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ORIGINAL ARTICLE

Ameliorative Effect of *Rumex pictus* extract on Paracetamol induced Hepatotoxicity in Rats

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ABSTRACT
The aim of the present study was to investigate the potential hepatoprotective activity of Rumex pictus (R. pictus) in a rat model of paracetamol (PCM)–induced liver damage. Liver injury was induced by PCM administration as a single dose(2 g/kg, orally). Wistar albino rats were administered with total ethanolic extract of R. pictus(100, 200 and 400 mg/ kg, p.o.) for 7 days. The animals were evaluated for various biochemical and histopathological studies.PCM administration caused severe hepatic damage in rats as evidenced by elevated serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (γ-GT) and serum level of total bilirubin (BRN), while decreased serum level of total protein (TP) and albumin (ALB). In liver homogenates, PCM elevated malondialdehyde (MDA) but decreased glutathione (GSH) level as well as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities.Administration of total ethanolic extract of R. pictus at100, 200 and 400 mg/ kg for 7 days before PCM inhibited the acute elevation of the serum activities of ALT, AST, ALP and γ-GT enzymes and level of BRN. Both doses of the extract increased the serum level of TP and ALB and attenuated PCM-induced lipid peroxidation. The tested extract increased the activities of the antioxidant enzymes (SOD, GPx and CAT) in the liver homogenates in addition to GSH concentration. Liver histopathology supported the biochemical findings. It was concluded that R. pictus possesses hepatoprotective activity that could be partly attributed to its antioxidant effect.
Keywords: *Polygonaceae, liver damage, paracetamol, hepatoprotection, malondialdehyde.*

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INTRODUCTION

Liver is a vital organ that has a wide range of functions, including detoxification, plasma protein synthesis, and production of biochemical necessary for digestion. Liver ailments remain a serious health problem.[1] Hepatotoxicity, an injury to the liver, is associated with impaired liver function caused by exposure to drugs, chemicals or noninfectious agents.[2]Several chemicals have been known to induce hepatotoxicity by producing the reactive species.PCM is an extensively used analgesic and antipyretic drug and is associated with significant hepatotoxicity when taken in overdose.[3] PCM-induced liver damage is a widely used model that resembles human liver failure. The hepatotoxicity of PCM has been attributed to the formation of toxic highly reactive metabolite n-acetyl parabenzoquineimine (NAPQI).
Conventional and synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is therefore necessary to search for alternatives to replace currently used drugs of doubtful efficacy and safety. Many natural products of herbal origin are in use for the treatment of liver ailments.[4]

The genus *Rumex*, belonging to the Polygonaceae includes more than 250 species, which are distributed worldwide.[5] Most Polygonaceae are perennial herbaceous plants with swollen nodes, but trees, shrubs and vines are also present. Plants of the genus Rumex are widely used as medicinal herbs and foods. They contain anthraquinones such as emodin and chrysophanol as active ingredients.[6] For centuries, *Rumex* spp. have been used in folk medicine for treating a wide range of ailments including; colds, sore throat, indigestion, scurvy, as well as a cooling drink for fevers. Also, they have been used to treat cancer, rheumatism, liver disorders, foul ulcers and skin conditions.[7] Moreover, roots have been made into poultices for treating nettle and bee stings and other inflammations.[8]*Rumexpictus* Forssk. "Veined dock" is a species that grow wild in Syria, Arabia and the Mediterranean coast Egypt.[9] It is an annual glabrous herb, 10-30 cm, stems decumbent and richly branched at the base. *R. pictus* is characterized by diversity of phenolic classes such as tannins, flavonoids, anthocyanins and anthraquinones.[10]No information has been found reporting the biological activity of *R. pictus* and very little information has been reported on its chemical composition.The present study investigates the potential antioxidant and hepatoprotective activities of *R. pictus* extract against PCM-induced hepatotoxicity in rats.

MATERIAL AND METHODS

Plant material

The whole well-grown *R. pictus* plant was collected from Hail region of Saudi Arabia, during March 2014.The sample was kindly identified and authenticated by an expert taxonomist. The collected plant material was air-dried in shade, reduced to fine powder, packed in tightly closed containers and stored at room temperature for biological studies.

Preparation of plant extract

The air-dried powder of *R. pictus* aerial parts (1 kg) was extracted by percolation in 70% aqueous ethanol at room temperature with occasional shaking for 2 days. The ethanol extract was filtered and the residues were re-percolated for four times. The total ethanol extract was concentrated under reduced pressure at a temperature not exceeding 35°C to yield a dry extract of 190 g. The total ethanolic extract of *R. pictus*was stored in the refrigerator and aliquot of the concentrations were prepared immediately before use.

Animals

Adult male albino rats of Wistar strain (180-200 g) were obtained from Lab Animal Care Unit, Pharmacy College, and Prince Sattam bin Abdulaziz University, Al-Kharj, KSA. The animals were housed in polypropylene cages and maintained at 22±2°C and light/dark (12/12 h) cycles. They were allowed free access to standard pellet diet and water *ad libitum*. The animals were allowed to acclimatize to the laboratory condition for one week before commencement of the experiment.

Acute toxicity study

Acute toxicity study for *R. pictus*extractwas carried in adult male Albino rats according to OECD-423 guidelines [11]. Animals were kept fasting providing only water, after which *R. pictus*extractwas administered orally by gastric tube in different gradual doses (1000-4000 mg/kg), and observed for any toxic symptoms and mortality for 72 h.

Hepatoprotective activity

Male rats were divided into 6 groups (n=6). Group I (normal control) and Group II (hepatotoxic control) received the vehicle (5 mL/kg b.wt). Group III received the reference drug, silymarin (50 mg/kg). Groups IV, V and VI received the ethanolic extract of*R. pictus*at doses of 100, 200 and 400 mg/kg, respectively. Rats of groups II-VI received a high dose of PCM (2 g/kg) as a single dose after 30 min of 7-day administration of the tested extract. The vehicle, silymarin, *R. pictus*extract and PCM were administered orally. After 48 h of PCM administration, rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Blood samples were collected from all rats by puncturing retro-orbital plexus into dry centrifuge tube and allowed to coagulate for 30 min at 37°. The clear sera obtained after centrifugation (3000 rpm for 15 min) were used for further biochemical estimation. After blood collection, all rats were sacrificed by cervical decapitation and livers were dissected out and divided into two portions. The first portion was kept in liquid nitrogen for estimation of the antioxidant status and the second part was fixed in buffered formalin 10% for histopathological examination.

Assessment of liver marker enzymes and biochemical parameters

The serum activities of liver marker enzymes **(**ALT, AST, ALP and γ-GT) and the biochemical parameters, such as total protein (TP) albumin (ALB) and BRN were estimated according to the instructor manual of commercially available kits.

Preparation of liver homogenate and determination of in vivo antioxidant status

Hepatic tissues were homogenized in 10% w/v 0.1 M phosphate buffer or 0.1 M tris buffer (pH 7.0) and centrifuged at 12,000·g for 10 min. The supernatant was used for the measurement of liver enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants were determined by estimating SOD [12], GPx[13] and CAT[14] activities in the hepatic tissue homogenate of all rats Moreover, the non-enzymatic antioxidants were determined by estimating the levels of MDA[15] and GSH[16] in the liver homogenate.

Histopathological examination

Liver samples were fixed in buffered formalin 10%, processed routinely, and embedded in paraffin. 5 μm thick sections were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation. The stained sections were examined and photographed under a light microscope.

Statistical analysis

The values are expressed as mean ± standard error of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Dunnett's test to determine the intergroup variability by using SPSS ver. 14.0. A comparison was made with the normal control and PCM-hepatotoxic groups. We took a P-value of <0.05 as our desired level of significance.

RESULTS

Acute toxicity study

The total ethanolic extract of *R. pictus* was subjected to acute toxicity testing in adult male Albino rats and animals were monitored for 72 h. The extract was found to be practically nontoxic when administered orally to rats at doses up to 4g/ kg. No mortalities observed with oral administration during 72 h of observation even at the highest dose. *R. pictus* extract did not produce any symptom of acute toxicity and none of the rats exhibit hyperactivity, convulsions, sedation, hypothermia, and respiratory distress. The oral LD_{50}value for *R. pictus* extract was indeterminable being in excess of 4 g/kg b. wt. and hence $1/40^{th}$, $1/20^{th}$ and $1/10^{th}$of the maximum dose administered (i.e. 100, 200 and 400 mg/kg, p.o.) were selected for the present study.

Hepatoprotective activity

No statistical difference in any parameters between hepatotoxic rats medicated with the ethanolic extract of *R. pictus* at 100 mg/ kg and PCM control rats was observed.

Effect on liver marker enzymes and biochemical parameters

The serum activities of liver marker enzymes (ALT, AST, ALP and γ-GT) and BRN levels after 48 h of oral administration of PCM at the dose of 2.0 g/kg are depicted in Tables 1 and 2. PCM-intoxicated rats developed significant hepatic damage as indicated by a significant increase in the activities of ALT, AST, ALP and γ-GT enzymes and BRN level by 93.85%, 140.34%, 146.12%, 114.22% and 69.44%, respectively compared to normal control rats. The serum of PCM-intoxicated group showed decrease in TP and ALB levels in comparison to the normal control rats. Treatment of rats with the total ethanolic extract of *R. pictus* (200 and 400 mg/kg) for 7 days before PCM administration restored serum activities of ALT, AST, ALP, γ-GT and levels of BRN, TP and ALB towards their normal values. Pretreatment with the standard hepatoprotective agent-silymarin also decreased all measured serum biochemical activities towards normalness.The efficacy of *R. pictus* extract was comparable with that of the standard drug silymarin and its activity was found to be dose dependent.

Effect on in vivo antioxidant status

Administration of PCM at the concentration of 2 g/kg to the rats caused a significant decline in the activities of SOD (-58.66%), GPx (-60.00%) and CAT (-42.51%) and level of GSH (-55.20%) in their liver homogenates as compared to normal control group. The results are shown in Table 3. The activities of these enzymes and level of GSH were significantly restored towards their normal values in rats pre-medicated with silymarin, or *R. pictus* extract (200 and 400 mg/kg) for 7 days prior to PCM administration. At 200 and 400 mg/kg of *R. pictus* extract, the recovery of enzyme activities recorded 90.04% and 96.26% (for SOD), 73.94% and 106.33% (for GPx) and 41.09% and 58.90% (for CAT), respectively compared to the normal control values. The levels of MDA as an index of lipid peroxidation in liver homogenate of PCM-control rats were significantly elevated by 170.33% when compared to normal control animals. Pretreatment with *R. pictus* (200 and 400 mg/kg) extract significantly reduced the MDA level in the liver tissues of PCM-medicated rats.

Histopathological analysis

The histological structure of liver in normal control rats showed normal hepatocytes with central vein and sinusoidal dilation (Figure 1-A). In contrast, the light microscopic study of PCM-hepatotoxic control rats revealed pathological changes of liver represented by cytoplasmic vacuolizations of hepatocytes, diffuse necrosis, and destruction of the lobular architecture and collections of inflammatory cells (Figure

1-B). The severity of these injuries was alleviated markedly in hepatotoxic rats treated with *R. pictus* extract (Figure 1-C) in a dose-dependent manner.

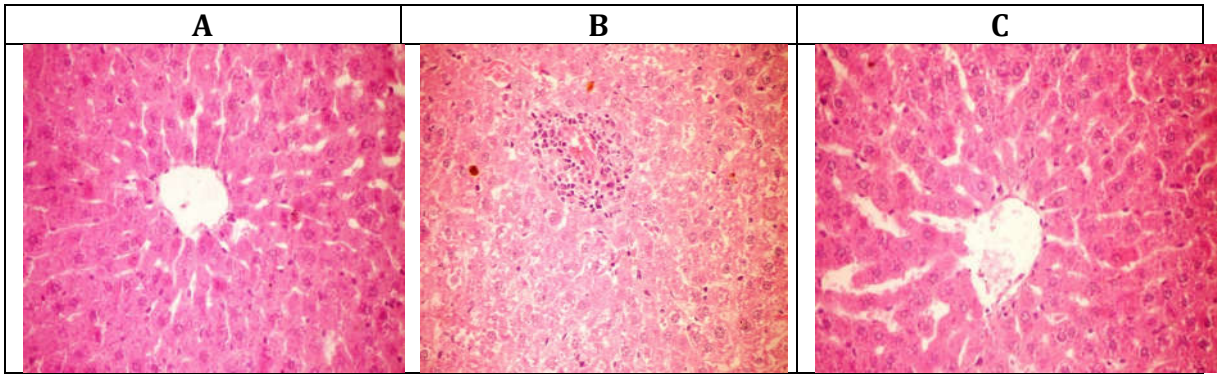

Fig 1: Photomicrographs of liver tissues of rats from different experimental groups. (A) Control rat showsnormal hepatic parenchymal architecture. (B) PCM-hepatotoxic control rat shows pathological changes of liver represented by cytoplasmic vacuolizations of hepatocytes, diffuse necrosis, and destruction of the lobular architecture and collections of inflammatory cells (C) PCM-hepatotoxic rat treated with *R. pictus* (400 mg/kg) shows features similar to the control and most of the hepatocytes were intact with no alteration except few vacuoles. (H & E, x200).

Table 1: Effect of ethanol extract of *R. pictus*on serum activity of liver marker enzymes in rats with PCM- induced hepatotoxicity.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	γ-GT (U/L)
Normal Control	59.86 ±1.25ǂ	86.83±2.56ǂ	112.63±3.26ǂ	13.85±0.26ǂ
PCM-hepatotoxic Control	116.04±3.27* (93.85%)	208.69±4.57* (140.34%)	277.21±4.38* (146.12%)	29.67±0.96* (114.22%)
Silymarin (50 mg/kg) +PCM	61.83±1.14ǂ (-46.71%)	91.27±2.20ǂ (-56.26%)	134.4±3.24ǂ (-51.51%)	16.40±0.45ǂ (-44.72%)
R. pictus(100 mg/kg) +PCM	110.15±3.17* (-5.07%)	203.64±4.33* (-2.41%)	272.57±4.26* (-1.67%)	28.42±0.58* (-4.21%)
R. pictus(200 mg/kg) +PCM	86.14±2.74*ǂ (-25.76%)	154.27±3.83*ǂ (-26.07%)	163.75±4.15*ǂ (-40.92%)	20.07±0.42*ǂ (-32.35%)
R. pictus(400 mg/kg) +PCM	75.55±2.64*ǂ (-34.89%)	129.25±3.28*ǂ (-38.06%)	155.95±4.16*ǂ (-43.74%)	19.68±0.66*ǂ (-33.67%)

Values are mean ± SEM, values between brackets means % changes(n=6);*P<0.05 as compared to normal control group, ǂP<0.05 as compared to hepatotoxic control group
% changes of PCM-hepatotoxic control group were compared to normal control group.
% changes of silymarin and extract-treated groups were compared to PCM-hepatotoxic control group.

Table 2: Effect of ethanol extract of *R. pictus*on serum levels of TP, ALB and BRN in rats with PCM- induced hepatotoxicity.

Groups	TP (g/dL)	ALB (g/dL)	BRN (mg/dL)
Normal Control	7.82±0.11ǂ	3.68±0.07ǂ	0.72±0.02ǂ
PCM-hepatotoxic Control	5.08±0.07* (-35.03%)	2.37±0.04* (-35.59%)	1.22±0.09* (69.44%)
Silymarin (50 mg/kg) +PCM	7.25±0.13ǂ (42.71%)	3.46±0.09ǂ (45.99%)	0.76 ±0.08ǂ (-37.70%)
R. pictus (100 mg/kg) +PCM	5.24±0.13* (3.14%)	2.58±0.06* (8.86%)	1.12±0.07* (-8.19%)
R. pictus (200 mg/kg) +PCM	6.35±0.15*ǂ (25.00%)	2.95±0.09*ǂ (24.47%)	0.82±0.06*ǂ (-32.78%)
R. pictus (400 mg/kg) +PCM	6.68±0.18*ǂ (31.49%)	3.18±0.04*ǂ (34.17%)	0.81±0.07*ǂ (-33.60%)

Values are mean ± SEM, values between brackets means % changes (n=6); *P<0.05 as compared to normal control group, ǂP<0.05 as compared to hepatotoxic control group
% changes of PCM-hepatotoxic control group were compared to normal control group.
% changes of silymarin and extract-treated groups were compared to PCM-hepatotoxic control group.

Table 3: Effect of ethanol extract of *R. pictus*on hepatic antioxidant profile, glutathione (GSH) and lipid peroxidation (MDA) in liver homogenate of rats with PCM- induced hepatotoxicity.

Groups	SOD (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)	GSH (µmol/ g tissue)	MDA (nmol/g tissue)
Normal Control	58.3±2.35ǂ	3.55±0.16ǂ	12.7±0.21ǂ	12.5±0.12ǂ	35.4±1.54ǂ
PCM-hepatotoxic Control	24.1±0.64* (-58.66%)	1.42±0.05* (-60.00%)	7.3±0.16* (-42.51%)	5.6±0.14* (-55.20%)	95.7±2.35* (170.33%)
Silymarin (50 mg/kg) +PCM	53.5±1.73ǂ (121.99%)	3.16±0.14ǂ (122.53%)	11.3±0.12ǂ (54.79%)	10.8±0.18ǂ (92.85%)	41.4±0.22ǂ (-56.73%)
R. pictus (100 mg/kg) +PCM	27.3±0.96* (13.27%)	1.55±0.11* (9.15%)	8.5±0.16* (16.43%)	6.2±0.13* (10.71%)	90.4±2.26* (-5.53%)
R. pictus (200 mg/kg) +PCM	45.8±1.74*ǂ (90.04%)	2.47±0.18*ǂ (73.94%)	10.3±0.18*ǂ (41.09%)	8.8±0.11*ǂ (57.14%)	61.7±1.50*ǂ (-35.52%)
R. pictus (400 mg/kg) +PCM	47.3±1.55*ǂ (96.26%)	2.93±0.17*ǂ (106.33%)	11.6±0.18*ǂ (58.90%)	9.6±0.18*ǂ (71.42%)	53.3±1.24*ǂ (-44.30%)

Values are mean ± SEM, values between brackets means % changes (n=6); **P*<0.05 as compared to normal control group, ǂ*P*<0.05 as compared to hepatotoxic control group

% changes of PCM-hepatotoxic control group were compared to normal control group.

% changes of silymarin and extract-treated groups were compared to PCM-hepatotoxic control group.

DISCUSSION

Evaluation of the potential toxicity of natural products is usually an initial step in screening for their pharmacological activities. In our study, oral administration of *R. pictus* extract at doses up to 4000 mg/kg did not produce any sign of acute toxicity and none of animals died during 72 h of observation. Accordingly, it suggested that its LD_{50} value was higher than 4 g/kg b.wt. In general, the higher the LD_{50} value, the lower toxic the compound. Therefore, the tested extract can be categorized as highly safe since substances possessing LD_{50} higher than 50 mg/kg are non-toxic [17]. In addition, substances with LD_{50} values higher than 4000 mg/kg by oral route are regarded as being safe or practically nontoxic.[18]

The primary function of the liver is to maintain body homeostasis, besides it plays a key role in metabolism, detoxification, and inflammatory response.[19] Therefore, damage to the liver inflicted by a hepatotoxic agent is of grave consequence. PCM is often considered as a safe painkiller drug, even though, overdoses of this drug lead to acute liver failureand hepatic cytolysis [20. Liver injury due to PCM in rats used as a successful experimental model to evaluate the efficacy of hepatoprotective agents [21].

The liver marker enzymes (ALT, AST, ALP and γ-GT), which are present in higher level in hepatocytes have still remained the gold standards for the assessment of liver injury [20]. Administration of PCM causes elevation of serum ALT, AST, ALP and γ-GT and BRN levels in rats. Due to liver injury caused by PCM overdose, the transport function of the hepatocytes gets disturbed resulting in the leakage of the plasma membrane,[20] thus causing an increase in serum enzyme levels. In addition, the elevated serum level of BRN is the usual indicator of hepatotoxicity.[22]The abnormal level of BRN in serum of PCM-intoxicated rats could be attributed to impaired hepatic clearance due to hepatic parenchymal damage and biliary obstruction.[23] This study demonstrated that *R. pictus* extract and silymarinhad reduced levels of liver marker enzymes and BRN level which were elevated by PCM administration. The return of ALT, AST, ALP and γ-GT activities toward normal may be due to the inhibitory effect of the tested extract on cytochrome P450 to reduce the production of the reactive metabolite (NAPQI) of PCM. This may result in stabilization of cellular membrane so preserving the structural integrity of hepatocytes as well as the restoration of hepatic injury induced by PCM.

ALB is the most abundant plasma protein produced by hepatocytes. Therefore, variation of serum TP or ALB levels can reflect liver health status.[24] In our study, the reduction in serum TP and ALB in PCM-control rats may be due to binding of the reactive metabolite of PCM (NAPQI) to the amino acid cysteine in proteins, forming PCM protein adducts.[25]Pretreatment with *R. pictus* extract showed a significant reversal of TP and ALB toward their normal levels and suggested the stabilization of endoplasmic reticulum that are responsible for protein synthesis. This assures the protective activity of *R. pictus* extract against PCM-hepatotoxicity.

The results of enzymatic antioxidants such as SOD, GPx and CAT and non-enzymatic antioxidants such as GSH and MDA indicate that the extract is non-toxic and safe.The formation of the highly reactive metabolite (NAPQI) by the hepatic cytochrome P450 is the first step in development of PCM hepatotoxicity.[26] NAPQI leads to oxidative stress that has been thought to be the major mechanism for PCM- hepatotoxicity. The body has an effective mechanism to prevent and neutralize the free radical

induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, GPx and CAT.In our study, administration of PCM at 2 g/kg to the rats reduced SOD, GPx and CAT activities in their liver homogenate. The pretreatment of rats with *R. pictus* extract (200 and 400 mg/kg) for 7 days provided protection against the depletion in activities of the anti-oxidant enzymes. This shows that *R. pictus* extract protects rats against oxidative injury by maintaining the levels of these enzymes even after PCM treatment.

GSH is widely distributed in hepatocytes for detoxification of free radicals. It plays a major role to protect cells against free radicals, peroxides and other toxic compounds.[27] GSH depletion increases the sensitivity of cells to various aggressions leading to tissue disorder and injury.[28]Accordingly, depletionof GSH is considered as one of the main biochemical markers for PCM-caused hepatotoxicity. In the present investigation, we have observed that GSH level was depleted significantly in the liver homogenate of PCM-treated rats compared to normal control group. PCM is primarily metabolized in the liver by glucuronidation and sulfation. A small proportion of the drug is metabolized by several of the cytochrome P450 enzymes into the reactive intermediate Nacetyl-p-benzoquinoneimine (NAPQI), which is normally detoxified by glutathione (GSH). In PCM-overdose, sulfation and glucuronidation become saturated and GSH is depleted by NAPQI.[29] The reduced levels of GSH which were observed in the liver homogenates of PCM-exposed rats were significantly restored towards normal values by treatment with *R. pictus* (200 and 400 mg/kg) extract.

Most hepatotoxic chemicals damage liver by inducing, directly or indirectly, lipid peroxidation. The cytotoxic aldehyde; MDA is one of the end products in the lipid peroxidation process.[30]Lipid peroxidation can reduce membrane fluidity and inactivate membrane-bound proteins. The inhibition of lipid peroxidation is thus a crucial property of antioxidant compounds. The mode of action of PCM on the liver is by covalent binding of its toxic metabolite, n-acetyl-p-benzoquinone-amine to the sulfhydryl group of protein resulting in cell necrosis and lipid peroxidation.[31]In our study elevation in levels of end products of lipid peroxidation in liver of rats treated with PCM were observed. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage.[32] Treatment with *R. pictus* significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of extract is due to its antioxidant effect.

Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress.[33]In accordance with these results, the protective effect of *R. pictus* extract against PCM may be attributed to the presence of phytochemicals. Flavonoids are considered as most responsible functional groups having antioxidant potential in plant source.[34]

Histopathological examination of rat liver treated with PCM showed marked hepatotoxicity characterized by diffuse necrosis, and destruction of the lobular architecture. It was also noted that the histopathological damage induced by PCM was improved in rat liver treated with the ethanolic extract of *R. pictus*. Extract treated groups showed normalization of the pathological changes of liver. The maximum protection against hepatic damage was achieved with *R. pictus* extract at a dose of 400 mg/kg.

CONCLUSIONS

In conclusion, the present study demonstrates that *R. pictus* has hepatoprotectiveactivity which is mainly attributed to its antioxidant property. The antioxidanteffect may be attributed, at least in part, to the presence of flavonoidsin the extract. Further detailed investigations are needed in order to identify and isolate the hepatoprotective components in the extract and to justify its use in the treatment of liver disorders.

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