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RESEARCH ARTICLE

Protective effect of Sharbat-e-Deenar against acetaminophen-induced hepatotoxicity in experimental animals

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Abstract

OBJECTIVE: To investigate the effect of Sharbat-e-Deenar (SD) on acetaminophen (APAP)-induced hepatotoxicity in rat model.

METHODS: Albino rats were treated with SD at the doses of 1, 2 and 4 mL/kg, p.o. against hepatotoxicity after APAP (2 g/kg, p.o. once only) intoxication. The blood, tissue biochemical parameters and histopathological observation were performed. The

RESULTS: APAP exposure in rats significantly increased the level of biochemical parameters such as aspartate aminotransaminase, alanine aminotransaminase, lactate dehydrogenase, serum alkaline phosphatase, bilirubin, urea and creatinine into blood circulation which were reversed towards normal by SD therapy at all doses. The tissue biochemical parameters such as lipid peroxidation, reduced glutathione, adenosine tri-phosphatase and glucose-6-phosphatase were significantly restored after SD treatment against hepatotoxity. Histological analysis confirmed that SD-treated rats significantly alleviated of liver damage and reduced lesions caused by APAP intoxication. The biochemical changes are in good correlation with the histopathological observations.

CONCLUSION: On the basis of these results, it can be concluded that SD exerts hepatoprotective activity against APAP-induced liver injury.

Keywords: Sharbat-e-Deenar; Acetaminophen; Hepatitis, toxic; Hepatoprotection

INTRODUCTION

Human beings are exposed on a daily basis to toxic chemicals and pathogens, which cause serious health problems. Liver diseases are still major global health problem and are mostly associated with oxidative stress and tissue damage. Drug-induced liver damage is responsible for 50% all acute liver failures.[1] The liver is the major site of detoxification and the primary target of drug exposure in the body. Acetaminophen known as paracetamol is widely used as analgesic and antipyretic agent which is known to cause hepatotoxicity in human and laboratory animals.[2] Herbal plants play an important role in the management of liver diseases such as hepatitis, fibrosis, cirrhosis, acute necrosis, jaundice, fatty liver etc.[3] Herbal plants are using extensively day by day due to relatively safe, lesser side effects and alternative treatment in comparison of modern medicines. About 80% world population rely on traditional system of medicine for primary health care.[4]

However, herbal medicines are rapidly becoming popular in recent years as an alternative medicine, and there is a resurgence of interest in herbal medicines for the treatment of various human diseases including hepatic diseases. In India, several herbal medicines, their active constituents and formulations, are used in the treatment of a wide variety of clinical diseases and provide benefit to societies. However, the active phytochemical constituents of individual plant are insufficient to achieve the desirable protection. When combining of the multiple plants in particular ratio, it will provide maximum therapeutic effects and reduce the toxicity. However, there are a number of herbal formulations such as Jigrin,[5] Triphla,[6] Liv 52 and Livomyn,[7] Majoon-e-Dabeed-ul-ward[8] have been reported to have hepatoprotective efficacy against drug-induced liver toxicity.

In the present study, Sharbat-e-Deenar (SD) is a Unani Herbal formulation which are prepared mixing of eight medicinal plants (Table 1) and standardized according to the National Formulary of Unani Medicine (NFUM). Some medicinal plants of this formulation such as Cichorium intybu,[10] Rheum emodi,[11] and Rosa damascene,[12] have been reported to have antioxidant and hepatoprotective activity. Therefore, the present study was aimed to evaluate the hepatoprotective effects of SD against acetaminophen-induced hepatotoxicity in rats, which have not been reported yet.

MATERIALS AND METHODS

Drug and chemicals

Therapeutic agent, Sharbat-e-Deenar (SD) was procured by the Central Council for Research in Unani Medicine (CCRUM), New Delhi (INDIA). Silymarin (Sigma Aldrich Chemicals Pvt Ltd., Bangalore, India), APAP from Smith-Kline Beecham Pharma, (Batch No. 0103) and other reagents were used of analytical grade.

Animals

Female albino rats of Wistar strain [(160 ± 10) g] were used in this study. Animals were housed under standard husbandry conditions [(25 ± 2) ℃ tempressure, 60%-70% relative humidity, and 12 h photoperiod] and had access to standard rat feed and drinking water ad libitum. Animals were treated and cared in accordance with the guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of doses and treatments

A suspension of APAP (2.0 g/5 mL/kg) was made in warm distilled water and administered orally to experimental animals.[13] SD is a semi liquid formulation prepared in distilled water [1, 2, 4 mL/5 mL/kg, post orally (p.o.)] were administered to the animals orally. Silymarin (50 mg/5 mL/kg, p.o.) was prepared in 1% gum acacia and used as positive control.[14]

Experimental design

Rats were divided into seven groups of six animals each and treated as follows: group 1: distilled water, control; group 2: SD (4 mL/kg, p.o.), treatment control; group 3: APAP (2 g/kg, p.o.) once only, experimental control; groups 4-6: APAP (as in group 3) + SD (1, 2, 4 mL/kg, p.o. respectively); group 7: APAP (as in group 3) + Silymarin (50 mg/kg, p.o.).

Animals were sacrificed 24 h after the last treatments; blood was collected and allowed to clot, and serum was separated at 2000 rpm for 20 min and stored at 20 ℃ in a deep freezer. Various blood, tissue biochemical parameters and histology were performed in serum and liver tissues.

Blood biochemical assay

Various biochemical parameters for assessment of the liver function markers like aspartate transaminase (AST); alanine transaminase (ALT),[15] and serum alkaline phosphatase (SALP); lactate dehydrogenase (LDH), bilirubin, and markers of kidney function test like urea and creatinine were measured in serum using diagnostic kits (E-Merck, Germany).

Tissue biochemical assay

Thio-barbituric acid reactive substance (TBARS) mea-

Table 1 Composition of Sharbat-e-Deenar

Unani name	Botanical name	Family name	Part used	Weight (g)
Post-e-bekh-e-Kasni	Cichorium intybus	Asteraceae	Root bark	170
Tukm-e-Kasoos	Cuscuta reflexa	Convolvulaceae	Seed	100
Tukm-e-Kasni	Cichorium intybus	Asteraceae	Seed	85
Guncha-e-Gul-e-Surkh	Rosa damascena	Rosaceae	Flower bud	85
Rewand chini	Rheum emodi	Polygonaceae	Root	60
Gul-e-Nilofar	Nymphaea alba	Nymphaeaceae	Flower	45
Gaozan	Borago officinalis	Boraginaceae	Leaves	45
Aab	Water	-	-	Q.S.
Qand Safaid	Sugar	-	A product from cane sugar	1200

sured lipid peroxidation,[16] dithionitrobenzoic acid determined reduced glutathione (GSH) level,[17] an enzyme activities of adenosine triphosphatase (ATPase),[18] and glucose-6-phosphatase (G-6-Pase),[19] were determined in tissue sample.

Histopathological study

For light microscopic observations, liver tissue was fixed in Bouin's fixative, 5 µm thick paraffin sections of liver were stained with hematoxylene and eosine (HE) which were examined under light microscope.

Statistical analysis

Results are presented as mean ± standard error of six animals used in each group. The data for various biochemical parameters were analyzed by one-way analysis of variance followed by Duncan's multiple range test[20] and Statistical Package for the Social Sciences (SPSS) software developed by International Business Machines corporation, New York, NY, USA). Percent protection (%) was also used for determination of the protective activity by treatment groups. The protective activity by treatment groups, expressed as percent protection, was calculated as follows: Percent protection was calculated by the following formula:

% Protection = 1-(D-C/T-C) × 100 (D: drug, C: control, T: toxicant).

RESULTS

Blood biochemical markers

The values for liver marker enzymes for APAP, SD & silymarin treated rats and control group are depicted in Table 2. APAP intoxication significantly increased the level of AST, ALT, LDH and SALP when compared to control group. SD treatment at three doses were significantly reduced the levels of these enzymes towards normal ($P \leq 0.05$). APAP exposure to animals significantly decreased the level of bilirubin, urea and creatinine which were significantly reversed by SD therapy in dose dependent manner (Table 3). No adverse effect was observed in the Treatment control group (4 mL/kg) when compared with control group.

Tissue biochemical parameters

Table 4 showed the effect of SD and APAP on lipid peroxidation, GSH and enzyme activities of ATPase and G-6-Pase in liver Lipid peroxidation was expressed in terms of thiobarbuturic acid reactive species (TBARS). APAP treatment significantly elevated TBARS level and a concomitant fall in GSH level in liver ($P \leq 0.05$). Therapy with SD significantly lowered TBARS level and alleviated GSH content as seen by statistical analysis ($P \leq 0.05$). In our study, the enzyme

Table 2 Protective effect of SD on liver marker enzymes against APAP-induced hepatic damage

Treatment	*n*	AST (IU/L)	ALT (IU/L)	LDH (U/L)	SALP (mg Pi/ h/100 mL)
Control	6	70±5	49±4	45±3	210±11
SD 4 mL/kg	6	69±5	50±3	44±4	209±12
APAP	6	280±17[a]	330±18[a]	158±9[a]	570±36[a]
APAP+SD 1 mL/kg	6	155±8[b] (59.5%)	180±10[b] (53.3%)	80±4[b] (69.0%)	300±20[b] (75.0%)
APAP+SD 2 mL/kg	6	140±9[b] (66.6%)	160±8[b] (60.4%)	70±4[b] (77.8%)	280±15[b] (80.5%)
APAP+SD 4 mL/kg	6	141±8[b] (66.1%)	150±9[b] (64.0%)	67±5[b] (80.5%)	275±18[b] (81.9%)
APAP+S 50 mg /kg	6	90±6[b] (90.4%)	80±7[b] (88.9%)	50±6 (95.5%)	230±17[b] (94.4%)
F value (at 5% level)	-	75.5	120.0	71.9	47.2

Notes: APAP: acetaminophen; SD: Sharbat-e-Deenar; S: silymarin; %: percent protection. % Protection = 1-(D-C/T-C) × 100 (D: drug, C: control, T: toxicant). Values are mean ± standard error. [a]$P \leq 0.05$ *vs* control, [b]$P \leq 0.05$ *vs* APAP. APAP: acetaminophen.

Table 3 Protective effect of SD on hepato-renal parameters against APAP-induced hepatic damage (mg/dL)

Treatment	*n*	Bilirubin	Urea	Creatinin
Control	6	0.141±0.081	18.012±2.031	0.221±0.044
SD 4 mL/kg	6	0.111±0.040	19.01±2.604	0.202±0.022
APAP	6	1.102±0.160[a]	47.022±3.455[a]	4.101±0.354[a]
APAP+SD 1 mL/kg	6	0.901±0.131 (20.8%)	45.012±5.004 (6.89%)	2.501±0.391[b] (41.2%)
APAP+SD 2 mL/kg	6	0.701±0.055[b] (41.6%)	35.013±2.441[b] (41.3%)	1.002±0.154[b] (79.8%)
APAP+SD 4 mL/kg	6	0.602±0.043[b] (52.0%)	30.021±3.242[b] (58.6%)	0.802±0.033[b] (85.0%)
APAP+S 50 mg /kg	6	0.225±0.011[b] (91.6%)	21.01±1.934[b] (89.6%)	0.351±0.051[b] (96.6%)
F value (at 5% level)	-	28.1	18.0	59.2

Notes: APAP: acetaminophen; SD: Sharbat-e-Deenar; S: silymarin; %: percent protection. % Protection = 1-(D-C/T-C) × 100 (D: drug, C: control, T: toxicant). Values are mean ± standard error. [a]$P \leq 0.05$ *vs* control, [b]$P \leq 0.05$ *vs* APAP.

activities of G-6-Pase and ATPase were declined in APAP-treated group. Therapy with SD was found to be effective in restoring these enzymatic parameters in dose-dependent manner ($P \leq 0.05$).

Histopathological study

Histopathological effects of SD against APAP induced toxicity are shown Figure 1. Figure1A showed the normal architecture of normal liver. The liver section of an experimental control group (APAP) showed cellular degeneration, prominent congestion in sinusoidal space and high accumulation of inflammatory cells and nuclear disintegration (Figure 1B). Therapy with SD (1 mL/kg) showed recovery with moderate reduction in histological change (Figure 1C). Treatment with SD at the doses 2 mL/kg and 4 mL/kg showed normal lobular pattern with well-formed cellular morphology having conspicuous nucleus, and wider sinusoidal spaces when compared to the control group (Figure 1C, D, E).

DISCUSSION

Acetaminophen is metabolized in liver *via* cytochrome P450 pathway (CYP2E1 enzyme) into a highly toxic metabolite, N-acetyl-p-benzoquinamine (NAPQI). Generally, this metabolite is detoxified through reaction with reduced glutathione (GSH) and excreted in urine.[21] Hepatotoxic dose of APAP, liver GSH levels are depleted and NAPQI covalently binds to macromolecules of the cell membrane and enhance lipid peroxidation which consequently induced oxidative stress resulting in hepatic toxicity.[22] Serum enzyme levels such as AST, ALT, SALP and LDH are commonly used as hepatic markers to assess drug-induced liver damage.[23]

In the present study, APAP-treated animals increased the level of these enzymes into the blood stream. These changes indicated hepatocellular damage by APAP intoxication.SD administration at all doses significantly reduced the level of these hepatic enzymes as compared to experimental control group. Results also confirmed that APAP treated animals showed marked elevations in the level of bilirubin, urea and creatinine in serum indicating hepatic and renal damage.[24] Treatment with SD significantly decreased the hepato-renal injury by restoring these parameters towards normal. Scientific studies have reported that medicinal plants and herbal formulations have potent hepatoprotective properties due to the presence of active phytochemical constituents.

Oxidative stress is a major mechanism underlying the pathogenesis of APAP-induced liver damage.[25] Lipid peroxidation is an oxidative modification of unsaturated lipids and is involved in the destructive processes that affect liver in APAP overdose and GSH plays an es-

Table 4 Effect of SD on acetaminophen induced alterations in tissue biochemistry

Treatment	*n*	Lipid peroxidation (nmoles TBARS/mg protein)	GSH (μmole/g)	ATPase (mg Pi/100 mL/min)	G-6-Pase (μmole Pi/min/g liver)
Control	6	0.22±0.03	8.10±0.72	1980.00±124.00	6.40±0.43
SD 4 mL/kg	6	0.20±0.03	8.20±0.80	1990.00±125.00	5.29±0.38
APAP	6	1.80±0.25[a]	4.80±0.71[a]	681.01±67.01[a]	2.86±0.31[a]
APAP+SD 1 mL/kg	6	0.86±0.09[b] (59.4%)	7.50±0.44[b] (81.8%)	1571.00±107.00[b] (68.5%)	4.58±0.38[b] (48.0%)
APAP+SD 2 mL/kg	6	0.65±0.08[b] (72.7%)	7.70±0.47[b] (87.8%)	1857.00±120.00[b] (90.5%)	4.85±0.59[b] (57.0%)
APAP+SD 4 mL/kg	6	0.62±0.04[b] (74.6%)	7.80±0.59[b] (90.9%)	1885.00±119.00[b] (92.6%)	4.98±0.72[b] (60.0%)
APAP+S 50 mg /kg	6	0.42±0.03[b] (87.3%)	8.00±0.48[b] (96.9%)	1940.00±99.01[b] (96.9%)	6.30±0.48[b] (97.2%)
F value (at 5% level)	-	30.6	4.4	23.4	6.8

Notes: APAP: acetaminophen; SD: Sharbat-e-Deenar; S: silymarin; TBARS: Lipid peroxidation; GSH: Glutathione; ATPase: Adenosine triphosphatase; G-6-Pase: Glucose-6-phosphatase; %: percent protection. % Protection = 1-(D-C/T-C) × 100 (D: drug, C: control, T: toxicant). Values are mean ± standard error. [a] $P \leq 0.05$ *vs* control, [b] $P \leq 0.05$ *vs* APAP.

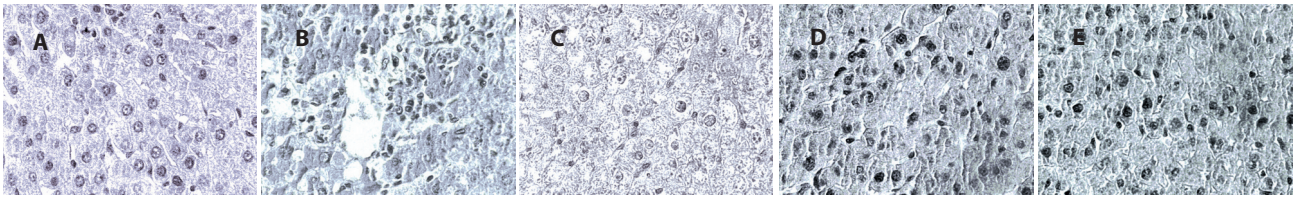

Figure 1 Histopathological observations of liver sections from different groups stained with Hematoxylin-Eosin (×100)
A: Rat liver section of control group showed normal liver architecture with well preserved nuclei. B: APAP (2 g/kg) treated rats showed hepatocytes disarrangement, deformed nuclei and cellular degeneration as compared with control liver. C: APAP + SD (1 mL/kg) group showed less improvement in hepatocytes. D: APAP + SD (2 mL/kg) and E: APAP + SD (4 mL/kg) treated groups showed less pathological changes with maximum restoration in liver morphology.

sential role in detoxification of NAPQI and prevention of APAP induced liver injury. However, hepatic lipid peroxidation, expressed as TBARS, and reduced GSH which are known as oxidative stress markers.[26] In present findings, the level of TBARS increased and the glutathione level in liver tissue which were restored the level of these parameter by SD treatment same as positive control, silymarin.

Research studies reported that mitochondrial damage is an early event of hepatic toxicity induced by APAP intoxication.[27] The cellular damage is linked to the lipid peroxidation which further leads to decrease the activity of ER membrane-bound enzymes such as G-6-Pase and ATPase.[28] SD treatment at all doses significantly reversed the level of these enzyme in dose-dependent manner indicating improvement in the physiological functions of liver and the biochemical results were comparable with the positive control, silymarin. The histological examination of rat liver treated with APAP showed significant structural changes as characterized by hypertrophy and cellular degeneration in hepatocytes.[29] SD treatment reversed these changes with improve cellular morphology towards control group. These findings were significantly supported by the biochemical findings. Based on biochemical analysis and histological observations, SD at doses 2 and 4 mL/kg showed significant and similar pronounced hepatoprotective effects against hepatotoxicity. Possible mechanisms of hepatoprotective effect of the SD formulation may be due to the presence of phenolic contents and its free radical scavenging activity.[30]

In conclusion, this data suggest that therapeutic administration of SD protects the liver and reduces hepatotoxicity induced by APAP intoxication in rats. Therefore, this formulation may be used as herbal remedy for the treatment of liver diseases.

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