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Hepatoprotective Activity of *Bauhinia forficata* Link. Against Paracetamol Induced Liver Damage in Rats.

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ABSTRACT

To investigate the effect of hepatoprotective activity of *Bauhinia forficata* Link. against paracetamol induced liver damage in rats. 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 V were treated with 300mg/kg PEBF and MEBF respectively. The biochemical markers like SGPT, SGOT, ALP, Bilirubin (total and direct), Total protein, triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (HDL-C), VLDL-Cholesterol (VLDL-C). The *In-vivo* antioxidant activity was determined by estimating the tissue levels of GSH, SOD, CAT and lipid peroxidation. Histopathology of liver was also carried out. The PEBF and MEBF (300 mg/kg) produced significant effect by decreasing the activity or level of SGOT, SGPT, ALP, Bilirubin 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 lipid peroxidation, while it significantly increased the levels of tissue GSH, SOD and CAT in a dose dependent manner. From the present study it can be concluded that Petroleum ether and Methanolic extracts of *Bauhinia forficata* Link. whole plant possesses hepatoprotective activity against paracetamol induced hepatotoxicity.

Key words: Hepatoprotective, Paracetamol, Silymarin, *In-vivo* antioxidant activity, *Bauhinia forficata* Link.

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INTRODUCTION

There are numerous plants and traditional formulations available for the treatment of liver diseases [1, 2]. About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations [3]. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy [4].

The liver is the chemical factory which regulates, synthesizes, stores and secretes important macromolecules in the body. It has a strategic anatomical location and large capacity for metabolic transformation of drugs and other toxins entering from the gastrointestinal tract. As a result of this, the healthy functioning of the liver determines the health status of an individual [5]. Liver diseases are a global problem and the synthetic drugs available for the treatment of liver disorders are believed to have serious adverse effects on biological systems [6]. Due to these facts, attention has been given to finding suitable curative agents for the treatment of liver diseases from natural product of plant origin [7]. Normally, paracetamol is metabolized by cytochrome P450 enzymes into an active intermediate, i.e. N-acetyl-p-benzoquinone imine (NAPQI), which is rapidly detoxified by conjugation with glutathione [8] Excessive production of NAPQI due to overdoses of paracetamol reduces the levels of free glutathione by saturating the glucuronidation and sulfation pathways, which ultimately leads to hepatic necrosis progressing to liver malfunction. Excess NAPQI binds to the mitochondrial proteins and also damages the mitochondria in hepatocytes, leading to extreme generation of free radicals followed by lipid peroxidation and finally hepatic cell death [8].

As the liver is actively involved in a variety of drug metabolism, protection of the liver from the deleterious effects of drug metabolites is of utmost importance. To treat such situations, various synthetic drugs have been developed, but most of them have numerous other side effects. From ancient times, man has always used herbs for various liver disorders as a treatment strategy, because natural remedies from traditional medicinal plants have proven to be effective alternative treatments in cases of liver injury. From various studies, it has been found that hepatoprotective effects are directly associated with phytoconstituents [9-11]. All around the world, and especially in developing countries, people use traditional herbal medicines; therefore, there is a strong need to develop such a hepatoprotective medicine using natural products that can pass the safety evaluation and screening in the early phase of drug discovery because most toxic compounds are metabolized in the [12-14].

Bauhinia forficata Link. (Fam. Leguminosae) commonly known as Pata de vaca, casco de vaca, mororo, pata de boi, unha de boi, unha de vaca, unha-de-anta. A crop plant of India, South africa, South America, Argentina, Bolivia, Brazil, Paraguay, Uruguay. The plant is a Pata de Vaca small tree growing from 5 to 9 meters in height with large divided leaves resembling a cow's hoof, that are distinctive to the Bauhinia genus. It produces large drooping white flowers and a brown seed pod which looks like the mimosa seed pod. *Bauhinia forficata* Link is a diabetes, hepatoprotective, as a diuretic for kidney and urinary disorders (including polyuria, cystitis and kidney stones); as a blood cleanser and to build blood cells; for high cholesterol.[15,16].

MATERIALS AND METHODS

Plant material

The whole plant of *Bauhinia forficata* Link most widely found in the India. The plant was collected from the forest near to Chittoor District (Andhra Pradesh). The plant was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India.

Preparation of plant extracts

Whole plant 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 by using different solvents. Solvents are used based on their increasing order of polarity i.e. Petroleum ether (54°C), methanol (65°C). Thereafter, the extracts 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 3 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. [17]

Experimental animals

Albino wistar rats weighing 180-220g was procured from Biogen, Bangalore. They were maintained in the animal house of East Point College of Pharmacy, for experimental purpose. Animals were maintained under controlled condition of temperature at 27° ± 2° C and 12 hr light-dark cycles for one 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 East Point College of Pharmacy, Bangalore (REF- IAEC/012/08/2013) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

***In vivo* hepatoprotective activity**

Evaluation of Hepatoprotective Activity in Paracetamol-Induced Hepatotoxicity [18]

In the dose response experiment, albino rats were randomly assigned into 5 groups of 6 individuals each.

Group-I: Animals (-ve control) were administered normal saline 1ml/kg p.o., for 7 days
Group-II: Animals (+ve control) were administered normal saline 1ml/kg p.o., for 7 day
Group-III: Animals were administered with silymarin 100 mg/kg p.o., for 7 days.
Group-IV: Animals were administered with PEBF 300 mg/kg p.o., for 7 days.
Group-V: Animals were administered with MEBF 300 mg/kg p.o., for 7 days.

On 5th day, 30 min after the administration of normal saline, 100 mg/kg silymarin, 300 mg/kg of PEBF and MEBF to Group-II, III, IV and V respectively, paracetamol 2g/kg was given orally. After 48 hours 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. gm/100gm [19].

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 [19].

***In-vivo* antioxidant activity**

Glutathione Estimation:[20]

Tissue samples were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in an Ultra Turrax tissue homogenizer. Glutathione measurements were performed using a modification of the Ellamn procedure (Aykae, et.all.). 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 Glutathione. Hence, % increase in OD is calculated.

Lipid Peroxidation

Procedure

Thiobarbituric acid reactive substances (TBARS), the last product in lipid peroxidation pathway, were measured using the modified method of Esterbauer and Cheeceman, 1990. Liver tissue (200mg) was homogenized in 10 volumes of ice-cold 50mM Phosphate buffer (pH 7.4) and the homogenates were centrifuged at 12,000rpm for 15min at 4°C. The supernatant was used for the assay. Protein concentrations of different homogenates were measured according to the method of Bradford. Protein (1mg) was incubated at 37°C for 1h and then 1ml 20% TCA and 2ml 0.67% TBA was added and heated for 30min at 100°C. Precipitate was removed by centrifugation at 1000g for 10min. The absorbance of the samples was measured at 535nm 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.[21]

Calculation

LPO = (Test O.D × Total Volume × 1) / (1.56 × 105× 10 9 × Sample Volume × mg protein per ml)

Unit: nmol MDA / min × mg protein

Catalase [22]

Principle

In U.V. range H_2 O_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 240nm. The difference in absorbance per unit is a measure of Catalase activity.

Procedure

The liver homogenates contain 5µg total protein was mixed with 700 µl, 5Mm hydrogen peroxide and incubated at 37^0C. The disappearance of peroxide was observed at 240nm for 15min. One unit of Catalase activity is that which reduces 1µmol of hydrogen peroxide per minute.

Observation

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

Calculation

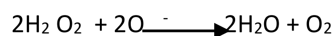
CAT = T × Dilution factor × 100 / (OD at 0 sec × mg protein per ml)

Unit: µmole of $H_2 O_2$ / sec/mg protein/ml

Superoxide Dismutase [23]

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. -Superoxide dismutase 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.



$$2H_2O_2 + 2O^- \longrightarrow 2H_2O + O_2$$

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 10µl of adrenaline solution (9mM). The change in absorbance was recorded at 480nm for 8-12 minutes. Throughout the assay, the temperature was maintained at 30^0 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 ± SEM. The data was analysed by using one way ANOVA followed by Tukey multiple comparison tests using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

Effect of PEBF and MEBF on Paracetamol Induced Hepatotoxicity

Wet Liver Weight and Wet Liver Volume

Paracetamol treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups were treated with Silymarin and PEBF and MEBF showed significant restoration of wet liver weight and wet liver volume nearer to normal. These values are tabulated in the Table No.1.

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Table No.1: Effect of PEBF and MEBF on Wet Liver Weight & Wet Liver Volumes in Paracetamol Induced Hepatotoxic Rats.

Groups	Treatment	Wet Liver weight (gm/100gm) (Mean ± SEM)	Wet Liver volumes (ml/100gm) (Mean ±SEM)
Group I	Negative Control (0.5ml saline)	2.785±0.735	2.75±0.375
Group II	Positive Control Paracetamol (2 g/kg p.o.)	3.53±0.510	3.50±0.150
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o.+ 100 mg/kg p.o.)	3.35±0.053***	3.4±0.053***
Group IV	Paracetamol + PEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	3.765±0.215**	3.60±0.12**
Group V	Paracetamol + MEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	3.56±0.150***	3.70±0.510***

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. PEBF: Petroleum ether extract of *Bauhinia forficata* Link, MEBF: Methanolic extract of *Bauhinia forficata* Link

Effect on Serum Marker Enzymes:

There is a marked increase in SGPT levels observed in Paracetamol treated group. However the SGPT levels were decreased by PEBF and MEBF dose dependently. In addition the standard silymarin has restored the SGPT levels significantly. Serum 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 PEBF and MEBF 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 PEBF and MEBF 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 ALP serum levels observed in Paracetamol treated group, and was remarkable decreased significantly by the PEBF and MEBF by dose dependent manner and standard silymarin treatment. The results are summarized in Table No.2

Table No.2: Effect of PEBF and MEBF on SGPT, SGOT, ALP, Direct Bilirubin, Total Bilirubin levels in Paracetamol Induced Hepatotoxic Rats

Groups	Groups	SGPT Levels (U/L) (Mean±SEM)	SGOT Levels (U/L) (Mean±SEM)	Total Bilirubin Levels (mg/dl) (Mean ±SEM)	Direct Bilirubin Levels (mg/dl) (Mean ±SEM)	ALP Levels (U/L) (Mean±SEM)
Group I	Negative Control (0.5ml saline)	58.77±0.990	56.07±1.027	0.913±0.022	0.515±0.155	122.6±0.513
Group II	Positive Control Paracetamol (2 g/kg p.o.)	264.9±1.363	314.5±1.306	4.875±0.059	1.508±0.0183	354.8±1.396
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o.+ 100 mg/kg p.o.)	80.29±1.563***	88.86±1.274***	1.078±0.004***	0.361±0.006***	106.3±1.075***
Group IV	Paracetamol + PEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	163.1±3.747***	126.4±1.248***	2.053±0.033***	1.048±0.058***	141.7±2.241***
Group V	Paracetamol + MEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	106.3±1.436***	96.28±0.981***	1.212±0.026***	0.476±0.017***	111.1±1.428***

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. PEBF: Petroleum ether extract of *Bauhinia forficata* Link, MEBF: Methanolic extract of *Bauhinia forficata* Link

Serum Total Protein

Paracetamol treatment considerably reduced serum total protein levels. Pretreatment with Silymarin and PEBF and MEBF showed a significant increase in total protein levels as compared with toxicant control group. The results are summarized in Table No.3

Table No.3: Effect of PEBF and MEBF on Serum Total Protein Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Total Protein levels (gm/dl) (Mean±SEM)
Group I	Negative Control (0.5ml saline)	5.065±0.1133
Group II	Positive Control Paracetamol (2 g/kg p.o.)	4.729±0.3144
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o.+ 100 mg/kg p.o.)	5.488±0.1266***
Group IV	Paracetamol + PEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	6.313±0.2833*
Group V	Paracetamol + MEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	5.763±0.2763***

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. PEBF: Petroleum ether extract of *Bauhinia forficata* Link, MEBF: Methanolic extract of *Bauhinia forficata* Link

Serum Lipid Profile

The lipid profile was evaluated by estimating triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (HDL-C), VLDL-Cholesterol (VLDL-C) in normal and Paracetamol induced hepatotoxic rats. The Paracetamol induced hepatotoxic rats showed a significant increase in the TG, TC, LDL-C and VLDL-C levels and suppression of HDL-C levels compared to control group (Table No.4). But after treatment with the 300mg/kg p.o dose of PEBF and MEBF and silymarin Paracetamol induced hepatotoxic rats showed decrease in the TG, TC, LDL-C and VLDL-C levels and increase in the HDL-C levels compared to untreated Paracetamol induced hepatotoxic rats.

Table No.4: Effect of PEBF and MEBF 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 (0.5ml saline)	111.9±1.283	165.6±1.031	48.42±1.41	30.36±1.616	33.12±0.206
Group II	Positive Control Paracetamol (2 g/kg p.o.)	184.4±1.108	205.5±0.988	27.56±1.82	115.74±2.018	41.10±0.198
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o.+ 100 mg/kg p.o.)	113.9±0.7641***	175.1±1.040***	45.89±1.14***	32.99±1.348***	35.02±0.208***
Group IV	Paracetamol + PEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	135.9±1.430*	185.2±0.902*	42.68±1.67*	56.15±1.857*	37.07±0.187*
Group V	Paracetamol + MEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	105.8±0.7878***	167.2±0.982***	42.61±1.70***	29.77±1.908***	33.42±0.208***

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. PEBF: Petroleum ether extract of *Bauhinia forficata* Link, MEBF: Methanolic extract of *Bauhinia forficata* Link

***In vivo* antioxidant activity**

Effect of PEBF and MEBF on GSH, Lipid Peroxidation Catalase 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 94.96%, PEBF and MEBF has shown a dose dependent increase in the levels of GSH. Paracetamol induced hepatotoxic rats exhibited significant lower catalase (27.34±0.19) as compared to those of negative control rats (93.60±3.412) treatment with the plant extract significantly elevated the reduced catalase levels. The 300mg/kg p.o dose of PEBF, MEBF and Silymarin showed a marked increase in the catalase levels (P<0.001) compared to the positive control. Paracetamol induced hepatotoxic rats exhibited significant lower SOD (4.16±0.075) as compared to those of negative control rats (13.90±0.566) treatment with the plant extract significantly elevated the reduced SOD levels. PEBF, MEBF and Silymarin showed a marked increase in the SOD levels (P<0.001) compared to the positive control. There is a dose dependent inhibition of *in-vivo* lipid peroxidation by PEBF and MEBF. 100 mg/kg silymarin has 41.63% inhibition whereas 300 mg/kg of PEBF and MEBF has 28.82% and 39.97% inhibition of lipid peroxidation. These values are tabulated in the Table No.5.

Table No.5: Effect of PEBF and MEBF on GSH, Lipid Peroxidation Catalase and SOD in Paracetamol Induced Hepatotoxic Rats

Groups	Groups	GSH (Mean±SEM)	CAT (Mean±SEM)	SOD (Mean ±SEM)	LPO (Mean ±SEM)
Group I	Negative Control (0.5ml saline)	0.912 ± 0.04	93.60±3.412	13.90±0.566	3.95±0.760
Group II	Positive Control Paracetamol (2 g/kg p.o.)	0.417 ± 0.06	27.34±0.19	4.16±0.075	9.68±0.950
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o.+ 100 mg/kg p.o.)	0.813 ±0.04*** (94.96)	77.93±0.871***	12.40±1.23***	5.65±0.055*** (41.63)
Group IV	Paracetamol + PEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	0.637 ±0.04*** (52.75)	31.64±0.144*	6.56±0.165*	6.89±0.061 (28.82)**
Group V	Paracetamol + MEBF (2 g/kg p.o.+ 300 mg/kg p.o.)	0.749±0.04*** (79.62)	85.62±0.917***	11.56±0.769***	5.81±0.83*** (39.97)

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. PEBF: Petroleum ether extract of *Bauhinia forficata* Link, MEBF: Methanolic extract of *Bauhinia forficata* Link

Histopathological Studies of the Liver in Paracetamol Induced Hepatotoxicity:

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.

Group-III: Section studied shows liver parenchyma with partially effaced architecture. Most of the sinusoids appear dilated and congested. Most of the hepatocytes show microsteatosis, while few show macrosteatosis. There is 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. The central veins and sinusoids appear congested. Intervening the hepatocytes are seen scattered mononuclear inflammatory cells.

Group-V: 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.

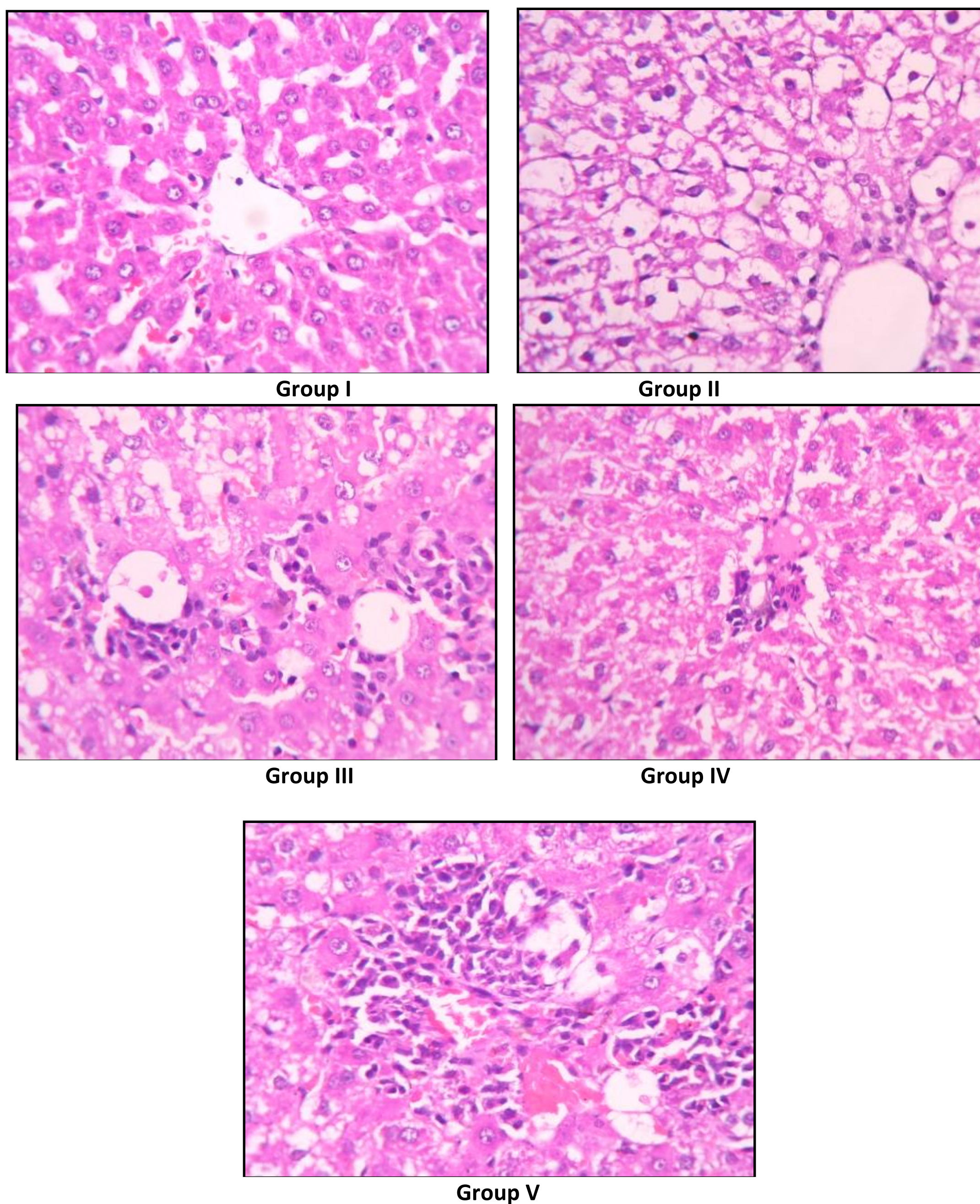

Figure No.1: Histopathological Studies of the Rat Liver in Paracetamol Induced Hepatotoxicity.

DISCUSSION

Paracetamol (acetaminophen) is a commonly and widely used analgesic and antipyretic agent. Hepatotoxic doses of acetaminophen deplete the normal levels of hepatic glutathione, when (N-acetylp-benzo-quinone imine) NAPQI covalently binds to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen adducts. The glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins. In living systems, liver is considered to be highly sensitive to toxic agents. The study of different enzyme activities such as SGOT, SGPT, SALP, total bilirubin and total protein have been found to be of great value in the assessment of clinical and experimental liver damage [24,25].

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. [26]

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.[27] It is reported that liver mass and volume are important parameters in ascertaining the hepatoprotective effect of the drugs. Treatment with PEBF and MEBF significantly reduced the wet liver weight and wet liver volumes of animals and hence it possesses statistically significant hepatoprotective activity.

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 triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), VLDL-Cholesterol (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 PEBF and MEBF 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 PEBF and MEBF. In the present study also paracetamol has increase tissue GSH, SOD, CAT and decrease the lipid peroxidation. Treatment with PEBF and MEBF has reversed the paracetamol induced elevated lipid peroxidation and decreased tissue GSH. These observations indicate that the PEBF and MEBF 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 PEBF and MEBF 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 lipid peroxidation and prevents the tissue damage.[28, 29]

CONCLUSION

According to the results of Physical parameters, Biochemical parameters, Functional parameters, Antioxidant parameters & Histopathology studies PEBF and MEBF was found to possess moderate hepatoprotective activity.

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