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ORIGINAL ARTICLE

Hepatoprotective activity of *Balsamodendron mukul* extract against Paracetamol-induced liver toxicity in rats: In vivo pharmacological and toxicological evaluation

Activité hépatoprotectrice de l'extrait de Balsamodendron mukul *contre la toxicité hépatique induite par le paracétamol chez le rat : évaluation pharmacologique et toxicologique in vivo*

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HIGHLIGHTS

- *Balsamodendron mukul* (*B. mukul*) extracts protected against Paracetamol-induced rat-liver toxicity.
- *B. mukul* extracts exhibited higher antioxidant potential.
- *B. mukul* extracts blocked inflammatory cytokines such as Tumor Necrosis Factor-α and Interleukin-1.
- Hydroxyl radical scavenging activity and lipid peroxidation inhibiting activity expressed higher hepatoprotective activities.

KEYWORDS
Hepatoprotective activity;
Serum enzymes;
Balsamodendron mukul;
Paracetamol induced hepatotoxicity;
Silymarin

Summary Overuse of the antipyretic agent Paracetamol (PCM) is linked to hepatotoxicity, which limits its clinical use. The goal of this investigation was to find out how well *Balsamodendron mukul* (*B. mukul*) extract protects the liver from acute PCM poisoning. *B. mukul* extract was procured from a standard crude drug supplier in the local market. The PCM-induced hepatotoxicity was screened in experimental animals. Animals that were treated only with excessive PCM (2 g/kg) had changes in their serum biomarkers (i.e., serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, and serum total bilirubin), oxidative stress, Tumor Necrosis Factor-α (TNF-α), and Interleukin-1 proteins. *B. mukul* extracts of 245 μg and 332 μg revealed 50% of hydroxyl radical scavenging and lipid peroxidation inhibiting, respectively, which was found to be more significant when compared to ascorbic acid treatment. The outcomes confirmed that *B. mukul* extract has strong antioxidant activity, which leads to the inhibition of reactive oxygen species (ROS). Treatment with *B. mukul* extract at doses of 300 and 600 mg/kg produced a dose-dependent reduction in the PCM-induced rise of the biochemical parameters. Silymarin at 100 mg/kg body weight significantly prevented such rise in the study. Finally, the findings confirmed that the *B. mukul* extract has more potent than silymarin and revealed higher antioxidant and hepatoprotective activity, which could consider a novel approach for the reduction of PCM-induced liver toxicity.

MOTS CLÉS
Activité hépatoprotectrice ;
Enzymes sériques ;
Balsamodendron mukul ;
Hépatotoxicité induite par le paracétamol ;
Silymarine

Résumé La surutilisation de l'agent antipyrétique paracétamol (PCM) est liée à une hépatotoxicité, ce qui limite son utilisation clinique. Le but de cette enquête était de déterminer dans quelle mesure l'extrait de *Balsamodendron mukul* (*B. mukul*) protège le foie contre l'empoisonnement aigu au PCM. L'extrait de *B. mukul* a été acheté auprès d'un fournisseur standard de médicaments bruts sur le marché local. L'hépatotoxicité induite par le PCM a été examinée chez des animaux de laboratoire. Les animaux qui ont été traités uniquement avec un excès de PCM (2 g/kg) ont présenté des modifications de leurs biomarqueurs sériques (c.-à-d. glutamate oxaloacétate transaminase sérique, glutamate pyruvate transaminase sérique, phosphatase alcaline et bilirubine totale sérique), stress oxydatif, facteur de nécrose tumorale-α (TNF-α) et les protéines Interleukine-1. Les extraits de *B. mukul* de 245 μg et 332 μg ont révélé respectivement 50 % de piégeage des radicaux hydroxyle et d'inhibition de la peroxydation lipidique, ce qui s'est avéré plus significatif par rapport au traitement à l'acide ascorbique. Les résultats ont confirmé que l'extrait de *B. mukul* a une forte activité antioxydante, ce qui conduit à l'inhibition des espèces réactives de l'oxygène (ROS). Le traitement avec l'extrait de *B. mukul* à des doses de 300 et 600 mg/kg a produit une réduction dose-dépendante de l'augmentation induite par le PCM des paramètres biochimiques. La silymarine à 100 mg/kg de poids corporel a significativement empêché une telle augmentation dans l'étude. Enfin, les résultats ont confirmé que l'extrait de *B. mukul* est plus puissant que la silymarine et a révélé une activité antioxydante et hépatoprotectrice plus élevée. Ce qui pourrait envisager une nouvelle approche pour la réduction de la toxicité hépatique induite par le PCM.

Introduction

Acetaminophen, commonly referred to as paracetamol (PCM), is a popular analgesic and antipyretic medication used around the globe [1,2]. However, the overdose of PCM that results in deadly liver damage. PC is transformed into N-acetyl-p-benzoquinone (NAPQI) by the cytochrome P450 enzyme system in the liver [3]. When NAPQI and hepatic glutathione are coupled, it leaves the body via urination [1,2]. The hepatic GSH reserves are exhausted by NAPQI buildup after a PCM overdose, which promotes the production of tumor necrosis factor-α (TNF-α) [4]. Reactive oxygen species (ROS), including hydroxyl radicals, and superoxide anions are produced as a result, which damages lipids and macromolecules like proteins and nucleic acids in hepatocytes in the liver [5,6]. Therefore, abnormal oxidative stress causes cells to experience inflammation and apoptosis, which results in the death of liver cells [7,8]. This toxicity further leads to liver cirrhosis, which is irreversible hepatic damage, which limits the clinical use of PCM. Thereby, hepatoprotective therapeutic is highly needed along with PCM.

In recent years, there has been a lot of interest in using natural products to make new medicines that are safe and cheap for therapy. *Balsamodendron mukul* (*B. mukul*), is a native plant in the Burseraceae family [9,10]. It is identical to *Commiphora mukul* (*C. mukul*) and is highly valued in Ayurveda, an Indian medical system used in India [11,12]. It is grown in the Rajputana Desert, Sindh, Baluchistan, Bellari, and Mysore. It is used to treat neurological ailments, pectoral and hepatic problems, cutaneous, urinary, and cardiac issues [13]. It can also help treat tumors, anemia, diabetes, leucoderma, obesity, gout, sciatica, facial paralysis, leprosy, helminthiasis, dyspepsia, cough, asthma, bronchitis, epilepsy, and dyspepsia. In Chinese medicine, it is also used to treat extra-dural hematomas [10].

Previous research reported that the *B. mukul* extract has highly effective against PCM-induced liver toxicity. Additionally, *B. mukul* is a naturally occurring bioactive medicine that is very safe and effective in treating hepatic disorders compared to synthetic medicines due to its biodegradable and biocompatible qualities. However, *B. mukul* extract demonstrated hepatoprotective activities by lowering the levels of alanine aminotransferase, aspartate aminotransferase, and total bilirubin in PCM-induced hepatotoxic animals. It potentially inhibited the different oxidative and inflammatory factors such as reactive oxygen species (ROS), oxidative stress factors, Tumor Necrosis Factor-α (TNF-α), and Interleukin-1 proteins [5,14]. Thus, the present research was conducted to evaluate the hepatoprotective and antioxidant activity of BM extract against PCM-induced hepatotoxic animals.

Materials and methods

Materials

Plant extract of *B. mukul* was procured from a standard ayurvedic drug supplier form local market as this extract is used in its natural form for treatment by ayurvedic practitioners. Silymarin was received as a gift sample from Microlabs, (Bangalore, India). Paracetamol and Ethanol were purchased from SD Fine chemicals (Mumbai, India). Animal feed was supplied by Amrut laboratories Pranava Agro Industries Ltd. (Sangli, India). Kits for estimation of selected biochemical parameters such as SGPT, SGOT, ALP and BIT were purchased from Swemed diagnostics (Bangalore, India).

Experimental animals

Albino rats (Wistar strain) of either sex weighing between 180—220 g were procured from the central animal house M.R. Medical College (Gulbarga, India). The animals were acclimatized for seven days under standard laboratory conditions with 24 h light or dark cycles. The animals were fed with a standard diet manufactured by Amrut laboratories Pranava Agro Industries Ltd. (Sangli, India). Water was allowed *ad libitum* under strict hygienic conditions. After obtaining prior permission from Institutional Animal Ethical Committee (IAEC HKECOP/IAEC/05/2008-09), all animal studies were performed in accordance with the guidelines of CPCSEA.

Determination of acute toxicity (LD_{50})

The acute toxicity of a *B. mukul* extract was determined by using female albino mice (20—30 g) maintained under standard husbandry conditions. The animals were fasted 4 h prior to the experiment and acute toxicity as per OECD guideline was determined. Animals were administered with a single dose of 2000 mg/kg extract and observed individually at predetermined time intervals during the first 24 h, with special attention given during the first 4 h followed by daily observation for a total of 21st days. During this period, the mortality and/or the moribund status of the animals were noted [3,5].

Antioxidant activity

The following methods were utilized to determine the antioxidant potential of *B. mukul* extract. An antioxidant formulation played the crucial role in the as hepatic tonic and protective, hence the following evaluations has been needed [5].

Analysis of hydroxyl radical scavenging activity by deoxyribose degradation method

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). Fenton reaction mixture consisting of 1 mL of ferrous sulphate ($FeSO_4.7H_2O$) (10 mM), 1 mL of EDTA (10 mM) and 1 mL of 2-Deoxy-D-Ribose (10 mM) and was mixed with 6 μL of phosphate buffer at pH 7.4 and 1 mL of various dilutions of extract (25—400 μg). Thereafter, 1 mL of H_2O_2 (10 mM) was added before the incubation at 37 °C for 1 h. Then 1 mL of this Fenton reaction mixture was treated with 0.2 mL of Sodium dodecyl sulphate (8.1%), 1.5 mL of thiobarbituric acid (0.8%) and 1.5 mL of acetic acid (20%). The total volume was then made to 5 mL by adding distilled water and kept in oil bath at 100 °C for 1 h. After the mixture had been cooled, 5 mL of 15:1 v/v butanol-pyridine mix-

ture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm by using UV—vis double beam spectrophotometer. A control was prepared using 0.1 mL of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of hydroxyl radicals by the extract was determined by comparing the absorbance values of the control and experimental tubes (Eq. (1)) [15].

$$\% \text{ Inhibition} = \frac{\text{Average of the control OD} - \text{Test sample OD}}{\text{Average of the control OD}} \times 100 \quad (1)$$

Determination of lipid peroxidation inhibiting activity by Fe^{2+}/ascorbate system

Inhibition of lipid peroxidation was determined by the method developed by Ohkawa et al., 1979. Rat liver tissue weighing 10 g was homogenized with a poly homogenate and centrifuged at 4000 rpm for 10 min. An aliquot of supernatant 0.1 mL was mixed with 0.1 mL of plant extract of different concentrations, followed by addition of 0.1 mL of potassium chloride (30 mM), 0.1 mL of ascorbic acid (0.06 mM) and 0.1 mL ammonium ferrous sulphate (0.16 mM) and incubated for 1 h at 37 °C. The reaction mixture was treated with 0.2 mL of Sodium dodecyl sulphate (8.1%), 1.5 mL of thiobarbituric acid (0.8%) and 1.5 mL of acetic acid (20%). The total volume was then made to 4 mL by adding distilled water and kept in an oil bath at 100 °C for 1 hour. After cooling, 1 mL of distilled water and 5 mL of 15:1 v/v butanol-pyridine mixture were added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured at 532 nm by using UV—vis spectrophotometer [16]. A control was prepared using 0.1 mL of the vehicle in place of plant extract/ascorbic acid. The percentage inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of control.

Paracetamol-induced hepatotoxicity

The hepatotoxicity was induced as previously reported methods [17,18]. Toxicity-induction was carried out using oral administration of PCM with a single dose of 2 g/kg BW. PCM was suspended in 0.5% carboxymethyl cellulose and then 2 mL of suspension was administered orally. The experimental animals were divided into 5 groups each ($n = 6$ animals in each group) as follows:

- Group-1: Control: 2% w/v gum acacia (1 mL/kg) p.o. once daily;
- Group-2: Toxicant: paracetamol (Negative control) (2 g/kg) p.o. once daily;
- Group-3: Standard: silymarin suspension (100 mg/kg) p.o. followed by paracetamol (2 g/kg) p.o.;
- Group-4: BM suspension (300 mg/kg) p.o. followed by paracetamol (2 g/kg) p.o.;
- Group-5: BM suspension (600 mg/kg) p.o. followed by paracetamol (2 g/kg) p.o.

The study was carried out for 21st days and on day 22nd, blood samples were collected from all treated animals by retro-orbital plexus method. Serum was separated by centrifugation (3000 rpm for 15 min) and subjected to estimation of biochemical parameters (Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic-pyruvic transaminase (SGPT), Alkaline phosphatase (ALP) and total bilirubin level using a standard kit (Swemed) and ARTOS semiauto analyzer).

Investigation of total bilirubin content, SGOT, SGPT, and ALP in rat serum

Analysis of total bilirubin level, SGOT, SGPT, and ALP in rat serum was performed by following previous studies [5]. The techniques and procedures are outlined as follows:

Estimation of SGOT activity

Accurately measured (100 μL) volume of serum was added to 1000 μL of reagent 1 SGOT (Tris, L-aspartate, MDH, LDH) and then incubated at 37 °C for a time duration of 5 min. Eventually, mixed with 250 μL of reagent 2 of AST (2-oxoglutarate, NADH). Then the absorbance was analyzed at 365 nm [3].

Estimation of SGPT activity

Accurately 100 μL of serum was mixed in 1000 μL of reagent 1 SGPT (Tris, L-alanine, LDH) then this mixture was incubated at 37 °C for 5 min. Simultaneously, 250 μL of reagent 2 SGPT (2-oxoglutarate, NADH) was blended with the mixture and homogenized. Finally, absorbance was measured at 365 nm [5].

Estimation of ALP activity

Accurately 20 μL of serum was mixed into 1000 μL of reagent 1 ALP (2-amino-2-methyl-1-propanol, magnesium acetate, zinc sulphate, HEDTA). Then this mixture was incubated at 37 °C for 5 min and added around 250 μL of reagent 2 of ALP (p-nitrophenylphosphate) then homogenized. Finally, the absorbance was recorded at 400—420 nm [5,19].

Estimation of direct serum bilirubin levels

Accuretaly 50 μL of serum was mixed in 1000 μL of reagent 1 direct bilirubin (EDTA-Na2 and sulphamic acid) and then this mixture was incubated at 37 °C for 3—5 min. Combined this mixture with 250 μL reagent 2 direct bilirubin (2.4-chlorophenyl diazonium, HCl and EDTA-Na2) then homogenized this blend. Finally, the absorbance was measured at 546 nm [5].

Histopathological screening in experimental animals

Freshly isolated liver sections from treated rats were incorporated in 10% formalin for viability. Prior to the study, each section of the liver was thoroughly washed, and removed all adhesive tissues and debris. Clean sections were again properly treated with alcohol and then dehydrated [20,21].

Further, the sections were stained via staining reagents like hematoxylin and eosin. Subsequently, the stained tissues were analyzed under a light microscope and analyzed for any necrosis and cellular damage during the treatment period [22].

Statistical analysis

The findings from the in vivo studies were assessed using the Student's *t*-test with ANOVA. The probability values ($^{*}P \leq 0.05$ and $^{**}P < 0.01$) were presented as statistically significant.

Results

Determination of acute toxicity (LD_{50})

The LD_{50} studies were conducted using OECD guidelines for *B. mukul* extracts. It was observed that the extract did not show mortality even at a dose of 2000 mg/kg, which confirmed that high dose of *B. mukul* extract was practically non-toxic in experimental animals. Finally, a dose of 2 g/kg was considered safe for treatment.

Screening of antioxidant activity

Experimental outcomes confirmed that the *B. mukul* extract effectively inhibits reactive oxygen species produced by PCM overdose, thereby preventing severe liver injury. Different concentrations (25—400 μg) of *B. mukul* extract and ascorbic acid were utilized for antioxidant activity. The outcomes of the study confirmed that the antioxidant potential of *B. mukul* extract and ascorbic acid have dose-dependent. If the dose of *B. mukul* extracts and ascorbic acid had enhanced, simultaneously, the hydroxyl radical scavenging and lipid peroxidation inhibiting activities also enriched (Table 1). However, the *B. mukul* extract was found more potent than the standard antioxidant (ascorbic acid), because the 400 μg dose of BM extract has potentially inhibited 75.29 ± 2.86 of free radicle and 60.81 ± 1.29 of lipid peroxidation activity, respectively, when compared to the inhibitory potential of ascorbic acid (i.e., 72.26 ± 2.08 and 58.79 ± 2.94).

Approximately, 245 μg of *B. mukul* extract was required for 50% inhibition (IC_{50}) of hydroxyl radicals that exhibit lower concentration than the ascorbic acid (Fig. 1). Whereas, 270 μg of ascorbic acid was needed for IC_{50} of hydroxyl radicals, which exists more than the concentration of BM extract. Simultaneously, Fig. 1 represented, 332 μg of *B. mukul* extract revealed IC_{50} for lipid peroxidation activity when compared to 342.5 μg of ascorbic acid. Hence, findings of the study proved the antioxidant potential of *B. mukul* extract against the free radicle and lipid peroxidation activities.

Investigation of total bilirubin content, SGOT, SGPT, and ALP in rat serum

As shown in Table 2, the statistical investigation of the SGPT, SGOT, ALP, and BIT levels in rat serum revealed a significant ($^{**}P < 0.01$) difference between the control and negative control groups. The findings demonstrated that 2 mg/kg PCM has been successfully causing hepatic intoxication or liver damage [5]. Acute changes in hepatic biochemical parameters were observed with an increase in SGPT, SGOT, ALP, and BIT levels in rat serum due to a high dose of paracetamol, resulting in severe liver damage.

Estimation of SGPT and SGOT activity

Study outcomes confirmed that the SGPT and SGOT levels in the serum of PCM-intoxicated rats were significantly greater than in control rats. According to statistical investigations, the normal and negative control group animals had substantially different SGPT and SGOT concentrations. Fig. 2(a) also demonstrated the potency of *B. mukul* extract on PCM-induced hepatotoxic animals. *B. mukul* extract successfully inhibited PCM intoxication and controlled the increment in the levels of SGOT and SGPT. According to the results of statistical analysis, there was a significant difference observed between the SGOT and SGPT activities of the control, negative control, and treated groups ($^{**}P < 0.01$ and $^{***}P < 0.005$). The average SGPT and SGOT activities in the negative control group after 21st day of treatment were found to be 163.8 ± 3.99 IU/L and 365.0 ± 5.60 IU/L, respectively. Whereas, the *B. mukul* extract (300 mg/kg and 600 mg/kg) treated groups were found to be SGPT: 57.4 ± 1.16 IU/L and 55.35 ± 0.69 IU/L, and SGOT: 97.53 ± 2.44 IU/L and 85.95 ± 1.79 IU/L, respectively, which were similarly effective to the standard (Silymarin 100 mg/kg) treated group (Table 2). Average percentage changes in serum biochemical parameters were shown in Table 3. Findings proved that the *B. mukul* extract had much more potent in the prevention of PCM-induced hepatotoxicity as compared to a negative control group.

Estimation of ALP activity

Administration of 100 mg/kg BW of Silymarin and 300 and, 600 mg/kg BW of *B. mukul* extract significantly reduced the ALP levels in PCM-intoxicated animals when compared with the negative control group (Fig. 2(a)). It found that the ALP activities treated with silymarin and BM extract were significantly different ($^{**}P < 0.01$). Those animals treated with 300 and 600 mg/kg BM extracts did not significantly vary from control animals in terms of their ALP activities (Table 2). The average percentage changes in ALP activities were shown in Table 3. It indicated that the maximum changes were obtained in the ALP concentration in negative control animals (345.09 ± 4.83), whereas the *B. mukul* extract treated groups 300 and 600 mg/kg treated groups significantly minimized the APL levels (45.75 ± 4.26 and 36.61 ± 1.57), respectively. Hence, the outcomes confirmed that *B. mukul* extract had more potent in the treatment of PCM-induced hepatotoxicity.

Estimation of direct serum bilirubin levels

Fig. 2(b) demonstrates a significant elevation in bilirubin total levels (3.18 ± 0.21 mg/dL) in the PCM-induced negative control rat group. Whereas, *B. mukul* extract 300 mg/kg, 600 mg/kg treated, and Silymarin 100 mg/kg treated groups showed 0.43 ± 0.10 mg/dL, 0.33 ± 0.12 mg/dL, and 0.26 ± 0.06 mg/dL BIT after treatment of 21 days (Table 2).

Table 1 Percentage hydroxyl radical scavenging and lipid peroxidation inhibiting activities by *Balsamodendron mukul* extract and ascorbic acid.
Pourcentage d'activité de piégeage des radicaux hydroxyles et d'inhibition de la peroxydation lipidique par l'extrait de Balsamodendron mukul et l'acide ascorbique.

Antioxidant activity	Compound	Quantity (μg)					
		25	50	100	200	300	400
Hydroxyl radical scavenging activity	*B. mukul* extract	6.88 ± 0.36	12.83 ± 1.49	23.78 ± 2.44	41.61 ± 2.56	59.23 ± 2.12	75.29 ± 2.86
	Ascorbic acid	6.05 ± 1.34	12.09 ± 2.07	21.27 ± 2.66	39.11 ± 1.37	56.10 ± 2.71	72.26 ± 2.08
Lipid peroxidation inhibiting activity	*B. mukul* extract	3.14 ± 0.56	7.39 ± 1.62	17.40 ± 2.90	30.46 ± 3.32	47.14 ± 2.48	60.81 ± 1.29
	Ascorbic acid	6.38 ± 0.28	10.30 ± 1.90	16.91 ± 2.97	31.02 ± 1.69	46.25 ± 2.06	58.79 ± 2.94

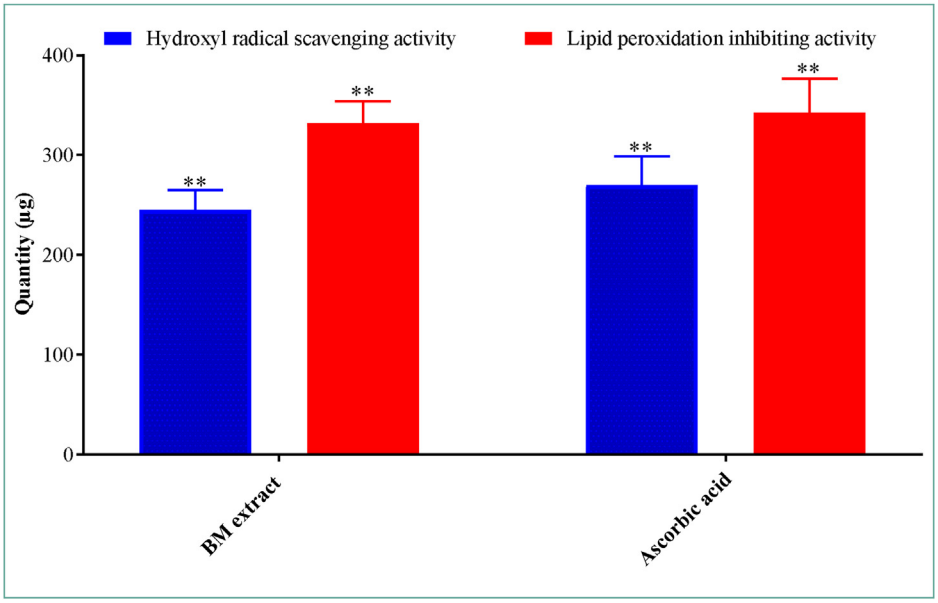

Figure 1. IC_{50} values of *Balsamodendron mukul* extract and ascorbic acid in the inhibition of hydroxyl radical and lipid peroxidation activities, data are expressed as mean ± SD.
Valeurs IC_{50} de l'extrait de Balsamodendron mukul et de l'acide ascorbique dans l'inhibition des radicaux hydroxyle et des activités de peroxydation lipidique, les données sont exprimées en moyenne ± ET.

A significant reduction in total bilirubin level was observed in extract and silymarin-treated rat groups when compared to a negative control rat group (**$P < 0.01$).

Histopathological screening in experimental animals

Histopathological evaluation was conducted utilizing the small sections of hepatic tissue to identify/determine any pathological changes or signs of severity/toxicity after intoxication via PCM and treatment via *B. mukul* extract and standard drug. The pathological images of control, negative control, standard treated, BM suspension 300 mg/kg treated, and *B. mukul* suspension 600 mg/kg treated after 21 days of the administration are represented in Fig. 3(a)—(e).

Histological illustrations revealed that no abnormal effects were observed in hepatic tissue structures in control animals. Whereas the negative control hepatic tissues suffer from severe damage, which revealed serious hepatic vein congestion and degeneration after 21 days of treatment with 2 g/kg PCM. However, the *B. mukul* extract and standard drug-treated hepatic tissues have reflected mild congestion in the hepatic vein but no severe abnormalities were found in structure when compared with control and negative control tissues.

According to the results shown in Fig. 4, the liver segment of the negative control group showed very high degrees of congestion and degeneration of liver tissues. While the treatment groups did not reflect any elevations in the hepatocyte degeneration and congestion scores. Hence, the *B. mukul* extract proved its safety and potency in the

Table 2 Influence of BM extract on selected serum biochemical parameters in PCM-induced hepatotoxic rats after 21st days of treatment. Data are expressed as mean ± SD for 6 animals in each group ($n = 6$). Various superscript symbols indicate significant difference (**$P < 0.01$) when compared with control and negative control groups.

*Influence de l'extrait de BM sur des paramètres biochimiques sériques sélectionnés chez des rats hépatotoxiques induits par PCM après 21 jours de traitement. Les données sont exprimées en moyenne ± SD pour 6 animaux dans chaque groupe (n = 6). Divers symboles en exposant indiquent une différence significative (**p < 0,01) par rapport aux groupes de contrôle et de contrôle négatif.*

Group	Treatment	SGPT (IU/L)		SGOT (IU/L)		ALP (IU/L)		BIT (mg/dL)	
		0 day	21st day	0 day	21st day	0 day	21st day	0 day	21st day
1	Control	40.54 ± 0.40	40.73 ± 1.12	63.45 ± 1.05	62.78 ± 1.94	143.7 ± 0.45	143.06 ± 1.01	0.746 ± 0.09	0.84 ± 0.10
2	Negative Control (PCM, 2 g/kg daily)	44.43 ± 0.72	163.8 ± 3.99**	64.63 ± 1.31	365.0 ± 5.60**	138.75 ± 1.71	617.56 ± 1.00**	0.67 ± 0.10	3.18 ± 0.21**
3	PCM + Standard Silymarin (100 mg/kg) treated	43.57 ± 0.39	53.51 ± 1.90**	68.42 ± 2.03	74.78 ± 1.18**	142.9 ± 1.98	188.28 ± 5.27**	0.24 ± 0.06	0.26 ± 0.06**
4	PCM + BM suspension (300 mg/kg) treated	42.98 ± 0.84	57.4 ± 1.16**	69.23 ± 0.67	97.53 ± 2.44**	143.22 ± 1.57	208.79 ± 5.42**	0.38 ± 0.18	0.43 ± 0.10**
5	PCM + BM suspension (600 mg/kg) treated	42.76 ± 2.03	55.35 ± 0.69**	71.32 ± 1.41	85.95 ± 1.79**	141.92 ± 0.99	196.72 ± 2.45**	0.31 ± 0.12	0.33 ± 0.12**

SGPT: Serum glutamic-pyruvic transaminase; SGOT: Serum glutamic-oxaloacetic transaminase; ALP: Alkaline phosphatase; BIT: Bilirubin total levels; PCM: Paracetamol (2 g/kg BW daily); BM: *Balsamodendron mukul*.

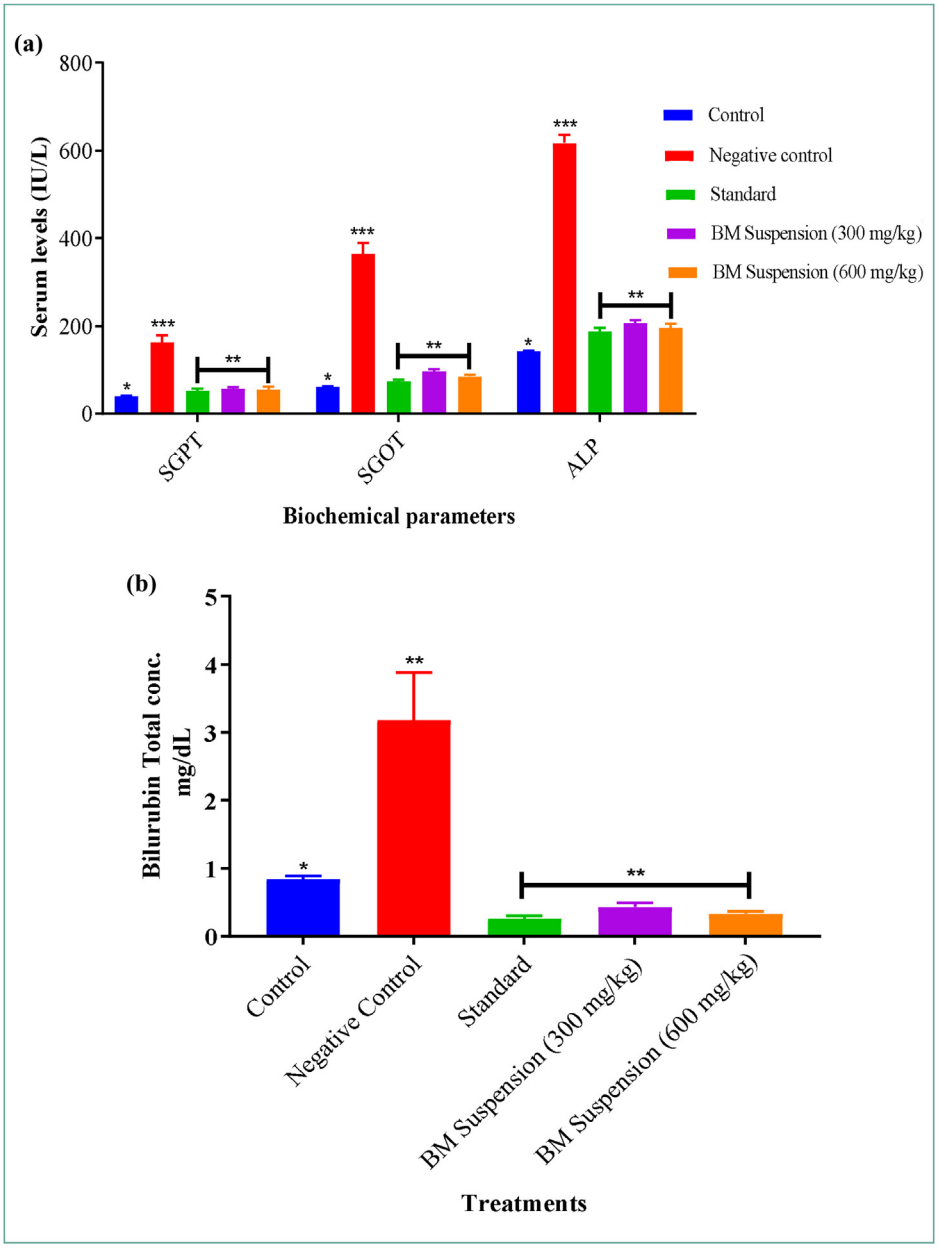

Figure 2. Effect of different treatments on hepatic serum biochemical parameters (a) SGPT, SGOT and ALP, and (b) total bilirubin concentration in PCM-induced hepatotoxic rats. Statistically significant differences were found $^{**}P < 0.01$ and $^{***}P < 0.005$ when compared with the control group.

Effet de différents traitements sur les paramètres biochimiques sériques hépatiques (a) SGPT, SGOT et ALP, et (b) concentration totale de bilirubine chez des rats hépatotoxiques induits par PCM. Des différences statistiquement significatives ont été trouvées $^{**}p < 0{,}01$ et $^{***}p < 0{,}005$ par rapport au groupe témoin.

treatment of PCM-induced hepatotoxicity and could be considered as an alternative therapy for the liver.

Discussion

B. mukul extract exhibited various medicinal activities viz anti-cancer, antioxidant, antiviral, antibacterial, anti-inflammatory, and hepatoprotective. It is highly effective against hydroxyl radical and lipid peroxidation activities. Liver participates in a variety of metabolic activities and hosts set of enzymes in this process it is exposed to many toxicants, chemicals and drugs with risk and impaired function. In the present study, paracetamol was used as a hepatotoxicants to induce liver damage. Recent study [5] indicates that oxidative stress is involved in the pathogenesis of liver diseases including drug induced hepatic damage, PCM-induced hepatitis and viral hepatitis or ischemic liver injury.

The *B. mukul* possesses antioxidant activity, which was assessed by hydroxyl radical scavenging and lipid peroxidation inhibiting activities, which are comparable with standard ascorbic acid. Outcomes of antioxidant study

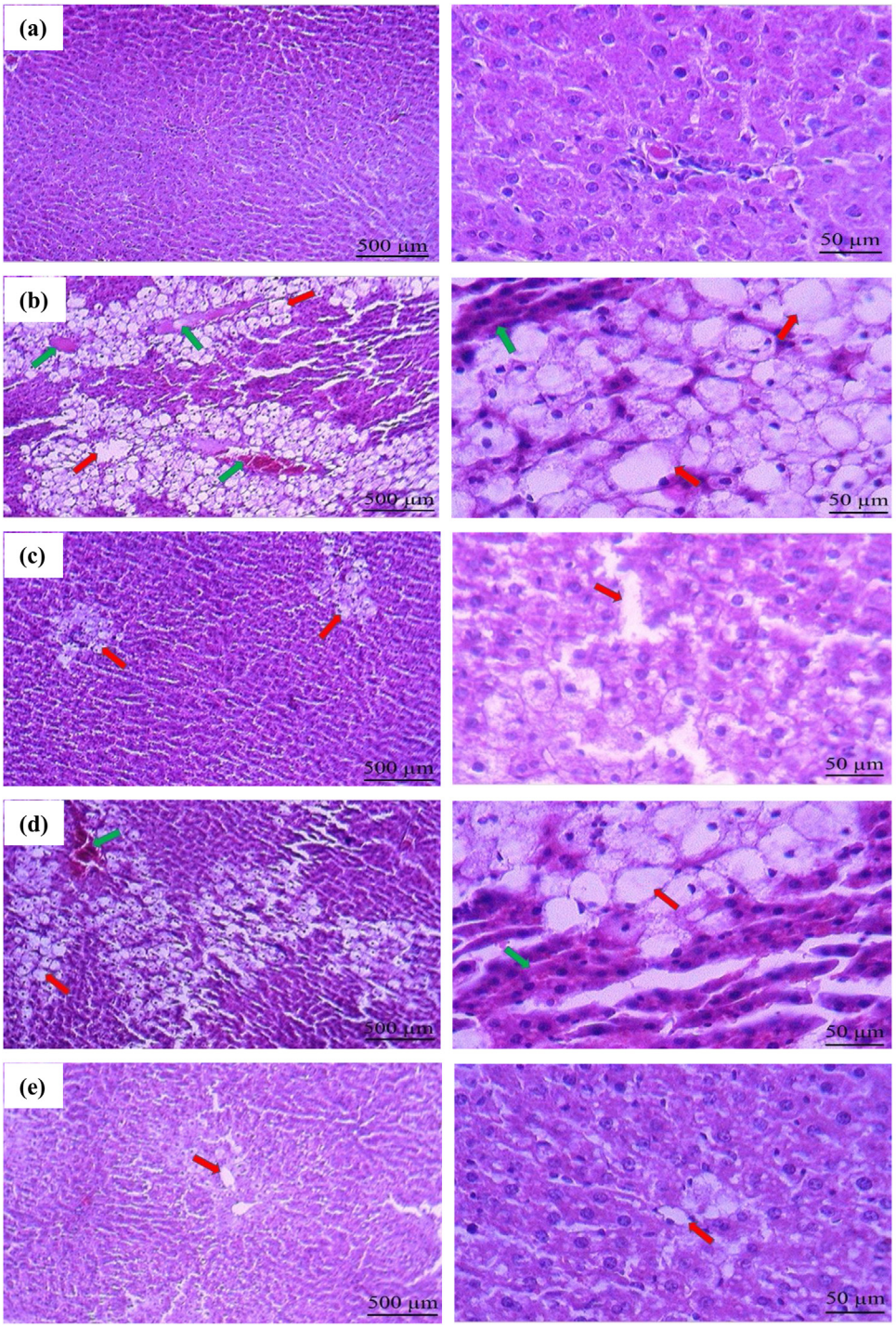

Figure 3. Treated histopathological sections of rat hepatic tissues revealing normal pathological signs in control animals (a), the negative control group (b) showed congested hepatic vein (green arrows) and severe degeneration of hepatic cells (red arrows), standard treated group samples (c) showed little degeneration of hepatic cells (red arrows), BM suspension treated samples (d) showed moderate congestion in hepatic vein (green arrows) and moderate degeneration of hepatic cells (red arrows), and BM suspension treated group (e) showed normal degeneration of hepatic cells (red arrows).
Coupes histopathologiques traitées de tissus hépatiques de rat révélant des signes pathologiques normaux chez les animaux témoins (a), le groupe témoin négatif (b) a montré une veine hépatique congestionnée (flèches vertes) et une dégénérescence sévère des cellules hépatiques (flèches rouges), des échantillons de groupe traités standard (c) a montré une faible dégénérescence des cellules hépatiques (flèches rouges), les échantillons traités avec la suspension de BM (d) ont montré une congestion modérée dans la veine hépatique (flèches vertes) et une dégénérescence modérée des cellules hépatiques (flèches rouges), et le groupe traité avec la suspension de BM (e) a montré une normale dégénérescence des cellules hépatiques (flèches rouges).

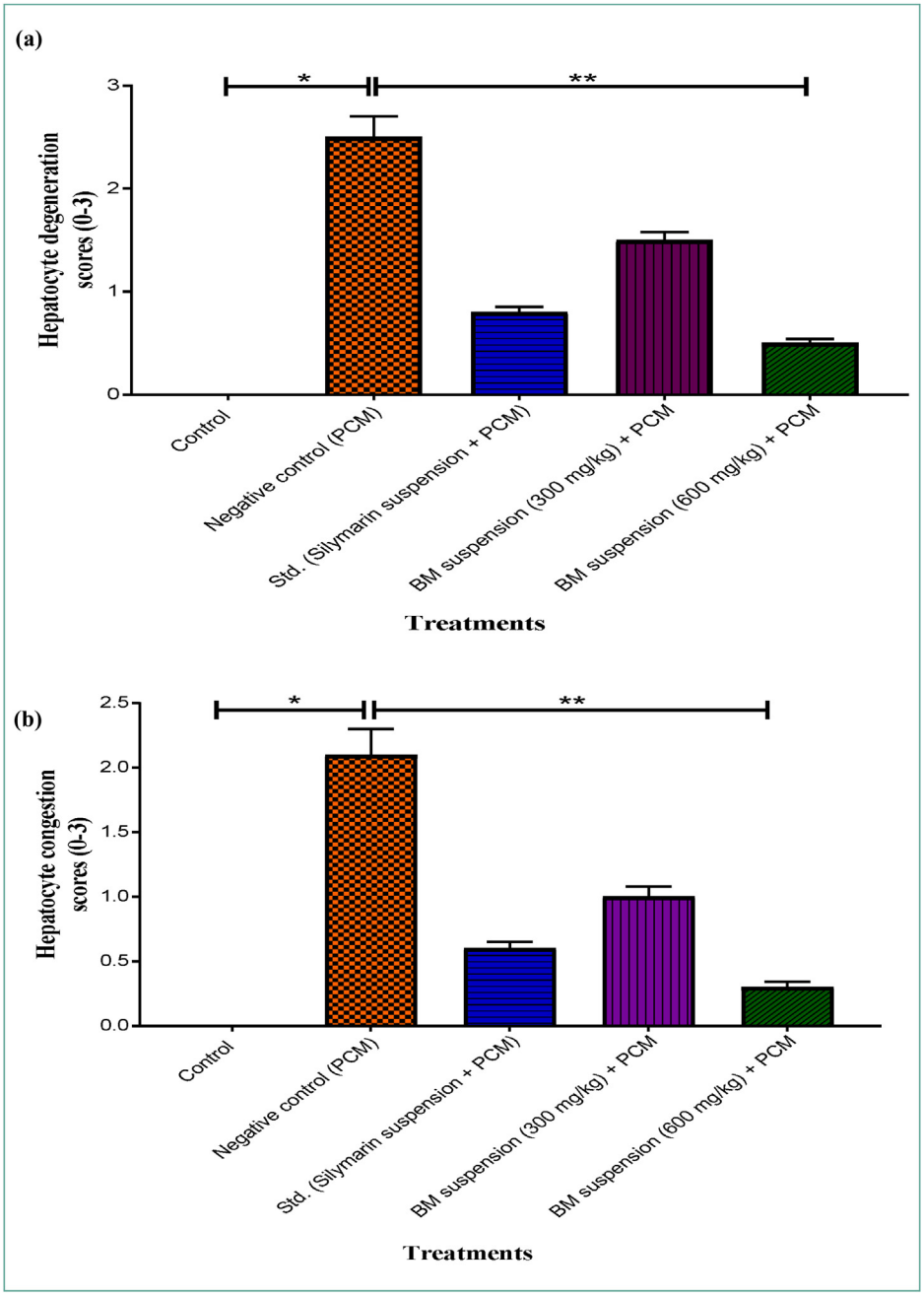

Figure 4. Statistical analysis of different hepatic samples for hepatocytic congestion (a) and hepatic degeneration (b) scores in the stained rat hepatic samples. Statistically significant differences were found *$P < 0.05$ and **$P < 0.01$ when compared with the control group.
*Analyse statistique de différents échantillons hépatiques pour les scores de congestion hépatocytaire (a) et de dégénérescence hépatique (b) dans les échantillons hépatiques de rat colorés. Des différences statistiquement significatives ont été trouvées *p < 0,05 et **p < 0,01 par rapport au groupe témoin.*

confirmed the hepatoprotective effect of *B. mukul* extract due to its free radical scavenging and lipid peroxidation inhibiting activities.

The increased level of AST, ALT, ALP, and bilirubin is conventional indicator of liver injury. Oxidative stress is one major factor in etiology of PCM-induced injury. Whereas, PCM-induced hepatotoxicity based on the conversion of reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQI), which causes glutathione depletion and the generation of PCM protein adducts, which initiates the hepatotoxicity. The function of the respiratory chain is altered by adduct formation on mitochondrial proteins, which resulting a rise in the amounts of free radicals like superoxide. However, excessive reactive oxygen species i.e., superoxide are highly responsible for liver injury, which is further converted into hepatitis.

The studies with *B. mukul* extract were evaluated in PCM-induced liver toxicity by the above-mentioned biochemical parameters. Acute administration of PCM in negative control group exhibited marked elevation of the serum biochemical parameters when compared with control group. Treatment with *B. mukul* extract at doses of 300 mg/kg and 600 mg/kg

Table 3 Average percentage changes in selected serum biochemical parameters into PCM-induced hepatotoxic rats, data are expressed as mean ± SD ($n = 6$). Various superscript symbols indicate significant difference (**$P < 0.01$) when compared with control and negative control groups.
*Variations moyennes en pourcentage des paramètres biochimiques sériques sélectionnés chez les rats hépatotoxiques induits par PCM, les données sont exprimées en moyenne ± ET ($n = 6$). Divers symboles en exposant indiquent une différence significative (**$p < 0,01$) par rapport aux groupes de contrôle et de contrôle négatif.*

Group	Treatment	SGPT	SGOT	ALP	BIT
1	Control	0.47 ± 2.56	1.06 ± 1.52	0.45 ± 0.85	12.16 ± 15.71
2	Negative Control (PCM, 2 g/kg daily)	268.67 ± 12.92**	464.75 ± 11.32**	345.09 ± 4.83**	374.6 ± 52.12**
3	PCM + Standard Silymarin (100 mg/kg) treated	22.81 ± 4.25**	9.29 ± 4.07**	31.76 ± 4.06**	6.67 ± 4.86**
4	PCM + BM Suspension (300 mg/kg) treated	33.55 ± 3.88**	40.88 ± 3.84**	45.75 ± 4.26**	13.16 ± 9.91**
5	PCM + BM Suspension (600 mg/kg) treated	29.44 ± 2.04**	20.51 ± 18.08**	36.61 ± 1.57**	7.10 ± 6.74**

SGPT: Serum glutamic-pyruvic transaminase; SGOT: Serum glutamic-oxaloacetic transaminase; ALP: Alkaline phosphatase; BIT: Bilirubin total levels; PCM: Paracetamol (2 g/kg BW daily); BM: *Balsamodendron mukul*.

resulted in a dose-dependent decrease in PCM-induced hepatic biochemical parameters. Simultaneously, the standard Silymarin (100 mg/kg) significantly prevented such rises in serum biochemical parameters.

Histopathological findings proved the *B. mukul* extracts (300 mg/kg and 600 mg/kg) treated animal groups reduced severe hepatic vein congestion against PCM-induced hepatotoxicity. Animals treated with *B. mukul* extracts and Silymarin (100 mg/kg) were mild congestion, degeneration, and inflammation than the negative control group according to the histological investigation. Therefore, this research shows that naturally biodegradable and safe *B. mukul* extract has the potential to treat liver toxicity induced by paracetamol.

Conclusion

BM extracts exhibited significant hepatoprotective activity against PCM-induced liver toxicity in rats. *B. mukul* tends to reduce and control the different unwanted chemical responses such as serum SGPT, SGOT, ALP, and BIT in the liver. PCM induced a noxious impact on the healthy liver cells, which causes the activation of reactive oxygen species (ROS). ROS has further responsible for liver toxicity. *B. mukul* extract also inhibits the activation of ROS, hydroxyl radical scavenging, and lipid peroxidation inhibiting, which proved its antioxidant activities. Thereby, the natural bioactive compounds with antioxidant properties could be played a significant role in PCM-induced/caused hepatotoxicity. Therefore, this investigation has proved that the natural biodegradable and safe *B. mukul* extract has a potential to treat Paracetamol-induced liver toxicities. Commercial and future prospect of this study requires inspecting the industrial scalability and feasibility of natural bioactive compound i.e., *B. mukul* extract.

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Disclosure of interest

The authors declare that they have no competing interest.

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