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Research Article

HEPATOPROTECTIVE ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF WHOLE PLANT OF *SOLANUM DULCAMARA* L. AND *NEPHROLEPIS CORDIFOLIA* (L) *C. PRESL* AGAINST PARACETAMOL INDUCE HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Objective: To investigate the effect of hepatoprotective activity of hydro-alcoholic extract of *Solanum dulcamara* L. and *Nephrolepis cordifolia* (L) *C. Presl* against paracetamol induced hepatotoxicity in rats.

Methods: Albino rats of either sex were divided into nine groups and treated for 7 days. Group I and II served as normal and toxic control, Group III were treated with Silymarin (100 mg/kg), and Group IV to IX were treated with 200, 400 and 600 mg/kg hydro-alcoholic (70%v/v) extract of *S. dulcamara* (HASD) and hydro-alcoholic (70%v/v) extract of *N. cordifolia* (HANC) respectively. The biochemical markers like serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), bilirubin (total and direct), total protein, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C). The *in vivo* antioxidant activity was determined by estimating the tissue levels of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO). Histopathology of liver was also carried out.

Results: The HASD and HANC (200, 400 and 600 mg/kg) produced significant effect by decreasing the activity or level of SGOT, SGPT, ALP, billirubin and total protein, where decrease in total protein level in liver, TG, TC, LDL-C and VLDL-C levels and increase in the HDL-C. And decrease tissue LPO, while it significantly increased the levels of tissue GSH, SOD and CAT in a dose-dependent manner.

Conclusion: From the present study it can be concluded that hydro-alcoholic extract of *S. dulcamara* L. and *N. cordifolia* (L) *C. Presl* whole plant possesses hepatoprotective activity against paracetamol induced hepatotoxicity.

Keywords: Hepatoprotective, *In-vivo* antioxidant activity, Paracetamol, *Solanum dulcamara* L. and *Nephrolepis cordifolia* (L) *C. Presl,* Silymarin.

INTRODUCTION

Experimental induction of liver injury, which would be predictable, reproducible in animal models cannot be attained with ease. One of the intrinsic hepatotoxin causing reproducible dose dependent toxicity in the liver is paracetamol [1]. Liver is the heaviest and the second largest gland and also a key organ regulating homeostasis in the body. Liver cells called hepatocytes, every second perform several complex biochemical and a number of important functions, including bile production, excretion of bilirubin, cholesterol, hormones and drugs. It is also responsible for metabolism of fats, proteins, carbohydrates, enzyme activation, storage of glycogen, vitamins, minerals and synthesis of plasma proteins such as albumin, globulin and clotting factors. Toxic liver injury produced by drugs and chemicals are similar to natural liver disease. Continuous use of agents such as paracetamol, tetracycline, antitubercular drugs, oral contraceptives of hormonal origin, chemicals used as food preservatives and agrochemicals are threatening the integrity of liver. Further addiction of alcohol and other drugs aggravated the problem and malnutrition also an important cause of liver damage [2].

Drug induced liver injury is an unresolved problem and often limits drug therapy in clinical practice. Liver injury follows with the inhalation, ingestion or parenteral administration of a number of chemical and pharmacological agents [3]. The nature and extent of liver damage varies depending on the type of stage of its disease. Not all liver diseases produce the same patterns of change, but many forms of chronic liver disease will ultimately lead to the typical clinical and histological picture of cirrhosis. The effects of liver disease on hepatic metabolism of drugs are complex and difficult to predict, particularly when multiple drugs are administered simultaneously [4].

It is well-known that drugs are structurally altered in the liver to form biologically inactive or active or toxic metabolites. Indiscriminate use of analgesics [5], anti-malarials [6], anti-tubercular drugs [7], oral contraceptives, antidepressants, anticonvulsants [8] etc. are potential threats to the integrity of liver. Quite often, certain drugs even in the therapeutic dose may cause hepatic damage in susceptible individuals. Toxic effects of drugs on the liver or its function may mimic any naturally occurring hepatic disease. The spectrum of drug induced liver injury ranges from asymptomatic increase in enzyme (markers of hepatic damage) levels to fulminant hepatic failure. It can occur in different forms including acute drug-induced hepatitis, steatohepatitis, cholestasis, chronic hepatitis and may lead to liver failure. Many drugs may cause more than one type of hepatic injury [9].

About 20,000 deaths found every year due to liver disorders, and hepatocellular carcinoma is one of the10 most common tumors in the world with over 2,50,000 new cases each year. In India, about 40 polyherbal commercial formulations are being used for hepatoprotection. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity. Liver protective herbals contain a variety chemical constituents like phenols, coumarins, lignans, essential oils, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids, and xanthenes. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of 25 different plants have been reported to cure liver disorders [10].

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulates liver function, offer protection to the liver from damage or help regeneration of hepatic cells except silybon, a recent synthetic drug [11] however there are numbers of drugs employed in traditional system of medicine for liver affections [12].

METHODS

Plant material

The whole plant of *S. dulcamara L* and *Nephrolepis cordifolia* (L) *C. Presl* most widely found in the India. The plants were collected from the forest near to Chittoor District (Andhra Pradesh). The plants were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India.

Preparation of plant extract

Whole plants was shade dried at room temperature and was chopped into small pieces. Dried plant were powdered and packed in air tight container. The coarse powder was packed in Soxhlet column and then extracted with 70% hydro-alcohol (75-80°C). Thereafter, the extract was concentrated using rotary flash evaporator (50°C).

Determination of acute toxicity (LD_{50})

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of three rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs [13].

Experimental animals

Albino Wistar rats weighing 150-200 g and Albino mice 20-30 g was procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy, for experimental purpose. Animals were maintained under controlled condition of temperature at 27±2°C and 12 hrs light-dark cycles for 1-week. They were housed in polypropylene cages and containing paddy husk as bedding. They had a free access to standard pellets and water *ad-libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore (REF - IAEC/012/12/2010) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

In vivo hepatoprotective activity

Evaluation of hepatoprotective activity in paracetamol-induced hepatotoxicity [12]

In the dose response experiment, albino rats were randomly assigned into 9 groups of 6 individuals each.

Group I: Animals (−ve control) were administered normal saline 1 ml/kg p.o., for 7 days.

Group II: Animals (+ve control) were administered normal saline 1 ml/kg p.o., for 7 days.

Group III: Animals were administered with silymarin 100 mg/kg p.o., for 7 days.

Group IV: Animals were administered with hydro-alcoholic (70%v/v) extract of *S. dulcamara* (HASD) 200 mg/kg p.o., for 7 days.

Group V: Animals were administered with HASD 400 mg/kg p.o., for 7 days.

Group VI: Animals were administered with HASD 600 mg/kg p.o., for 7 days.

Group VII: Animals were administered with hydro-alcoholic (70%v/v) extract of *N. cordifolia* (HANC) 200 mg/kg p.o., for 7 days.

Group VIII: Animals were administered with HANC 400 mg/kg p.o., for 7 days.

Group IX: Animals were administered with HANC 600 mg/kg p.o., for 7 days.

On 5th day, 30 minutes after the administration of normal saline, 100 mg/kg silymarin, 200, 400 and 600 mg/kg of HASD and HANC to Group II, III, IV, V, VI, VII, VIII and IX respectively, paracetamol 2 g/kg was given orally. After 48 hrs of paracetamol feeding rats were sacrificed under mild ether anaesthesia. Blood samples were collected for evaluating the serum biochemical parameters and liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and proceeded for histopathology to evaluate the details of hepatic architecture in each group microscopically. The blood so collected was centrifuged immediately to get clear serum and was subjected to various biochemical studies.

Physical parameters

Determination of wet liver weight

Animals were sacrificed, and livers were isolated and washed with saline and weights determined by using an electronic balance. The liver weights were expressed with respect to its body weight i.e. g/100 g [14].

Determination of wet liver volume

After recording the weight, all the livers were dropped individual in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded [14].

In-vivo antioxidant activity

Glutathione (GSH) estimation [15]

Tissue samples were homogenized in ice cold trichloroacetic acid (1 g tissue plus 10 ml 10% TCA) in an Ultra Turrax tissue homogenizer. GSH measurements were performed using a modification of the Ellamn procedure (Aykae *et al*.). Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogenphosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added, and the absorbance at 412 nm was measured immediately after mixing. % increase in OD is directly proportional to the increase in the levels of GSH. Hence, % increase in OD is calculated.

Lipid peroxidation (LPO)

Procedure

Thiobarbituric acid reactive substances (TBARS), the last product in LPO pathway, were measured using the modified method of Esterbauer and Cheeceman, 1990. Liver tissue (200 mg) was homogenized in 10 volumes of ice-cold 50 mM phosphate buffer (pH 7.4) and the homogenates were centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was used for the assay. Protein concentrations of different homogenates were measured according to the method of Bradford. Protein (1 mg) was incubated at 37°C for 1 hr and then 1 ml 20% TCA and 2 ml 0.67% TBA was added and heated for 30 minutes at 100°C. Precipitate was removed by centrifugation at 1000 g for 10 minutes. The absorbance of the samples was measured at 535 nm against a blank that contains all the reagents except the sample. TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA which is 1.56 × 10 mmol cm as 99% of TBARS is MDA [16].

Calculation:

LPO = (Test OD×Total volume×1)/(1.56×105×109×Sample volume×mg protein per ml)

Unit: nmol MDA/min×mg protein

Catalase (CAT) [17]

Principle

In UV range H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit is a measure of CAT activity.

Procedure

The liver homogenates contain 5 µg total protein was mixed with 700 µl, 5 mm hydrogen peroxide and incubated at 37°C. The disappearance of peroxide was observed at 240 nm for 15 minutes. One unit of CAT activity reduces 1 µmol of hydrogen peroxide per minute.

Observation

Check absorbance at time interval of (0, 15, 30, 45, 60, 75, 90, 105, and 120 seconds).

Calculation:

CAT = T×Dilution factor×100/(OD at 0 sec × mg protein per ml)

Unit: µmole of H_2O_2/sec/mg protein/ml

Superoxide dismutase (SOD) [18]

Principle

The enzyme is necessary for survival in all oxygen metabolizing cells. It is found in the cytosol and intermembrane space of mitochondria of eukaryotic cells. It contains copper and zinc. In normal cells, this radical alone is the precursor of hydrogen peroxide. SOD scavenges the super oxide (O^-_2) and thus provides a first catalyze the dismutation of super oxide anion (O_2) to hydrogen peroxide and molecular oxygen in the following manner.

$$2H_2O_2 + 2O \rightarrow 2H_2O + O_2$$

In the erythrocytes, the super oxide anion (O_2) interacts with peroxides to form hydroxyl radicals (OH), which causes heamolyses in the absence of SOD activity. SOD measurement was carried out on the ability of SOD to inhibit spontaneous oxidation of epinephrine to adrenochrome.

Procedure

2.8 ml of sodium carbonate buffer (0.05 mM) and 0.1 ml of tissue homogenate or sucrose (Blank) was incubated at 30°C for 45 minutes. Then, the absorbance was adjusted to 0 to sample. Thereafter, the reaction was initiated by adding 10 µl of adrenaline solution (9 mM). The change in absorbance was recorded at 480 nm for 8-12 minutes. Throughout the assay, the temperature was maintained at 30°C. Similarly, SOD calibration curve was prepared by taking 10 units/ml as standard solution. 1 units of SOD produce approximately 50% of inhibition of auto-oxidation of adrenaline. The results are expressed as unit (U) of SOD activity per mg of tissue.

Calculation:

SOD = (C×Total volume×1000)/(50×Sample volume×mg protein per ml)

Unit: Units/mg protein.

Statistical analysis

The values are expressed as mean±standard error of the mean. The data was analyzed by using one-way ANOVA followed by Tukey multiple comparison tests using Graphpad prism software. Statistical significance was set at $p \leq 0.05$.

RESULTS

Effect of HASD and HANC on paracetamol induced hepatotoxicity

Wet liver weight and wet liver volume

Paracetamol treatment in rats resulted in enlargement of the liver, which was evident by an increase in the wet liver weight and volume. The groups were treated with silymarin and HASD and HANC showed significant restoration of wet liver weight and wet liver volume nearer to normal (Table 1).

Effect on serum marker enzymes

There is a marked increase in serum glutamic-pyruvic transaminase (SGPT) levels observed in paracetamol treated group. However the SGPT levels were decreased by HASD and HANC dose dependently. In addition the standard silymarin has restored the SGPT levels significantly. Serum glutamic oxaloacetic transaminase (SGOT) levels have been also elevated in the paracetamol treated groups. Treatment with standard silymarin has brought back the SGOT levels to the near normal levels. However treatment with the HASD and HANC has decreases the SGOT levels in a dose dependent manner, which statistically significant. In case of total and direct bilirubin there is a noticeable rise in serum levels on paracetamol treatment observed. Treatment with HASD and HANC has reversed the total and a direct bilirubin serum level by dose dependent manner, which is statistically significant when compared with Paracetamol treated group. The reversal by treatment with standard silymarin which was also significant. Rise in alkaline phosphatase (ALP) serum levels observed in paracetamol treated group, and was remarkable decreased significantly by the HASD and HANC by dose dependent manner and standard silymarin treatment (Table 2).

Serum total protein

Paracetamol treatment considerably reduced serum total protein levels. Pretreatment with silymarin and HASD and HANC showed a significant increase in total protein levels as compared with toxicant control group (Table 3).

Serum lipid profile

The lipid profile was evaluated by estimating triglycerides (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) in normal and paracetamol induced hepatotoxic rats. The paracetamol induced hepatotoxic rats showed a significant increased in the TG, TC, LDL-C and VLDL-C levels and suppression of HDL-C levels compared to control group (Table 4). But after treatment with the 200 mg/kg, 400 mg/kg, 600 mg/kg p.o. dose of HASD and HANC and silymarin paracetamol induced hepatotoxic rats showed a decrease in the TG, TC, LDL-C and VLDL-C levels and increase in the HDL-C levels compared with untreated paracetamol induced hepatotoxic rats.

***In vivo* antioxidant activity**

Effect of HASD and HANC on GSH, LPO CAT and SOD in paracetamol induced hepatotoxic rats

There is a marked depletion of GSH levels in paracetamol treated group. 100 mg/kg silymarin has increased it by 95.18%, HASD and HANC has shown a dose dependent increase in the levels of GSH. There is a dose dependent inhibition of ***in-vivo*** LPO by HASD and HANC. 100 mg/kg silymarin has 66.13% inhibition whereas 600 mg/kg of HASD and HANC has 64.28% and 65.34% inhibition of LPO. Paracetamol induced hepatotoxic rats exhibited significant lower CAT (26.01±1.67) as compared to those of negative control rats (91.44±2.16) treatment with the plant extract significantly elevated the reduced CAT levels. The 200 mg/kg, 400 mg/kg, 600 mg/kg p.o dose of HASD, HANC and silymarin showed a marked increase in the CAT levels ($p<0.001$) compared to the positive control. Paracetamol induced hepatotoxic

Table 1: Effect of HASD and HANC on wet liver weight and wet liver volumes in paracetamol induced hepatotoxic rats

Groups	Treatment	Mean±SEM	
		Wet liver weight (g/100 g)	Wet liver volumes (ml/100 g)
Group I	Negative control (0.5 ml saline)	2.990±0.169	3.030±0.038
Group II	Positive control paracetamol (2 g/kg p.o.)	3.807±0.101	3.792±0.072
Group III	Paracetamol+standard (silymarin) (2 g/kg p.o.+100 mg/kg p.o.)	3.137±0.064***	3.197±0.065***
Group IV	Paracetamol+HASD (2 g/kg p.o.+200 mg/kg p.o.)	3.405±0.099^{ns}	3.437±0.085*
Group V	Paracetamol+HASD (2 g/kg p.o.+400 mg/kg p.o.)	3.222±0.057**	3.253±0.039***
Group VI	Paracetamol+HASD (2 g/kg p.o.+ 600 mg/kg p.o.)	3.162±0.056***	3.235±0.047***
Group VII	Paracetamol+HANC (2 g/kg p.o.+200 mg/kg p.o.)	3.390±0.062^{ns}	3.428±0.070*
Group VIII	Paracetamol+HANC (2 g/kg p.o.+400 mg/kg p.o.)	3.305±0.105*	3.382±0.099**
Group IX	Paracetamol+HANC (2 g/kg p.o.+600 mg/kg p.o.)	3.177±0.085***	3.225±0.083***

Values are mean±SEM (n=6) one-way ANOVA followed by Tukey-Karmer's test. Where, ***p<0.001, **p<0.01, *p<0.05 and ns represents not significant. All the values are compared to paracetamol treated group. HASD: Hydro-alcoholic (70%v/v) extract of *S. dulcamara*, HANC: Hydro-alcoholic (70%v/v) extract of *N. cordifolia. N. cordifolia: Nephrolepis cordifolia, S. dulcamara: Solanum dulcamara*, SEM: Standard error of the mean

Table 2: Effect of HASD and HANC on SGPT, SGOT, ALP, direct bilirubin, total bilirubin levels in paracetamol induced hepatotoxic rats

Groups	Treatment	Mean±SEM				
		SGPT levels (U/L)	SGOT levels (U/L)	Total bilirubin levels (mg/dl)	Direct bilirubin levels (mg/dl)	ALP levels (U/L)
Group I	Negative control (0.5 ml saline)	48.33±2.612	86.27±3.191	0.925±0.020	0.195±0.016	128.8±3.475
Group II	Positive control paracetamol (2 g/kg p.o.)	287.6±8.304	412.9±6.915	4.377±0.267	1.520±0.140	249.9±3.209
Group III	Paracetamol+standard (Silymarin) (2 g/kg p.o.+100 mg/kg p.o.)	57.62±4.333***	119.4±4.547***	1.278±0.185***	0.386±0.025***	89.27±3.641***
Group IV	Paracetamol+HASD (2 g/kg p.o.+200 mg/kg p.o.)	120.2±3.553***	232.8±2.502***	2.100±0.064***	0.796±0.032***	147.2±2.823***
Group V	Paracetamol+HASD (2 g/kg p.o.+400 mg/kg p.o.)	89.86±3.454***	170.2±4.340***	1.673±0.119***	0.656±0.038***	119.7±3.996***
Group VI	Paracetamol+HASD (2 g/kg p.o.+600 mg/kg p.o.)	64.87±3.639***	141.1±4.304***	1.397±0.150***	0.493±0.027***	97.09±4.430***
Group VII	Paracetamol+HANC (2 g/kg p.o.+200 mg/kg p.o.)	133.2±2.343***	241.6±3.232***	2.140±0.0840***	0.823±0.034***	160.2±3.970***
Group VIII	Paracetamol+HANC (2 g/kg p.o.+400 mg/kg p.o.)	96.39±3.197***	163.8±4.617***	1.565±0.131***	0.675±0.027***	123.4±2.573***
Group IX	Paracetamol+HANC (2 g/kg p.o.+600 mg/kg p.o.)	72.22±2.745***	133.7±2.433***	1.348±0.064***	0.473±0.022***	99.33±4.179***

Values are mean±SEM (n=6) one-way ANOVA followed by Tukey-Karmer's test. Where, ***p<0.001, **p<0.01, *p<0.05 and ns represents not significant. All the values are compared to paracetamol treated group. HASD: Hydro-alcoholic (70%v/v) extract of *S. dulcamara*, HANC: Hydro-alcoholic (70%v/v) extract of N. cordifolia. *N. cordifolia: Nephrolepis cordifolia, S. dulcamara: Solanum dulcamara*, SEM: Standard error of the mean, SGPT: Serum glutamic-pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphatase

Table 3: Effect of HASD and HANC on serum total protein levels in paracetamol induced hepatotoxic rats

Groups	Treatment	Total protein levels (g/dl) (mean±SEM)
Group I	Negative control (0.5 ml saline)	8.122±0.251
Group II	Positive control paracetamol (2 g/kg p.o.)	3.788±0.191
Group III	Paracetamol+standard (silymarin) (2 g/kg p.o.+100 mg/kg p.o.)	8.018±0.093***
Group IV	Paracetamol+HASD (2 g/kg p.o.+200 mg/kg p.o.)	5.805±0.120***
Group V	Paracetamol+HASD (2 g/kg p.o.+400 mg/kg p.o.)	7.702±0.381***
Group VI	Paracetamol+HASD (2 g/kg p.o.+600 mg/kg p.o.)	7.925±0.293***
Group VII	Paracetamol+HANC (2 g/kg p.o.+200 mg/kg p.o.)	6.072±0.105***
Group VIII	Paracetamol+HANC (2 g/kg p.o.+400 mg/kg p.o.)	7.533±0.260***
Group IX	Paracetamol+HANC (2 g/kg p.o.+600 mg/kg p.o.)	7.778±0.151***

Values are Mean±SEM (n=6) one-way ANOVA followed by Tukey-Karmer's test. Where, ***p<0.001, **p<0.01, *p<0.05 and ns represents not significant. All the values are compared to paracetamol treated group. HASD: Hydro-alcoholic (70%v/v) extract of *S. dulcamara*, HANC: Hydro-alcoholic (70%v/v) extract of *N. cordifolia. N. cordifolia: Nephrolepis cordifolia, S. dulcamara: Solanum dulcamara*, SEM: Standard error of the mean

rats exhibited significant lower SOD (4.17±0.28) as compared to those of negative control rats (14.06±0.77) treatment with the plant extract significantly elevated the reduced SOD levels. HASD, HANC and Silymarin showed a marked increase in the SOD levels (p<0.001) compared to the positive control (Table 5).

Histopathological studies of the liver in paracetamol induced hepatotoxicity (Fig. 1)

Group I: Section studied shows liver parenchyma with intact architecture. Most of the perivenular hepatocytes, periportal hepatocytes and midzonal hepatocytes appear normal. Within the hepatic parenchyma, the sinusoids appear normal.

Group II: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show macrosteatosis, while some show degenerative changes. There are seen focal aggregates of mononuclear inflammatory cells within the parenchyma. Scattered mononuclear inflammatory infiltration within the parenchyma.

Group III: Section studied shows liver parenchyma with partially effaced architecture. Some of the sinusoids appear congested. Most of the hepatocytes show macrosteatosis, while few show microsteatosis. There are seen scattered mononuclear inflammatory infiltration within the parenchyma.

Group IV: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show microsteatosis, while some show macrosteatosis. Some of the hepatocytes show degenerative changes. Intervening the hepatocytes are seen aggregates of mononuclear inflammatory cells.

Table 4: Effect of HASD and HANC on lipid profile levels in paracetamol induced hepatotoxic rats

Groups	Treatment	Serum lipid profile mg/dl				
		TC	TG	HDL-C	LDL-C	VLDL-C
Group I	Negative control (0.5 ml saline)	113.4±4.725	121.7±3.379	39.66±1.015	49.44±4.674	24.35±0.675
Group II	Positive control paracetamol (2 g/kg p.o.)	181.4±3.753	175.4±3.916	28.23±1.153	118.1±3.192	35.09±0.783
Group III	Paracetamol+standard (silymarin) (2 g/kg p.o.+100 mg/kg p.o.)	119.1±4.119***	126.0±2.128***	37.56±1.282***	56.40±4.125***	25.19±0.425***
Group IV	Paracetamol+HASD (2 g/kg p.o.+200 mg/kg p.o.)	155.4±3.381***	161.1±3.538^{ns}	32.35±1.182^{ns}	90.84±2.902***	32.22±0.707^{ns}
Group V	Paracetamol+HASD (2 g/kg p.o.+400 mg/kg p.o.)	131.5±3.365***	140.2±3.011***	36.46±1.438**	66.99±2.732***	28.03±0.602***
Group VI	Paracetamol+HASD (2 g/kg p.o.+ 600 mg/kg p.o.)	122.9±3.613***	130.1±3.199***	37.13±1.721***	59.77±4.169***	26.01±0.639***
Group VII	Paracetamol+HANC (2 g/kg p.o.+200 mg/kg p.o.)	163.7±3.784*	155.0±2.902**	31.67±1.137^{ns}	101.1±4.403^{ns}	31.00±0.580**
Group VIII	Paracetamol+HANC (2 g/kg p.o.+400 mg/kg p.o.)	136.9±3.368***	138.3±2.404***	36.10±1.222**	73.17±4.090***	27.66±0.480***
Group IX	Paracetamol+HANC (2 g/kg p.o.+600 mg/kg p.o.)	124.9±2.435***	128.6±3.400***	37.31±1.809***	61.84±±2.040***	25.72±0.679***

Values are Mean±SEM (n=6) one-way ANOVA followed by Tukey-Karmer's test. Where, ***p<0.001, **p<0.01, *p<0.05 and ns represents not significant. All the values are compared to paracetamol treated group. HASD: Hydro-alcoholic (70%v/v) extract of *S. dulcamara*, HANC: Hydro-alcoholic (70%v/v) extract of *N. Cordifolia. N. cordifolia: Nephrolepis cordifolia, S. dulcamara: Solanum dulcamara*, SEM: Standard error of the mean, TC: Total cholesterol, TG: Triglycerides, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol

Table 5: Effect of HASD and HANC on GSH, LPO CAT and SOD in paracetamol induced hepatotoxic rats

Groups	Groups	Mean±SEM			
		GSH	LPO	CAT	SOD
Group I	Negative control (0.5 ml saline)	0.916±0.030	0.246±0.013	91.44±2.160	14.06±0.774
Group II	Positive control paracetamol (2 g/kg p.o.)	0.415±0.015	0.378±0.015	26.01±1.673	4.17±0.287
Group III	Paracetamol+standard (silymarin) (2 g/kg p.o.+100 mg/kg p.o.)	0.810±0.032***	0.128±0.017***	85.84±2.470***	12.82±0.418***
Group IV	Paracetamol+HASD (2 g/kg p.o.+200 mg/kg p.o.)	0.578±0.027**	0.195±0.014***	43.47±2.155***	7.13±0.370**
Group V	Paracetamol+HASD (2 g/kg p.o.+400 mg/kg p.o.)	0.735±0.019***	0.153±0.015***	65.87±3.581***	9.91±0.427***
Group VI	Paracetamol+HASD (2 g/kg p.o.+600 mg/kg p.o.)	0.803±0.041***	0.135±0.013***	81.59±2.798***	11.25±0.583***
Group VII	Paracetamol+HANC (2 g/kg p.o.+200 mg/kg p.o.)	0.583±0.022**	0.223±0.018***	40.20±2.067**	8.240±0.718***
Group VIII	Paracetamol+HANC (2 g/kg p.o.+400 mg/kg p.o.)	0.716±0.023***	0.166±0.010***	61.32±1.646***	10.60±0.401***
Group IX	Paracetamol+HANC (2 g/kg p.o.+600 mg/kg p.o.)	0.795±0.038***	0.131±0.010***	79.74±2.940***	12.33±0.261***

Values are Mean±SEM (n=6) one-way ANOVA followed by Tukey-Karmer's test. Where, ***p<0.001, **p<0.01, *p<0.05 and ns represents not significant. All the values are compared to paracetamol treated group. HASD: Hydro-alcoholic (70%v/v) extract of *S. dulcamara*, HANC: Hydro-alcoholic (70%v/v) extract of *N. cordifolia. N. cordifolia: Nephrolepis cordifolia, S. dulcamara: Solanum dulcamara*, SEM: Standard error of the mean, LPO: Lipid peroxidation, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase

Group V: Section studied shows liver parenchyma with intact architecture. Most of the hepatocytes, central veins and sinusoids appear normal. Also seen are scattered regenerative hepatocytes. There are seen focal aggregates of mononuclear inflammatory cells within the parenchyma.

Group VI: Section studied shows liver parenchyma with intact architecture. The sinusoids and central veins appear congested. Also seen are few scattered hepatocytes with macrosteatosis. Intervening the hepatocytes are seen focal aggregates of mononuclear inflammatory cells within the parenchyma.

Group VII: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show degenerative cahnges, while some show regenerative changes. The central veins and sinusoids appear dilates. There are seen periportal and perivenular aggregates of mononuclear inflammatory cells.

Group VIII: Section studied shows liver parenchyma with intact architecture. Few of the central veins and sinusoids appear congested. Intervening the hepatocytes are seen scattered mononuclear inflammatory cells within the parenchyma.

Group IX: Section studied shows liver parenchyma with partially effaced architecture. Some of the central veins and sinusoids appear congested. Also seen are few epithelioid granulomas within the parenchyma.

DISCUSSION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury. Liver damage is always associated with cellular necrosis, increase in tissue LPO and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like AST, ALT, TG, cholesterol, bilirubin, ALP are elevated. In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for the treatment of hepatic disorders. However there are several herbs/herbal formulations claimed have possess beneficial activity in treating hepatic disorders [19].

There are reports that paracetamol induced hepatotoxicity is due to activation of PCM to a toxic electrophile N-acetyl p-benzoquinine amine (NAPQI) by a number of iso enzyme of CYP-450 namely CYP $2E_1$, $CYP1A_2$, $CYP2A_6$, $CYP3A_4$, $CYP2D_6$. Normally PCM is eliminated from the body as sulphate and glucuronide to the extents of 95% before oxidation. However, 5% of PCM is undergoing bioactivation by above mentioned isoenzymes of CYP to a highly reactive NAPQI [20].

In case of toxic liver, Wet liver weight and wet liver volumes are increased. Toxicants induced hepatotoxicity produce fatty changes and also it is observed that there is a fall in serum lipids in another series of experiments. In this case water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume [21]. It is reported that liver mass and volume are important parameters in ascertaining the hepatoprotective effect of the drugs. Treatment with HASD and HANC significantly reduced the wet liver weight and wet liver volumes of animals and hence it possesses statistically significant hepatoprotective activity.

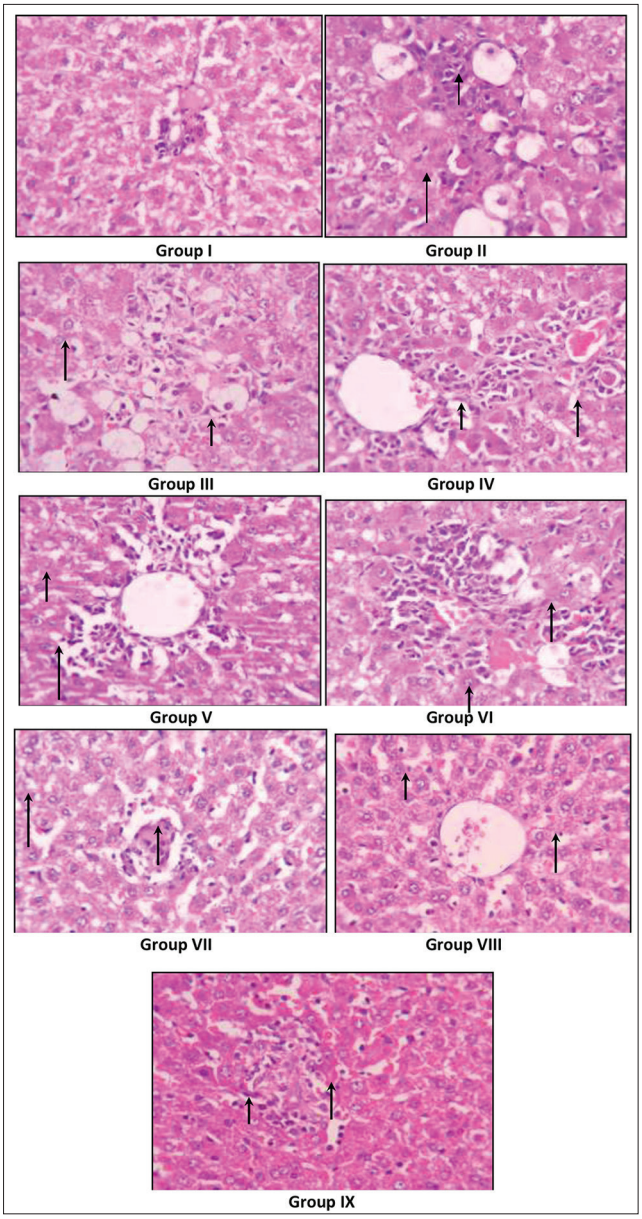

Fig. 1: Histopathological studies of the rat liver in paracetamol induced hepatotoxicity

Paracetamol induced hepatotoxicity model, paracetamol 2 g/kg b.w. bolck injection caused hepatotoxicity as indicated in the elevation of biochemical markers like SGPT, SGOT, total protein, bilirubin (total and direct TG, TC, HDL-C, LDL-C, VLDL-C and ALP). In addition PCM administration has disrupted the liver architecture as similar to CCl_4 model. There findings are in conformity with the earlier reports.

Treatment with HASD and HANC reversed the elevated levels of all the biochemical markers to the near normal levels in this model. The histopathological parameters of PCM induced hepatotoxicity were normalized by the treatment HASD and HANC. In the present study also paracetamol has increase tissue GSH, SOD, CAT and decrease the LPO. Treatment with HASD and HANC has reversed the paracetamol induced elevated LPO and decreased tissue GSH. These observations indicate that the HASD and HANC possess hepatoprotective activity against PCM induced hepatotoxicity.

After the over dosage of paracetamol, routs of sulphation and glucuronidation saturates. As a consequence oxidation of PCM, CYP-450 iso enzymes are increased leading to the increased concentration of NAPQI. This NAPQI further loses one electron resulting into the toxic radical. This radical interact covalently with membrane macromolecules and damage the membrane. However this reaction is countered by inbuilt tissue antioxidants systems like GSH. Excessive concentration of NAPQI radical over powers the inbuilt protecting mechanisms thereby damages the cell membrane. This results into the leakage of biochemical markers into the serum. It is apparent from the results that treatment with HASD and HANC prevents the formation of one electron reduced metabolite of NAPQI (which mediates cytotoxic effects of NAPQI) due to it's antioxidant property i.e. hydroxyl and superoxide anion scavenging activities. Further, this may be helpful in retaining the membrane GSH contents, reduced LPO and prevents the tissue damage [22,23].

CONCLUSION

According to the results of Physical parameters, Biochemical parameters, Functional parameters, Antioxidant parameters and Histopathology studies HASD and HANC was found to possess moderate hepato protective activity.

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