneous use.

Prophylactic i.e. prior administration of *ocimum sanctum* leaves filtrate at two higher doses could well combat acetaminophen challenge at sublethal dose.

Several scattered reports have confirmed that *ocimum sanctum* leaves are excellent antioxidant which could have mainly boosted up natural antioxidant potential of body which in turn could have prevented acetaminophen induced hepatorenal damage in mice .This assumption is based on the following reports.

Paracetamol induced histophysiological changes and depleted GSH level could be appreciably prevented with prior administration of leaf extract of *ocimum sanctum* in rats [2].Oral administration with *ocimum sanctum* leaf extrat for 15 days enhanced activities of CYT P450, CYTb5, aryl hydrocarbon hydroxylase, glutathione-S-

transfere, extra hepatic glutathione-S-transferese, GSH in lung ,liver and stomach in mice. All these help in detoxification of carcinogens and mutagens [12].*Ocimum sanctum* filtrate could protect mouse liver against radiation induced lipid peroxidation which was attributed to increased level of GSH, GSH transferase, GSH peroxidase, reductase and SOD [13]. Pretreatment with aqueous extract of *ocimum sanctum* to mice reduced lipid peroxidation in kidney and enhanced liver GSH content [14].Authors suggested that protective action of *ocimum sanctum* was via free radical scavenging ability and via enhancing detoxifying enzymes.

Pre and post treatment with *ocimum sanctum* leaf extract could prevent mercury induced lipid peroxidation in kidney [15.].Euganol and essential oils of *Ocimum sanctum* leaves are known to have membrane stabilized properties [16].*Ocimum sanctum* extract has been found to be nitric oxide scavenging agent [17].*Ocimum sanctum* oil could protect Hep G2 cells *in-vitro* against ethanol, like silimarin [18].

Dried leaf power of *Ocimum sanctum* has been suggested to inhibit lipid peroxidation *in-vivo* and *in-vitro* due to presence of group of polyphenolic falconoids [19].Leaf extract of *ocimum sanctum* prevented cell carcinogenesis via acting as an antioxidant by modulating phase I & phase II enzymes [20].*Ocimum sanctum* extract is suggested to protect against DMBA induced genotoxicity & oxidative and up-regulatory antioxidant stress by reducing lipid & protein oxidation and up–regulatory antioxidant defence [21].

It is concluded that hepatorenal ability of leaves of *ocimum sanctum* can be attributed to its antioxidant potential.

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