Ancient Science of Life, Vol. 30, No.3 (2011) Pages 72 - 77

Prevention of acetaminophen induced hepatorenal damage in mice with rhizomes of *Glycyrrhiza glabra* A histophysiological study

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ABSTRACT

Protective role of Gycyrrhiza glabra,Linn. rhizomes (roots) at three dose levels (100, 75, & 50 mg/kg/bw) against sublethaldose (300 mg/kg/bw) of acetaminophen (paracetamol) induced hepatorenal damage has been assessed in mice. Parameters of study were glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), billirubin, alkaline phosphatase (ALP) as liver function tests, creatinine and urea as kidney function tests and histology for pathology. G.glabra could antagonize acetaminophen induced both,hepato and nephrotoxicity in dose dependent manner. No protection provided by a single dose of G.glabra (1.5 gm/kg/bw) against lethal dose of acetaminophen (1g/kg/bw). Probable protective role is discussed.

Key words:*Glycyrrhiza glabra, acetaminophen /paracetamol, liver-kidney, mice, antioxidants.*

Introduction:

Analgesic drug acetaminophen (paracetamol) available *without prescription* in several parts of the world is used to cure pain and discomfort. Over dosage of acetaminophen mainly causes dose dependent, fatal hepatic necrosis but renal tubular necrosis and hypoglycemic coma may also occur (1-2). Acetaminophen overdose causes peroxidative damage (1,3-4) and licorice is a natural antioxidant which is found to protect liver and kidney against various toxicants individually (5-10), but not simultaneously. In the present study both, liver and kidneys are included to find how licorice protects them against sub lethal dose of acetaminophen because herbal compounds have been screened mostly for their ability to reduce and/or nullify acetaminophen induced hepatotoxicity but kidney was badly ignored (11).

In India people believe in Ayurveda and consume licorise alongwith acetaminophen. It is not known whether it will enhance or reduce toxicity of acetaminophen. Present study is an attempt to answers this question.

Materials and methods

(i) Animal Model:

Male Swiss Albino mice of 20-25 g were maintained on prepared food (soya flex 8.50%,maize 10.50%, wheat 50%,gram 30% ,1% NaCl and tap water *ad-libitum*. They were kept in the departmental animal house under natural day/night (ligh/dark) cycle.Animals were purchased from Biological Production Division of Government Veterinary College,Mhow (MP). Department has yet to get license from IAEC hence biochemical work was performed in Shri Pathological Lab,Ujjain hence only experimentation and histology were performed in the department.

(ii) Herbal drug:

Glycyrrhiza glabra rhizome (dry roots) was obtained from local herbal shop. It was authenticated by both, Prof D. Amritphale,HOD Botany and Prof U.S. Nigam HOD Medicine,Govt Ayurvedic College,Ujjain. Roots were powdered and 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 dose of drug was selected from described values in the literature (12)

(iii) Experiment I: Protection at lethal dose

Preliminary experiments were performed on mice to estimate the protective effect of this herbal compound against lethal dose of paracetamol (1g/kg). Animals were divided into two groups of 10 animals each. One group was treated orally with the test drug *Glycyrrhiza glabra* at maximum dose (1.5 g/kg/bw) and followed after 1 hour by intraperitoneal injection of paracetamol. Another group was administered distilled water instead of drug. The mortality was observed 24 hour after paracetamol administration in both groups. Percentage protection against lethal effect of paracetamol was calculated.

(iv) Experiment II: Protection at sub lethal dose

Hepatic and renal injury was induced in mice by subcutaneous administration of single sublethal dose (300 mg/kg/bw) of paracetamol injection (Intas, India). Details are shown in experimental design.

(v) Biochemical Observations

On day 9, 48 hours after paracetamol administration blood sample of each animal of each group was taken directly from heart under mild chloroform anesthesia and biochemical parameters GOT, GPT and billirubin, AP as liver function tests and creatine and urea as kidney function tests were evaluated using ready to use available kits made by standard companies (Beacon,Agappe,Merck and Span diagnostics).

(vi) Histopathological Observation

Also, on day 9, pieces of liver and kidney from each animal were fixed in Bouins fluid for routine histopathology. Hematoxylin-eosine stained sections were observed for histopathological study.

(vii) Statistical Analysis

Experiments were done twice. The results are expressed as mean S.E.M. and all statistical comparisons are made by means of student's t-test.

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Experimental Design

Group I	*Control group:06* Mice were treated orally with distilled water daily for 7 days followed by injection of benzyl alcohol 2 h after last treatment (vol. equal to that of injection of paracetamol used in next group) on 7th day.
Group II	*Paracetamol treated group: 06* Mice were given distilled water orally for 7 days and subcutaneous injection of sub lethal dose (300mg/kg/bw) of paracetamol on 7th day, 2 h after last treatment.
GroupIII IV ,V	*Drug treated and Paracetamol challenged groups: 06* Mice were treated with the test herbal compound (*Glycyrrhiza glabra* roots) in distilled water at three doses (100, 75 & 50mg/kg/bw), orally daily for 7 days followed by paracetamol injection on 7th day 2 h after last treatment.

Results

Lethality test :
(Experiment I):*Glycyrrhiza glabra* could not afford protection against lethal dose of acetaminophen (Table-1).

Table 1: Protection against lethal dose of acetaminophen by *Glycyrrhiza glabra*

S.No.	Group	Total number of mice used	Mortality out of 10	Percentage protection
1.	Paracetamol treated group	10	10 (100%)	0 (0)%
2.	Paracetamol challenge to licorice treated group	10	09 (90%)	1 (10)%

(1) Experiment II Protection at sub lethal dose
(2) (A) Histological Observations: Self explanatory figures and captions are given in plates I and II.

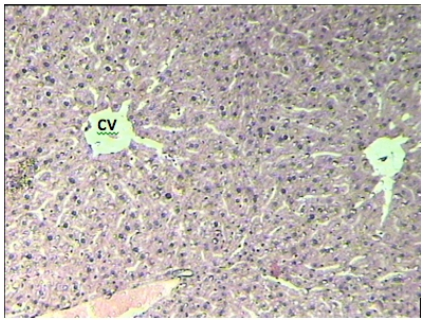

Fig.1: Showing normal histology of liver of control group of mice. Radiating chords of hepatocytes arounds central vein (cv) indicate well organized histoarchitecture. No inclusion and no infiltration.

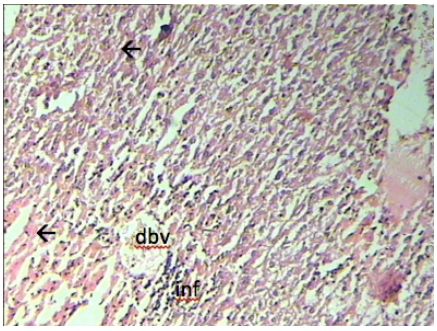

Fig.2: Showing severe disorganizatio of mice liver at 48 hr after single injection of acetaminophen (300 mg/kg i.p.).Damaged hepatocytes are seen as eosinophilic spots(←). Damaged & collapsed blood vessel (dbv) is seen with rough margins. Severe infiltration is evident (inf).

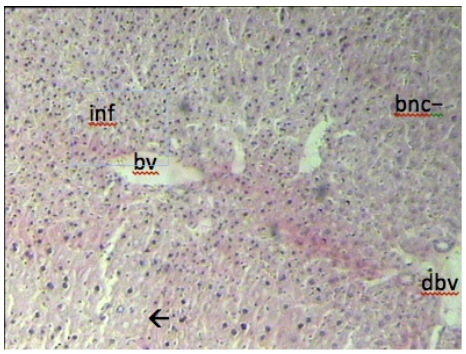

Fig.3: Showing liver of mice at 48 hr after single dose of acetaminophen (300 mg/kg i.p.) after 7 days pretreatment with *Glycyrrhiza glabra* root at highest dose (100 mg/kg) showing both normal hepatocytes as well as slightly affected ones. Mild damaged blood vessels,infiltration and binucleated cells (bnc) are seen. Histoarchitecture is quite better than what is seen in figure.2. Drug could reduce paracetamol toxicity appreciably.

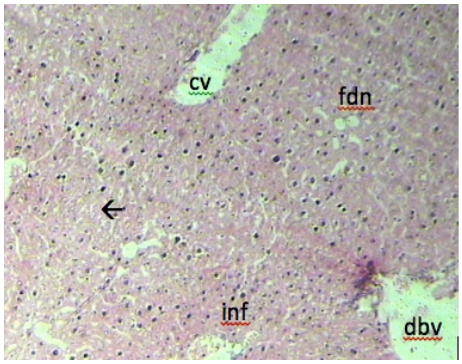

Fig.4: Showing liver of mice at 48 hr after single dose of acetaminophen (300 mg/kg i.p.) after 7 days pretreatment with *Glycyrrhiza glabra* root at lower test dose (75 mg/kg). Infiltration and damaged hepatocytes (←) are seen. Fatty degeneration is also evident (fdn). Damage is more pronounced than what is seen in earlier figure. Still drug could reduce acetaminophen toxicity as better histology is seen than figure 2. Drug afford partial protection.

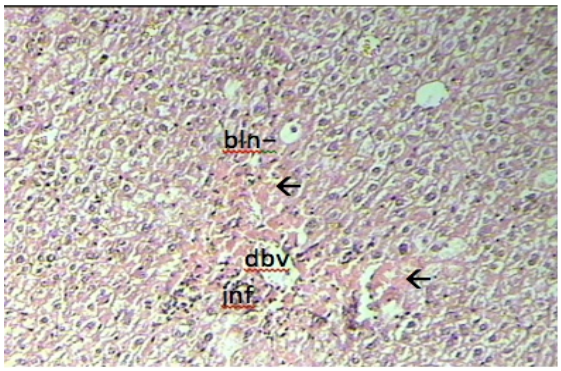

Fig.5: Showing liver of mice at 48 hr after single dose of acetaminophen (300 mg/kg i.p.) after 7 days pretreatment with *Glycyrrhiza glabra* root at lowest test dose (50 mg/kg). Severe infiltration , damaged hepatocytes (←) and damaged blood vessels are seen. Balloning of hepatocytes (bln) is also evident. Figure is like that figure 2. Drug could not afford protection.

Plate : I Histology of Mice Liver HE 150 X

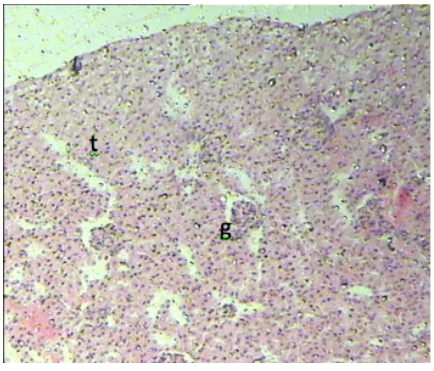

Fig.1: Showing normal histology of kidney of control group of mice, with well organized glomeruli (g) and tubules (t).

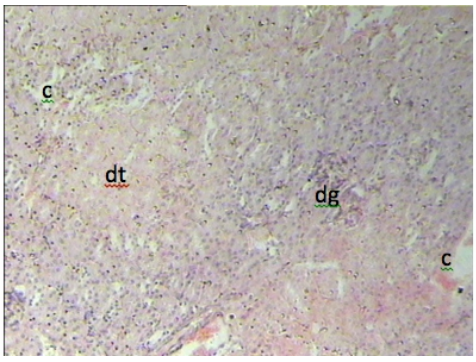

Fig.2: Showing severe disorganization of mice kidney at 48 hr after single injection of acetaminophen (300 mg/kg i.p.). Damaged glomeruli (dg), dilated tubules (dt) are seen. Dead tubules are also seen as cast (c).

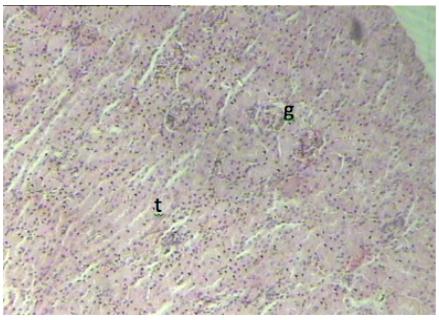

Fig.3: Showing mice kidney at 48 hr after single injection of acetaminophen (300 mg/kg i.p.) after 7 days pretreatment with *Glycyrrhiza glbra* root at highest test dose (100 mg/kg). Showing control like histoarchitecture. Drug could afford appreciable protection

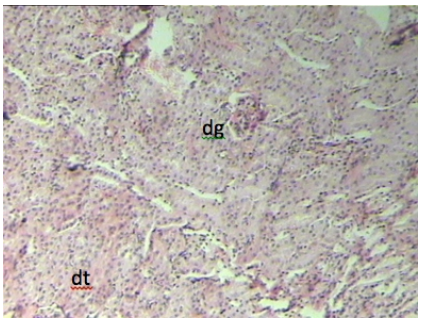

Fig.4: Showing mice kidney at 48 hr after single injection of acetaminophen (300 mg/kg i.p.) after 7 days pretreatment with *Glycyrrhiza glbra* root at lower test dose (75 mg/kg). Tubular dilation with disorganized glomeruli are seen. Drug could afford very little protection.

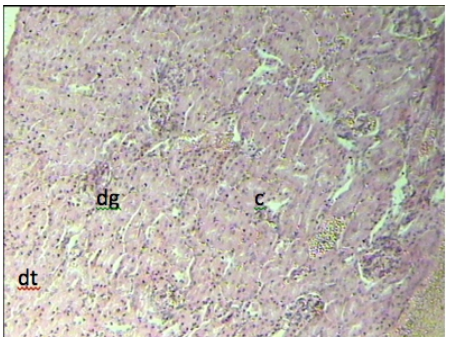

Fig.5: Showing mice kidney at 48 hr after single injection of acetaminophen (300 mg/kg i.p.) after 7 days pretreatment with *Glycyrrhiza glbra* root at lowest test dose (50 mg/kg). Severe tubular dilation, disorganized glomeruli and few casts are seen like that of figure 2. Drug could not afford protection.

Plate: II Histology of Mice Kidney HE 150 X

(B)Physiological Observations (Table:2):

Parameters of kidney & liver injury remained unaffected among mice that were pretreated with highest dose (100 mg/kg) of *Glycyrrhiza glabra* before paracetamol challenge, however, Pparacetamol injection caused sharp rise in these indicator markers. Lower dose (75 mg/kg) of Glycyrrhiza *glabra* could keep level of serum enzymes significantly lower than that the values obtained in Group II (Paracetamol exposed). At lowest dose (50 mg/kg) *Glycyrrhiza glabra dose not* provide protection against paracetamol induced hepatotoxicity and renal toxicity. Histological findings and Physiological observations corroborate each other.

Discussion

Results of present experiments indicate facts that rhizomes of *Glycyrrhiza glabra* did not enhance toxicity of paracetamol on the contrary it could reduce paracetamol induced histophysiological impairment of liver and kidneys at sublethal dose.

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Toxicity of paracetamol in mice is an established fact (13). Several earlier reports in human and in animal studies have cemented this fact. Due to this reason paracetamol is used as experimental toxin to induce liver and kidney damage in experimental studies.

Pretreatment i.e. prophylactic administration of aqueous suspension of powdered *Gycyrrhiza glabra roots* at three different doses for 07 days to mice could provide appreciable protection against acetaminophen (paracetamol) challenge on 8th day in sublethal experiments.*Glycyrrhiza glabra roots* a natural antioxidant might have enhanced endogenous antioxidant system of mice, however, it could have also played protective role via other routes which are discussed subsequent paragraphs.

Glycyrrhiza glabra rhizomes and its constituents enhance detoxification and excretion of medicines including acetaminophen in rat liver (14,8,7). Licorice constituents stabilize integrity of hepatic lysosomes and mitochondria (15, 16).

Glabridin, pyranoisoflavan isolated from *Glycyrrhiza glabra* could prevent nephritis in mice (17). *Glycyrrhiza glabra* and lipoic acid could prevent gentamicin induced experimental nephrotoxicity (10).

Glycyrrhizin could prevent lead acetate induced hepatic oxidative stress and hyperproliferative activity is wistar rats. Pretreatment to rats orally with glycyrrhizin decreased hepatic microsomal lipid peroxidation and increase in the level of GSH content and its dependent enzymes (glutoathione-reductase, glutothione-s-transferase and glutoathione peroxidase) and lowered DNA synthesis (18).

Table 2: Effects of pretreatment with different doses of *Glycyrrhiza glabra* roots against acetaminophen induced changes in serum enzymes in mice (Mean ± SEM; n=6)

S. NO.	GROUPS	LIVER FUNCTION TESTS				KIDNEY FUNCTION TESTS	
		AST (U/L)	ALT (U/L)	ALP (U/L)	BILIRUBIN (MG/DL)	CREATININE (MG/DL)	UREA (MG/DL)
1.	Group I (Controls)	61.83±0.94	52.46±0.68	122.18±1.01	0.31±0.03	0.40±0.04	52.65±0.95
2.	Group II (Acetaminophen treated, 300 mg/kg bw)	128.58[a]±1.94 [107.95%↑]	151.13[a]±1.52 [188.08%↑]	170.96[a]±1.62 [39.92%↑]	0.82[a]±0.04 [164.51%↑]	1.18[a]±0.09 [195.00%↑]	101.48[a]±1.01 [92.74%↑]
3.	Group III (Pretreated with higher dose 100 mg/kg of *G.glabra* + acetaminophen treated, 300 mg/kg bw)	66.71[b]±1.80 [07.89%↑]NS [48.11%↓]	57.83[b]±2.14 [10.23%↑]NS [61.73%↓]	127.55[b]±2.01 [04.39%↑]NS [25.39%↓]	0.41[b]±0.03 [32.25%↑]NS [50.00%↓]	0.51[b]±0.03 [27.50%↑]NS [56.77%↓]	55.93[b]±1.35 [06.22%↑]NS [44.88%↓]
4.	Group IV (Pretreated with lower dose 75 mg/kg of G.glabra+ acetaminophen treated, 300 mg/kg bw)	106.80[a,b]±1.91 [72.73%↑] [20.39%↓]	102.08[a,b]±2.03 [94.58%↑] [32.45%↓]	153.02[a,b]±2.18 [25.24%↑] [10.49%↓]	0.62[a,b]±0.05 [100.00%↑] [24.39%↓]	0.76 [a,b]±0.04 [90.00%↑] [35.59%↓]	82.67[a,b]±1.31 [57.01%↑] [18.53%↓]
5.	Group V (Pretreated with lowest dose 50 mg/kg of *G.glabra*+ acetaminophen treated, 300 mg/kg bw)	125.62[a]±1.72 [103.16%↑] [02.30%↓]NS	149.39[a]±1.96 [184.76%↑] [01.15%↓]NS	167.84[a]±2.12 [36.73%↑] [01.82%↓]NS	0.81[a]±0.07 [161.29%↑] [01.21%↓]NS	1.15[a]±0.07 [187.50%↑] [02.54%↓]NS	99.02[a]±1.42 [88.07%↑] [02.42%↓]N

'a'= Significant Group I vs all groups; ↑ =Rise as compared to Group I
'b'= Significant Group II vs Group III, Group IV, Group V ; ↓= Decline as compared to Group II . NS= Non significant

18-Betaglycyrrhetinic acid could prevent CCl_4- induced liver injury in mice by inhibiting depletion of hepatic GSH. This component of *Glycyrrhiza glabra* also showed antioxidant effect upon $FeCl_2$- ascorbate induced lipid peroxidation in mice liver homogenate and upon superoxide radical scavenging activity (6). Oxidative stress in one mechanism of acetaminophen toxicity (19). During formation of NAPQI by cytochrome P450, the superoxide is formed, with dismutation leads to hydrogen peroxide. Exogenous N-acetyl cysteine, GSH, ascorbate is known to prevent acetaminophen toxicity (20), hence this property of *Glycyrrhiza glabra* can be held responsible for protecting both liver and kidneys of mice against acetaminophen in the present case.

Glycyrrhiza glabra could attenuate peroxynitrite induced renal oxidative damage through inhibition of protein nitration (9). Antioxidant capacity of licorice is used to treat kidney or urinary system based on oxygen radical absorbance capacity method (21). Possible role of Nrf_2 (Nuclear erythroid 2p45-related factor, a key transcription facator of phase II drug metabolizing enzymes in renal celluar defense against oxidative stress has been suggested in mice (22). An indirect antioxidant 3H-1, 2-dithiole-3-thione (D3T) which protected cells from oxidative damage by up regulating the expression of antioxidative genes through the transcription factor Nrf_2 pathway as has been reported against cisplastin (23). Possiblity of such action of *Glycyrrhiza glabra* not be ruled out.

It is concluded that *Glycyrrhiza glabra* could reduce acetaminophen-induced hepatorenal damage in mice under laboratory conditions probably by strengthening endogenous antioxidant defense in the liver and kidneys of mice.

Acknowledgements

Authors respectfully thank Council of Scientific and Industrial Research, New Delhi for providing NET senior fellowship and grants to first author. Departmental Facilities are also acknowledged.

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