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Original Research Article

EVALUATION OF HEPATOPROTECTIVE EFFECT OF *LENZITUS BETULINA* AGAINST PARACETAMOL INDUCED HEPATIC DAMAGE IN RATS

C. Sundaramoorthi, R. Jayaraman, Johnson, Vipul, Riyas, Kamal Kumar Dhariya*

Department of Pharmacology, KMCH College of Pharmacy, Coimbatore
Dr. M.G.R University, Chennai, India.

Abstract: In present work preliminary phytochemical analysis and pharmacological evaluation methanolic extract of *lenzitus betuline* (MELB) was done. Phytochemical analysis was done for *lenzitus betulina*. Against toxicity produced by administering a combination of antipyretic drugs in paracetamol (PCM) 200mg/kg each for 21 days in male Wister rats. MELB was administered in two graded doses 0f 200 and 400mg/kg orally. Hepatoprotective effect of MELB WAS evident in the doses of 200 and 400 mg/kg as there was a significant decrease in SGOT, SGPT, ALP, Total and direct bilirubin levels treated groups in comparison to only induced groups without treatment. The reverse condition is seen in total protein level. The glutathione content and the activity of antioxidant enzyme such as superoxide dismutase, catalase, glutathione peroxidise and glutathione reductase were estimated in liver which shows that treatment with mushroom extract brought the enzyme levels close to normality. Histopathology of the liver section of the animals with the MELB further confirms the hepatoprotective activity.

Key Words: Lenzites betulina, paracetamol, Hepatoprotective effect, lipid peroxidation, Mushroom.

Introduction: Mushroom nature is a wonderful creation of great almighty, which has bestowed with many treasures for the use of mankind .The human being, is a very complex multi cellular organism and life in it depends upon a number of biochemical, physiological and psychological activities. In 1948, World Health Organization(WHO) defined health as a state of complete physical, mental and social well being and not merely the absence of disease or infirmity.[1] The WHO defines health's as "A state of complete physical ,mental and social well-being'' now is conventional wisdom.

The uses of mushrooms have been mentioned by all cultures. In those days, people collected information on fungus mushrooms methodically and scientifically and developed well-defined herbal, fungus are pharmacopoeias.[2]

For Correspondence:
kamalkumardhariya@gmail.com
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Lentinus polychrous Lev is local edible mushroom that found in northern and north-eastern part of Thailand. These been traditionally used as a folk medicine in the treatment of dyspepsia.[3]

Liver is the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental and chemotherapeutics agent. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxin, and abused by poor drug habits, and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease.[4, 5]

Liver is capable of detoxifying toxic substances and synthesizing useful ones. Hepatotoxic agents can cause very serious damages to the liver as they may deprive the liver from its principal functions. Hepatotoxicity chemicals cause the liver damages which are induced by lipid peroxidation and other oxidative damage.

Hepatotoxicity implies chemical driven liver damage. Certain medicinal agents when taken in overdose and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Any substances that cause liver injury are called hepatotoxin.

ALD is the number one cause of cirrhosis in the western world. Although this review is targeted at ALD, alcohol has been causally related to over 60 different medical conditions; the extent of which is related to type and volume of consumption. Studies have shown a threshold level (in men this threshold is 80g/day and women 20g/day) that once exceeded for a long period of time increases the risk of hepatoxicity dramatically.

Drug induced liver injury is responsible for 5% of all hospital admission and 50% of all acute liver failures.[6,7] India's system of medicine offers a number of traditional herb, fungal mushroom to improve liver health. Beside directly helping the body cops with liver problems, including jaundice, hepatitis, and cirrhosis, these herb, fungus mushrooms help the liver to eliminate toxins and microbial infections.[8] Since all the drugs used in the treatment of tuberculosis are shown to have hepatotoxicity effects, studies have been performed to prevent or reduce the toxicity by the use of natural wild fungal mushrooms drugs and synthetic compounds, without interfering with the therapeutic actions of the drugs.

It is important to note that the inhibition of CYTP4502E1 and antioxidant action seem to be the common mechanism of action of fungal mushrooms drugs.

In view of undesirable side effects of synthetic agents and absence of reliable liver protective drugs in the modern medicine, there is growing focus to follow systematic research methodology and to evaluate scientific basic for the use of traditional mushroom medicines which are claimed to possess hepatoprotective activity. About 70-80% of world populations rely on the use of traditional medicines, which is predominantly based on mushroom materials.

In modern medicine corticosteroids and immunosuppressant are commonly used to treat liver disease in allopathic form of medicine. But these drugs are associated with adverse effects such as immune suppression and bone marrow depression.

Attempts are being made globally to get scientific evidences for this traditionally reported herbal, mushroom drugs.[9] however there are the several herbs claimed to have beneficial activity in treating hepatic disorders.[10] But they need to be validated in the light of science to ensure their ability to conserve therapeutic effectiveness in the formulation form.

However there are a number of drugs in traditional system of medicine for liver affections. About 600 commercial preparations with claimed liver protecting activity are available all over the world. About 100 Indian

medicinal plants belonging to 40 families are used for herbal formulation. A few reports on the hepatoprotective activity are cited here.

eg. Apium graveolense Linn. (Umbelliferae),Ganoderma applanatum (Ganodermae), Rubia cordifolia (Rubiaceae).

Functions of the liver [11]

The liver is responsible for important functions, which includes:

- Secretion and excretion of bile
- Excretion of bilirubin, cholesterol, hormones and drugs
- Metabolism of carbohydrates, fats, protein and various chemicals including drug.
- Synthesis of fibrinogens, prothrombin and heparin.
- Enzyme activation
- Storage of glycogen, vitamins and minerals (iron and copper).
- Synthesis of plasma proteins, such as albumin, globulin and clotting factors.
- Blood detoxification and purification.

Materials and Methods: Collection and authentication of fungus mushroom: The mushroom materials were collected in month of the December from Chhattisgarh in janjagir champa district, India. The mushroom was authenticated by Dr. A.S.Krishnamoorthy (ICAR) All India coordinate Research Project on Mushroom. Tamilnadu Agriculture university Department of Plant Pathology, Coimbatore. Tamilnadu, India.

Collection and purification: After authentification the whole mushrooms parts were collected in large quantities and adhered earthy matters were removed, essential wholes fruiting bodies parts of mushrooms by distilled water to remove residual compost on sample surface. The mushroom was left to dry in oven at 60º C for 7days to ensure complete dryness.

Preparation of extract: The powdered fungus mushroom material (400g) was extracted successively with 95% aqueous methanol in Soxhlet extraction apparatus. The methanol extract was filtered and evaporated to dryness in water bath (40°C). The dry extract was kept in vaccum desiccators until use. Preliminary phytochemical analysis revealed the presence of mainly tannins, flavonoids and carbohydrates in MELB.[12]

Drugs and chemicals: Paracetamol: Ranbaxy Pharmaceutical Ltd. Indore, india; Silymarin: Micro Pharmaceutical Ltd. Indore, India; 2,2-azinobis (3-ethylbenzoline -6-sulfonic acid) ABTS salt, Glutathione reduced, Gallic acid: Sigma Aldrich Laboratories, USA; Ascorbic acid, Tris buffer: SISCO Research Laboratory, Mumbai, India; Sodium carboxy methyl cellulose, Thiobarbiturate acid: Loba chamie, Mumbai, India. All the other reagents used were of analytical reagent grade obtained commercially.

Animal and management: Male Wister rats of 200-300 g body weight were procured from animal house, KMCH College of pharmacy, Coimbatore. All rats were kept at room temperature and allowed to acclimate in standard condition at 12 hr light/12 hr dark cycle in the animal house. All the rats were fed with commercial pellet diet and water ad libitum freely throughout the experimental study. The experimental procedure was approved by IAEC (Institution animal ethical committee). Studies were performed in accordance with the CPCSEA guidelines.

Experimental animals: Male Wister rats, weighing 200-300 g were used for the hepatoprotective study. For acute toxicity study, male nulliparous, male Wister rats (200- 300g) were used. All animals were obtained from the animal house, KMCH College of Pharmacy, Coimbatore, Tamil Nadu. They were allowed food and water *ad libitum* up to the experimentation period. Prior to use, the mice were housed in polypropylene cages in group of five animals under standard environmental conditions (20-250c), natural light-dark (12h: 12h) cycle. Each animal was used only once under standard laboratory conditions. All the observations were made at room temperature in a noiseless diffusely illuminated room and were

made between 9.00 to 17.00 h in the experimental room. All the experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India.

Acute toxicity study

Acute oral toxicity was performed as per Organization for economic co-operation for development (OECD) guideline 423. The MELB was administered in a single dose by gavage using specially designed rat oral needle. Animals were kept overnight fasting prior to dosing (food was withheld but not water). Following the period of fasting animals was weighed and the test substance was administered orally at a dose of 2000 mg kg-1. After the LB extract administration, food was withheld 2 h in rats. Animals were observed individually at least once during the first 30min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter for a total of 14 days.

Treatment schedule: Male wistar rats of weight between 200-300g were divided into five groups with five animals each. Group I is maintained as normal control, were administrated carboxyl methyl cellulose 1ml/kg, p.o. Group II were maintained as positive control of paracetamol 200mg/kg and 1% CMC given through p.o. Group III were maintained as standard group (silymarin 140mg/kg). Group IV were maintained as low dose of methanol extract of mushroom (200mg/kg, p.o). Group V were maintained as high dose of methanol extract of mushroom (400mg/kg, p.o). Form group II, III, IV, V, were administrated paracetamol 1ml/kg in 1% CMC given through by p.o for 7 days for inducting hepatotoxicity. On the 8th day treatment was started group I and II receives tween 80 (2.5ml/kg, orally) for 21 days. Group III receives standard drug silymarin 140mg/kg suspended in 0.7 ml of tween 80 was administrated orally for 21 days. Group IV receives low dose of methanol extract of mushroom (200mg/kg) suspended in 1.5 ml of tween 80 was administrated orally for 21 days. Group V receives high dose of methanol extract of mushroom (400mg/kg) suspended in 1.5 ml of tween 80 was administrated orally for 21 days. Groups On the 22th day animal were anesthetized using aesthetic ether and the blood was collected by retro orbital puncture.

Serum was separated for the estimation of biochemical parameters like SGOT, SGPT, ALP, Direct bilirubin and Total bilirubin. Then the animal was sacrificed and the liver were separated, washed with ice -cold saline and weighed. Small piece of the liver tissue was collected and kept in 10% formalin solution for histopathological studies. The liver were collected was homogenized in a Teflon coated homogenizer in ice –cold 0.1 M Tris-hcl buffer pH 7.4 to get 10% homogenate. The homogenate was collected and centrifuged at 10000rpm for 10 minutes at 5^0C and the supernatant was collected and used for *in vivo* studies.

Serum biochemical parameters: Animal blood was collected from Retro-orbital sinus by using fine capillary tube for serum biochemical parameters. The blood was allowed to clot in room temperature and the serum was separated and collected by centrifuged at 10000 rpm for 10 minutes. The separated serum was used for the estimation of following parameters.

- Serum glutamic oxaloacetic transaminase (SGOT)
- Serum glutamic pyruvic transaminase (SGPT)
- Alkaline phosphatise (ALP)
- Direct bilirubin
- Total bilirubin

Were estimated by using a commercially available kits. Serum total protein was estimated according to the reported method.

Liver biochemical parameters: Liver samples were isolated and washed with normal saline and stored 12 h for in vivo antioxidant studies. The separated liver was homogenized with motor driven Teflon coated homogenizer in ice-

cold 0.1 M Tris-HCL buffers PH 7.4 to get 10% homogenate. The homogenate was centrifuged at 10000 rpm for 10 min at 5ºC. The supernatant was collected and used for the assay of catalase (CAT) activity was expressed as µmoles of hydrogen peroxide decomposed/min/mg of liver tissue [13], superoxide dismutase (SOD) activity was expressed as min/mg of liver tissue [14], glutathione peroxides (GPx) activity was expressed as µmoles of glutathione oxidized min/mg of liver tissue [15], glutathione (GSH) activity was expressed as µg/mg of liver tissue [16], proteins was determined by the method of Lowry *et al* [17]

Histopathological study: For histological study the liver tissue was collected and fixed in 10% formalin for two days and dehydrated with isopropyl alcohol (70-90%) for using a absolute alcohol with three changes for 12 hr each.Then finally dehydrated by using chloroform and chloroform is removed by paraffine infilteration. The clearing was done by using chloroform with two changes for 15 to20 minutes each. After paraffin infiltration the liver pieces were subjected to automatic tissue processing unit.

Statistical analysis: The data for various biochemical parameters were analysis using of variance (ANOVA) followed by Dennett's multiple comparison tests.

Result:

Acute toxicity: The oral LD_{50} value of the MELB in male Wister rats was 200-300mg/kg body weight.

Serum biochemical parameters:

Biochemical parameters like SGOT, SGPT, ALP and serum bilirubin in the paracetamol control groups were significantly ($p<0.001$) elevated as compared to the normal saline groups. Treatment with MELB at the dose 200 and 400mg/kg significantly ($p<0.001$) reduced the SGOT, SGPT, ALP and serum bilirubin levels towards the normal values in dose dependent manner. The total protein was found to be significantly decreased in the paracetamol control group as compared with the normal saline group ($p<0.001$). Administration of MELB at the dose of 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) in paracetamol-intoxicated rats significantly increased the total protein content as compared with the paracetamol (Table no.1)

Table 1. Effect of MELB on serum biochemical parameters of normal and paracetamol – intoxicated rats

Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (g/dl)	Total protein (g/dl)
Normal saline (5ml/kg)	45±2.48	38±1.4	83±3.9	0.67±0.04	8.35±0.1
PCM control (200 mg/kg)	189.8±4.7***	125.5±2.1**	228.8±6.7***	1.55±0.17***	5.25±0.1***
PCM (200 mg/kg) + MELB (200 mg/kg)	147.3±8.4**	103.8±1.2**	216.4±1.7^{ns}	1.47±0.08^{ns}	5.75±0.06**
PCM (200 mg/kg) + MELB (400 mg/kg)	100.8±1.2***	103.3±4.8**	203.5±2.7**	1.15±0.1^{ns}	6.25±0.06***
PCM (200 mg/kg) + Silmarin (140 mg/kg)	81.75±5.2***	82.75±5.4***	181±7.1***	0.77±0.13**	7.2±0.05***

Value are mean ± SEM (n=5), PCM : Paracetamol. PCM control group vs. saline group, $p < 0.001$. Treated groups vs. PCM control group, ** $p < 0.05$ where the significance was performed by one-way analysis of varience (ANOVA) followed by Dunnett's test.

Liver biochemical parameters: The level of reduced GSH was significantly depleted in paracetamol control group ($p < 0.001$) as compared with normal control group. Reduced GSH level was found to be significantly elevated towards normal level on administration of MELB at 200 ($p < 0.05$) and 400 mg/kg ($p < 0.001$) as compared with paracetamol control group. There were significant ($p < 0.001$) reduction in superoxide dismutase and catalase activities in paracetamol control groups compared with normal control group. Administration of MELB at 200 ($p < 0.05$) and 400 mg/kg significantly ($p < 0.001$) recovered SOD, CAT, GPx and GSH activities towards normal value when compared with paracetamol control animals (Table no.2)

Table 2. Effect of MELB on liver SOD, CAT, GPx, GSH. Value represented as means ± SEM (n=6) from four independent observations.

Enzymatic antioxidant activity	Control	Only PCM (200mg/kg)	PCM+MELB (200mg/kg)	PCM+MELB (400mg/kg)	PCM+MELB (140mg/kg)
SOD	6.5±0.64	3.07±0.41	4.4±0.43	5.5±0.36	5.8±0.37
CAT	532.3±15.72	364.4±13.63	378.6±8.9	407±4.2	490±11
GPx	6.7±0.37	3.85±0.22	4.58±0.27	5.15±0.28	5.65±0.33
GSH	6.17±0.30	4.5±0.45	5.15±0.26	5.45±0.22	5.6±0.30

Histopathological study:

Group I (Control) group liver section shows normal lobular architecture. The portal tracts are unremarkable without any inflammation. The hepatic parenchyma is normal. The sinusoids and central vein show normal morphology. there is no lobular inflammation (Fig .1)

Group II (Only PCM) group liver section shows marked dilation of central vein and sinusoids. There is extensive fatty change seen in the hepatocytes. There is lobular and portal tracts inflammation. There no fibrosis (Fig. 2)

Group III (PCM +Ext 1) liver section shows normal lobular architecture. There is portal tracts inflammation. The hepatic parenchyma shows mild lobular inflammation and granular formation. Dilation of central vein and sinusoids is mild. There is no fibrosis and fatty change (Fig. 3)

Group IV (PCM + Ext 2) liver section shows normal lobular architecture. There is portal tracts inflammation. The hepatic parenchyma shows mild lobular inflammation. Dilation of central vein and sinusoids is mild. There is no fibrosis and fatty change (Fig. 4)

Group V (PCM + STD) liver section shows normal lobular architecture. There was mild portal tracts inflammation. The hepatic parenchyma shows mild lobular inflammation. Dilation of central vein and sinusoids is mild. There is no fibrosis and change (Fig. 5)

Liver tissue of paracetamol induced group

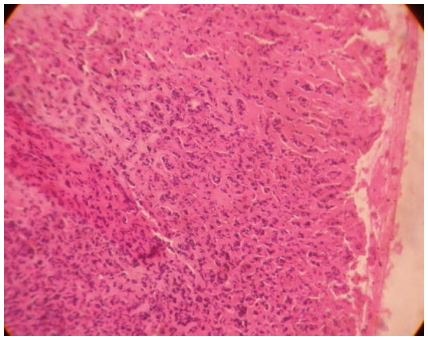

Fig.1. Control group

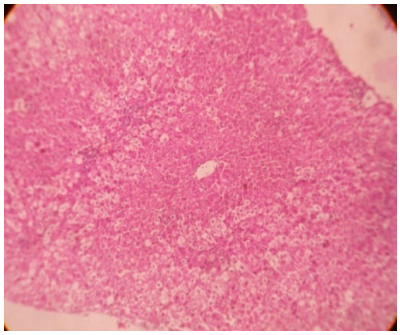

Fig.2. Toxicity PCM

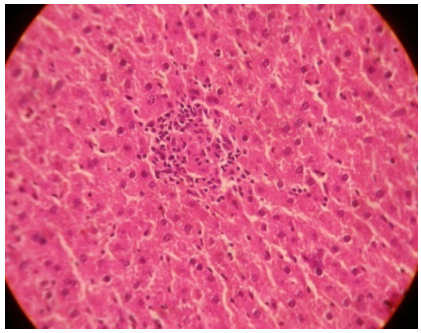

Fig.3. MELB + Toxicity PCM

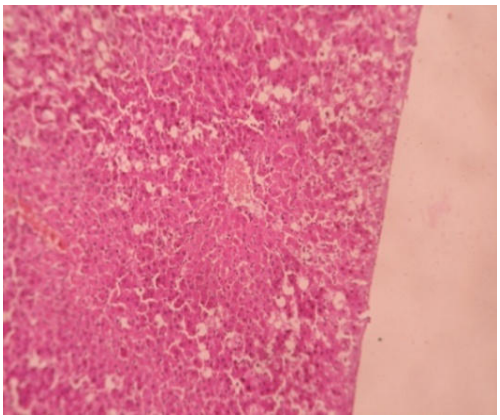

Fig.4. Silymarin + PCM induced

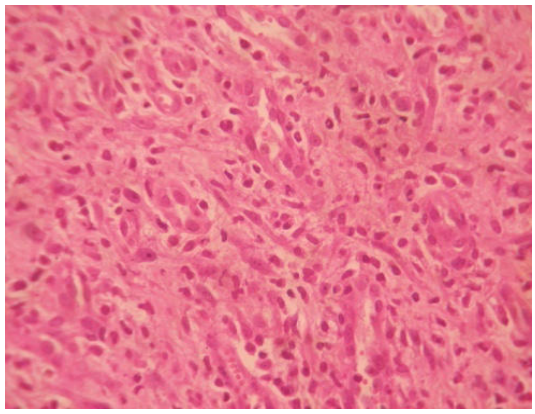

Fig.5. Mushroom extract

Discussion: Liver participates in a variety of metabolic activities perhaps by virtue of presence of number of enzyme and thus may self expose too many toxicants, chemical and drugs which could injury it. A number of pharmaceutical and chemical agent's act as hepatotoxins can produce a variety of liver ailment. In our hepatoprotective study, paracetamol were used as hepatotoxin to induce liver damage.

Hepatic and cirrhosis may occur because of enhanced lipid per oxidative reaction during the microtonal metabolism of paracetamol. The effects of the paracetamol have been suggested to be a result of the enhanced generation of oxygen free radicals during its oxidation in liver. Paracetamol inhibits glutathione peroxides, decrease the activity of catalase, superoxide dismutase, and along with increase in levels of glutathione in liver. The decreases in activity of antioxidant enzymes superoxide dismute, glutathione peroxidise are speculated to be due to the damaging effect of free radicals produced.

In the present study paracetamol induced liver damage in clearly evidenced by the increase in the levels of marker enzyme like a SGOT, SGPT, and ALP. In the (Table no.1). clearly shows the marked rise of SGOT, SGPT, and ALP in the paracetamol induced animal when compared to control groups. Whereas the extracts treated groups there was a significant decrease in level of marker enzymes which indicated the hepatoprotective nature of MELB extract. Likewise the same treated was observed in the total bilirubin levels also. (Table no. 1)

shows the liver weight of animal. The paracetamol induced groups showed significant increase in the liver weight than that of control. The treatment with MELB showed the liver weight which is near to normal values.

Free radicals may be defined as any species that is capable of independent existence and possessing one or more unpaired electrons. The radical can react with other molecules in many ways. The net effect is that radicals donates its unpaired electron to another molecule and these molecules then becomes radical the same is applicable to Paracetamol which is a powerful hepatotoxins capable to produce vast number of free radicals and have a capacity to initiate the lipid peroxidation. Administration of MELB showed almost significant decreases in the liver lipid peroxidation. The result was shown in (Table no. 2)

Super oxide dismutase is an enzyme it dismutase's the super oxide radical to hydrogen peroxide and oxygen. Simultaneously catalase enzyme reduced the hydrogen peroxide to oxygen and water because hydrogen peroxide has a mutagenic action. The treatment groups shows the significant increase in both SOD and catalase activity which reflects the antioxidant property of the extract (Table no. 2)

The depletion of GSH, GPx lead to generation and oxidative stress with cascade of effects there by affecting fuctional as well as structural integrity of cell and organell membrane. GSH in the nucleus maintains the redox state of critical protein sulphydrals that are necessary for DNA repair and expression. In this study these enzyme levels were drastically decreased in paracetamol treated groups. Administration of MELB increases the activities of GPx and GSH when compared with only induced rats. The results of GSH and GPx showed in (Table no. 2)

The induced group's animal's shows significant increase in their protein levels and MELB treated animals significantly reduces their protein levels to animal the results of protein estimation was showed in (Table no.2)

The histopathological reports showed the extent of reduction in liver damage by MELB when compared to control groups (Fig. 1, 2, 3, 4, 5)

The present investigation highlights the hepatoprotective nature of MELB extract in paracetamol induced hepatotoxicity which might be due to the synergistic effect of phytomolecules present in the extract.

Conclusion: The results from the present study highlights the hepatoprotective nature of MELB extract in paracetamol induced hepatotoxicity. The rise in serum marker enzyme such as SGOT, SGPT, and ALP, direct and total bilirubin was adjusted to near normal range in MELB treated group. which reveals that MELB can restore the activity of antioxidant defence system like GSH, GPx, SOD and CAT possibly could reducing generation of free radicals. Also MELB abolishes the activity of liver injury by decreasing lipid peroxidation. The might be due to the synergistic effect and presence of flavanoids in the extract.

The present investigation highlights the hepatoprotective nature of MELB extract in paracetamol induced hepatotoxicity which might be due to the synergistic effect of phytomolucules and flavanoid constituents present in the extract.

From the results obtained it may be concluded that *lenzitus betulina* has significant protective effect upon paracetamol induced hepatic damage in male Wister rats. Further studies should be intiated for identifying the phytomolecule and establishing its mechanism of action for its hepatoprotectivity.

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