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Thioacetamide-induced acute liver toxicity in rats treated with *Balanites roxburghii* extracts

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ABSTRACT

Objective: To evaluate the antioxidant and thioacetamide induced liver toxicity using extracts of aerial parts of *Balanites roxburghii* (*B. roxburghii*).

Methods: The *in-vitro* antioxidant activity was estimated for different extracts on superoxide, hydroxyl and 1,1- diphenyl-2-picrylhydrazyl free radicals and the hepatoprotective activity of the selected plant extracts was evaluated by using thioacetamide-induced liver intoxication in rats model.

Results: There were no visible signs of toxicity, mortality and no behavioral changes were observed for the selected plant extracts (hydroalcoholic, ethyl acetate and hexane) at 2000 mg/kg body weight. The plant extracts showed dose-dependent effectiveness in the inhibition of free radicals generation and hepatoprotective nature. The hydroalcoholic extract showed more scavenging activity as compared to other extracts with IC_{50} values of (332.50 ± 1.30) μg, (302.00 ± 1.50) μg and (230.40 ± 1.10) μg on superoxide, hydroxyl and 1,1- diphenyl-2-picrylhydrazyl free radicals. Aerial parts of *B. roxburghii* at three dose levels such as 125 mg/kg, 250 mg/kg and 500 mg/kg showed dose dependent percentage protection as standard drugs and on enhancement activities of serum biomarker enzymes (aspartate aminotransferase, alanine transaminase, alkaline phosphatase, total bilirubin and total protein) observed in thioacetamide-induced hepatotoxicity. The hydroalcoholic extract showed significant results at 500 mg/kg body weight as compared to other extracts ($P < 0.05$) with 65.65%, 62.39%, 59.89%, 55.90% and 54.61% on aspartate aminotransferase, alanine transaminase, alkaline phosphatase, total bilirubin and total protein levels.

Conclusions: It was observed that the *B. roxburghii* plant extracts are non-toxic and have capacity to restore the physiological changes caused by the hepatotoxic compounds.

1. Introduction

Liver is a very important organ in the body playing a vital role in the metabolic functions. Over the last few decades, the morbidity and mortality of different types of liver diseases have increased around the world[1]. Liver diseases include alcoholic, non-alcoholic, microorganisms, genetic variation (autoimmune) and finally drugs toxicity[2]. All these liver diseases affect persons of all ages, especially people with ages from 40 to 60 years, and cause the alterations in the normal physiological functions of the body[3]. The untreated liver diseases and long term usage of medication sometimes cause side effects and finally lead to death[4]. So, researches on liver diseases are critically important in these days to bring the major toll of liver diseases under control for human health.

Medicinal plants are used in the natural medicines such as Chinese traditional medicine, Indian Ayurveda and Unani medicine in middle east countries[5]. Many of the currently available drugs are derived either directly or indirectly from medicinal plants. In recent times, many plants have been used for a wide spectrum of liver diseases so far to recovery processes of the intoxicated livers[6]. But there are many medicinal plants available to provide scientific evidence for their biological activities which have been used in traditional

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All the experimental procedures involving animals were conducted according to OECD guidelines and approved by the institutional animal ethics committee, Andhra University.

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or herbal medicine for treating diseases including liver diseases[7]. One of those medicinal plants is *Balanites roxburghii* (*B. roxburghii*)[8].

B. roxburghii which is commonly known as hingot belongs to the family Zygophyllaceae. It is commonly found in sandy plain areas like Western Rajastan, West Bengal, Maharashtra and drier parts of India [9]. Different parts of the *B. roxburghii* have been used to treat different diseases in traditional medicine. For example, the fruits are used in the treatment of whooping cough, skin diseases and antifertility; leaves are used for the treatment of jaundice and the bark is used for swelling in body organs and inflammation and used to treat snake-bite and dog bite[9–12]. But there are few scientific evidences regarding its biological activities. So, the author selected the aerial parts of *B. roxburghii* to evaluate its antioxidant activity on superoxide, hydroxyl and 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radicals and the hepatoprotective activity against thioacetamide (TAA)-induced liver toxicity.

2. Materials and methods

2.1. Drug and chemicals

Chemicals used for the study were of analytical grade. Silymarin, TAA and DPPH were purchased from Sigma Chemicals, USA, Nitroblue tetrozolium was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. Riboflavin was purchased from Loba Chemie Pvt Ltd., Mumbai. The kits for the assessment of different biochemical parameters like aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin and total protein were purchased from Span Diagnostics Ltd., Gujarat, India.

2.2. Plant material and preparation of extracts

The plant material was collected at Andhra Pradesh, India on July, 2012. The authentication of the plant was done by Rtd. Prof. M. Venkaih, Department of Botany, Andhra University, Visakhapatnam. The plant material was dried under shade and powdered for extraction separately using maceration process with hexane, ethyl acetate and hydroalcoholic (ethanol 70%), and the extracted solvents were concentrated to dryness under vacuum using rotavapour.

2.3. In vitro antioxidant activity

In-vitro antioxidant activity was assessed by using the prepared extract of *B. roxburghii* using dimethyl sulphoxide as the vehicle on superoxide, hydroxyl and DPPH free radicals[13,14]. The percentage of inhibition and IC_{50} values were calculated.

2.3.1. Superoxide radical scavenging activity

The superoxide scavenging activity of the selected plant extracts was evaluated as per standard methods[13]. It was by the absorption of light at 560 nm induction of superoxide free radical generation by riboflavin and corresponding reduction by nitroblue tetrazolium.

2.3.2. Hydroxyl radical scavenging activity

The scavenging activity of selected plants extracts on hydroxyl radical was measured as per established methods[14]. It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe^{2+}/ethylene diamine tetraacetic acid/H_2O_2 system.

2.3.3. DPPH radical scavenging activity

The DPPH radical scavenging activity was measured as per methods[13,14]. This method is based on measure of color absorbance of alcoholic DPPH solution (blue color) after addition of antioxidant solution (extract/compound). If antioxidants present in the test compound blue color due to DPPH changes to yellow color.

2.3.4. Calculation of percentage inhibition

The percentage inhibition of the superoxide production by the extract was calculated using the formula:

$$\text{Inhibitory ratio} = (A_0 - A_1) \times 100/A_0$$

where A_0 was the absorbance of control and A_1 refered to the absorbance of plant extract or/and ascorbic acid.

2.3.5. IC_{50} calculation form percentage inhibition

The optical density obtained with each concentration of the extract/ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ascorbic acid.

2.4. Selection of animals

Healthy albino rats of either sex weighing between 180 and 250 g aging from 60 to 90 days were used for the study. The rats were taken care of at standard light and humidity by supplying proper food and water.

2.5. Acute toxicity studies

The acute toxicity study was conducted for *B. roxburghii* extracts as per Organisation for Economic Co-operation and Development (OECD) guidelines 420 (OECD. 2001) and regulations of the Institutional Animal Ethics Committee (No. 516/01/A/CPCSEA).The albino mice of single sex were selected into three groups ($n = 6$). They were maintained for one week before the experiment under room temperature and allowed free access to water and diet. The animals were subjected for acute toxicity study using each extract at a dose of 2000 mg/kg orally in 2 groups at regular intervals of time, *i.e.*, 1, 2, 4, 8, 12 and 24 h. During this time, the animals were under observation to note different conditions like skin changes, morbidity, aggressiveness, oral secretions, sensitivity to sound and pain, respiratory movements and finally their mortality.

2.6. Assessment of hepatoprotective activity of *B. roxburghii*

The selected plant extracts were tested for their hepatoprotective nature using TAA-induced liver toxicity in rats[15]. For this experiment, animals were grouped into twelve groups ($n = 6$). Animals in group I were treated with normal saline (vehicle) for one week through oral administration at 2 mL/kg body weight. Rats in group II and group III were treated with TAA as a 2% w/v solution in water on the first day at 50 mg/kg body weight subcutaneously, then group II was

continuously treated with saline and group III with silymarin at a dose of 25 mg/kg body weight orally for three weeks. Groups IV to XII were treated with TAA as a 2% w/v solution in water on the first day at 50 mg/kg body weight subcutaneously, then groups IV, V and VI were treated with the hydroalcoholic extract, and groups VII, VIII, IX were treated with the ethyl acetate extract and groups X, XI, XII were treated with the hexane extract at a doses of 125, 250, 500 mg/kg body weight orally for three weeks. Animals of all groups were anaesthetized using chloroform after 48 h of final dose administration of extracts. The blood samples were collected from animal groups by retro-orbital plexus, then samples were centrifuged at 2400 r/min for quarters of an hour. After centrifugation, clearly separated serum was used for measuring the different biochemical parameters using autoanalyzer with the help of reagent kits[16,17]. All the experimental procedures involving animals were conducted according to OECD guidelines and approved by the institutional animal ethics committee, Andhra University.

3. Results

3.1. Antioxidant activity

In the present study, the tested extracts of *B. roxburghii* were found to possess concentration dependent antioxidant activity on superoxide, hydroxyl and DPPH free radicals.

Superoxide anion radical (O_2^-) are formed by activated phagocytes, such as monocytes, macrophages, eosinophils and neutrophills, and the production of O_2^- is an important factor in the killing of bacteria by phagocytes. In the present method, superoxide anion was derived from riboflavin and reduced by nitroblue tetrazolium. The decrease in absorbance at 560 nm with tested extracts/compounds indicated the utilization of superoxide anion in the reaction mixtures. The mean IC_{50} values on superoxide radical for hydroalcoholic, ethyl acetate and hexane extracts of *B. roxburghii* were found to be (332.50 ± 1.30) μg, (356.00 ± 0.80) μg and (486.00 ± 0.62) μg, respectively. The mean IC_{50} value of ascorbic acid was found to be (54.40 ± 1.10) μg. The hydroalcoholic extract at a concentration of 640 μg showed the better scavenging activity on superoxide free radical (Table 1, Table 2 and Figure 1).

Hydroxyl radical is the main reactive oxygen species (ROS) with very short life span damaging every part of the living cell as compared to other ROS. These molecules sometimes bind with the nucleotides in the genetic materials of the body causing cancers, mutations and toxicity. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/ethylene diamine tetraacetic acid/H_2O_2 system (Fenton reaction). The mean IC_{50} values on hydroxyl radical of hydroalcoholic, ethyl acetate and hexane extracts of *B. roxburghii* were found to be (302.00 ± 1.50) μg, (260.0 ± 1.20) μg and (503.0 ± 0.76) μg, respectively. The mean IC_{50} value of ascorbic acid was found to be (68.00 ± 1.30) μg. The hydroalcoholic extract had better antioxidant activity on hydroxyl radical at 640 μg as compared to other extracts (Tables 2 and 3, Figure 1).

DPPH is a stable free radical having the highest absorption at 517 nm. DPPH is easily stabilized by combining with the electrons or hydrogen radicals. The antioxidants have the ability of donating hydrogen. This makes the reduction of absorbance by DPPH, because of its stabilization with hydrogen ions (reaction mixtures' purple color changed into yellow color). So, DPPH free radical scavenging activity is commonly used for the identification of antioxidant activity of different extracts/compounds. The tested extracts (hydroalcoholic, ethyl acetate and hexane) of *B. roxburghii* were found to be dose dependent percentage of inhibition on DPPH free radical with IC_{50} values of (230.40 ± 1.10) μg, (359.50 ± 0.68) μg and (507.80 ± 0.24) μg, respectively. The hydroalcoholic extract have more inhibition, *i.e.*, (78.25 ± 1.80) μg, at 640 μg (Tables 2 and 4, Figure 1).

Table 2
IC_{50} of different extracts of *B. roxburghii* against superoxide, hydroxyl and DPPH radicals.

Name of the extracts	IC_{50} (μg)		
	Superoxide radical	Hydroxyl radical	DPPH radical
Hydroalcoholic	332.50 ± 1.30	302.00 ± 1.50	230.40 ± 1.10
Ethyl acetate	356.00 ± 0.80	260.00 ± 1.20	359.50 ± 0.68
Hexane	486.00 ± 0.62	503.00 ± 0.76	507.80 ± 0.24
Ascorbic acid	54.40 ± 1.10	68.00 ± 1.30	22.00 ± 0.50

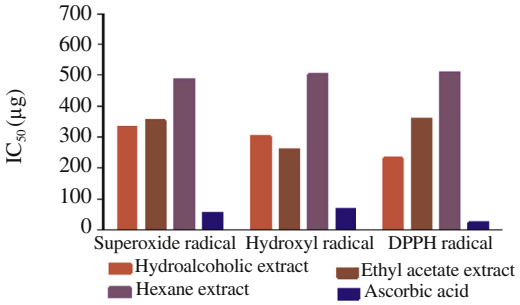

Figure 1. IC_{50} of different extracts of *B. roxburghii* against superoxide, hydroxyl and DPPH radicals.

Table 1
Concentration dependent percentage inhibition of different extracts of *B. roxburghii* on superoxide radical.

Name of the extracts	Concentration (μg/0.1 mL)					
	20	40	80	160	320	640
Hydroalcoholic	8.50 ± 0.30	18.50 ± 1.00	30.40 ± 1.10	42.51 ± 1.20	56.15 ± 1.20	69.30 ± 0.50
Ethyl acetate	9.20 ± 0.50	18.60 ± 0.23	27.45 ± 0.15	38.62 ± 0.34	48.18 ± 0.25	61.92 ± 0.35
Hexane	7.24 ± 0.80	15.20 ± 0.60	24.80 ± 1.00	32.90 ± 0.30	44.25 ± 1.40	54.80 ± 0.50
Ascorbic acid	28.15 ± 0.50	43.19 ± 1.50	56.87 ± 1.40	74.46 ± 0.70	80.72 ± 2.10	84.41 ± 1.20

Table 3
Concentration dependent percentage inhibition of different extracts of *B. roxburghii* on hydroxyl radical.

Name of the extracts	Concentration (μg/0.1 mL)					
	20	40	80	160	320	640
Hydroalcoholic	8.40 ± 0.50	16.50 ± 1.10	25.40 ± 0.50	36.10 ± 1.20	51.30 ± 1.00	62.80 ± 1.10
Ethyl acetate	10.20 ± 0.30	21.58 ± 0.50	32.24 ± 0.30	43.60 ± 0.50	52.98 ± 0.50	66.46 ± 0.30
Hexane	7.28 ± 0.20	15.86 ± 0.24	23.48 ± 0.46	34.52 ± 0.32	45.42 ± 0.84	53.88 ± 0.72
Ascorbic acid	24.32 ± 1.00	35.12 ± 0.40	55.61 ± 1.10	65.31 ± 1.20	76.25 ± 1.20	82.11 ± 0.70

Table 4
Concentration dependent percentage inhibition of different extracts of *B. roxburghii* on DPPH radical.

Name of the extracts	Concentration (μg/0.1 mL)					
	20	40	80	160	320	640
Hydroalcoholic	9.70 ± 0.40	15.80 ± 1.10	20.60 ± 0.50	32.70 ± 1.10	56.80 ± 1.20	78.25 ± 1.80
Ethyl acetate	8.58 ± 0.38	17.50 ± 0.14	22.68 ± 0.28	31.88 ± 0.62	48.28 ± 0.14	62.46 ± 1.22
Hexane	7.54 ± 0.13	16.36 ± 0.34	21.56 ± 0.82	29.58 ± 0.36	41.78 ± 1.20	54.62 ± 0.80
Ascorbic acid	48.00 ± 0.50	88.08 ± 1.00	90.68 ± 0.30	93.63 ± 0.50	94.21 ± 0.30	94.74 ± 1.10

3.2. Acute toxicity studies

There were no visible signs of toxicity, mortality and no behavioral changes such as alertness, motor activity, breathlessness, restlessness, diarrhea, tremor, convulsion and coma were observed at the administered doses. The animals were physically active and no death was recorded even at the dose of up to 2000 mg/kg body weight. Hence, all the tested extracts were considered as safe and non-toxic.

3.3. Hepatoprotective activity

We examined the aerial parts of *B. roxburghii* at three dose levels, 125 mg/kg, 250 mg/kg and 500 mg/kg, and assessed by measuring liver-related biochemical parameters [serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), ALP, total serum bilirubin and total protein] levels for their hepatoprotective nature using TAA-induced hepatotoxicity in rats (Table 5 and Figures 2–6).

Table 5
Percentage protection (%) of enzymes levels due to the effect of *B. roxburghii* extracts at different doses on TAA-induced liver toxicity in rats.

Extract of *B. roxburghii*	Quantity of the extract (mg/kg body weight)	Percentage protection on enzymes levels of TAA-induced hepatotoxicity				
		SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)	Total protein (g/dL)
Silymarin	50	96.24	95.25	93.90	97.83	96.14
Hydroalcoholic	125	37.70	35.33	37.24	23.42	24.97
	250	51.85	49.43	47.86	36.24	37.48
	500	65.65	62.39	59.89	55.90	54.61
Ethyl acetate	125	36.13	31.20	30.20	28.55	28.92
	250	47.08	43.30	41.72	40.51	41.44
	500	59.17	58.55	55.01	57.61	56.59
Hexane	125	32.86	29.91	26.12	22.56	22.33
	250	44.10	40.88	38.30	35.38	34.85
	500	56.76	54.27	52.49	52.48	50.66

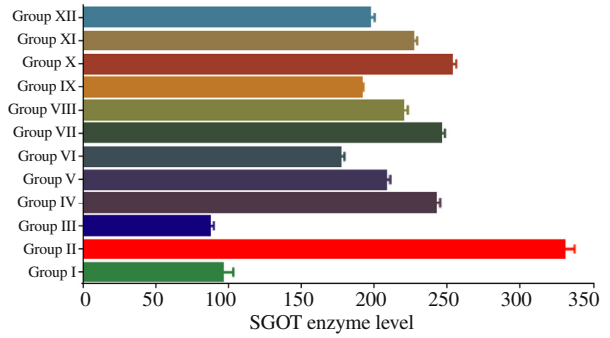

Figure 2. SGOT enzyme levels due to the effect of *B. roxburghii* extracts at different doses on TAA-induced liver toxicity in rats.

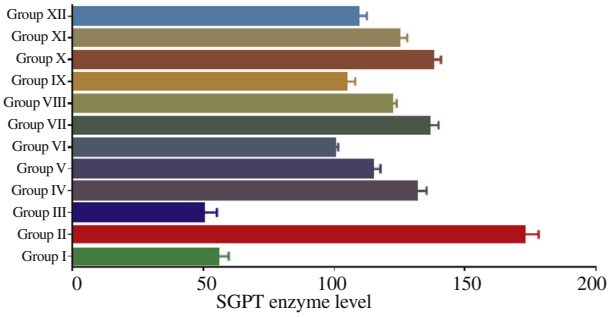

Figure 3. SGPT enzyme levels due to the effect of *B. roxburghii* extracts at different doses on TAA-induced liver toxicity in rats.

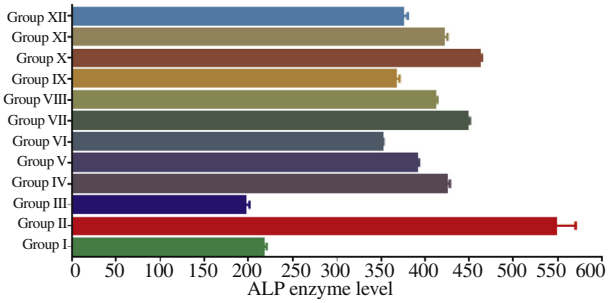

Figure 4. ALP enzyme levels due to the effect of *B. roxburghii* extracts at different doses on TAA-induced liver toxicity in rats.

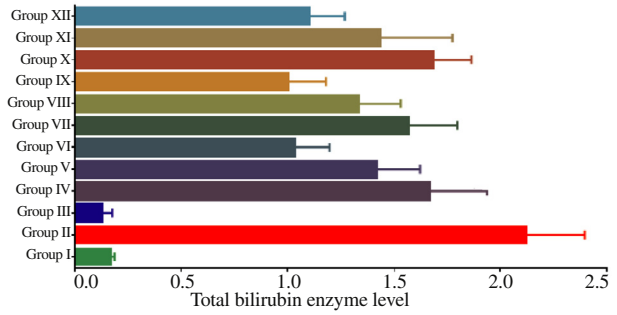

Figure 5. Total bilirubin enzyme levels due to the effect of *B. roxburghii* extracts at different doses on TAA-induced liver toxicity in rats.

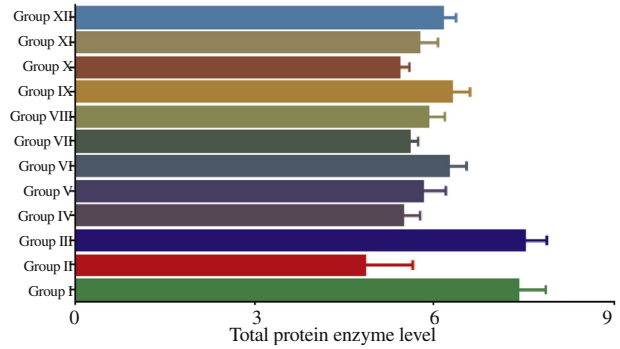

Figure 6. Total protein enzyme levels due to the effect of *B. roxburghii* extracts at different doses on TAA-induced liver toxicity in rats.

Group I treated with vehicle showed no significant changes in the biomarkers of liver enzymes (AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein) levels. Group II treated TAA showed significant changes in levels of biomarker enzymes. The animals of group III were administered with TAA and then silymarin. There were significant changes in biomarker enzymes levels as compared to group II and the percentage protection offered by the silymarin against the changes in AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 96.24%, 95.25%, 93.90%, 97.83% and 96.14%, respectively.

The percentage protection produced by the hydroalcoholic extract (Groups IV, V and VI) on the enhancement of AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 37.70%, 35.33%, 37.24%, 23.42% and 24.97%, 51.85%, 49.43%, 47.86%and 36.24% and 37.48%, 65.65%, 62.39%, 59.89%, 55.90% and 54.61% at doses of 125, 250 and 500 mg/kg body weight, respectively.

The percentage protection produced by the ethyl acetate extracts (Groups VII, VIII and IX) of the enhancement of AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 36.13%, 31.20%, 30.20%, 28.55% and 28.92%, 47.08%, 43.30%, 41.72%, 40.51% and 41.44%, 59.17%, 58.55%, 55.01%, 57.61% and 56.59% at doses of 125, 250 and 500 mg/kg body weight, respectively.

The percentage protection produced by the hexane extracts (Groups X, XI and XII) on the enhancement of AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 32.86%, 29.91%, 26.12%, 22.56% and 22.33%, 44.10%, 40.88%, 38.30%, 35.38% and 34.85%, 56.76%, 54.27%, 52.49%, 52.48% and 50.66% at doses of 125, 250 and 500 mg/kg body weight, respectively.

The decrease in the SGPT, SGOT, ALP and total bilirubin levels, increase in levels of protein to normal and percentage protection produced by the higher dose of the extracts were comparably similar to silymarin. Among all extracts, hydroalcoholic extract of the selected plants showed a better activity.

4. Discussion

The liver participates in different physiological metabolisms of the body, such as the exclusion of body wastage. So, the protection of liver is very important for the human wealth. However, in recent years, liver diseases are one of the most severe diseases causing deaths around the world. Hepatotoxic agents can react with the basic cellular components and consequently induce acute liver failure[18]. TAA is a thiono-sulfur containing compounds and has been used as a fungicide, organic solvent. At the same time, it is hepatotoxic. TAA causes centrilobular necrosis with a subsequent regenerative response of liver leading to liver cirrhosis and hepatocarcinoma[19,20].

TAA induces the formation of reactive metabolites (free radicals) derived from thioacetamide-S-oxide and by ROS generated as intermediates, which leads to the cell death from apoptosis and necrosis[16]. The metabolites produce are covalently attached to the cellular macromolecules induce the oxidative stress[17]. ROS production resulting from TAA administration was followed by lipid peroxidation, glutathion depletion and reduction in the SH-thiol groups and TAA also affects the calcium movement from intracellular parts[21]. All these effect the cellular permeability like different cell organelles, mainly effect the function of mitochondria, power house of the cell, by inhibiting the respiration[22].

The results of our experiment provide evidence for the hepatoprotective and antioxidant activities of *B. roxburghii*. The elevated enzymes level after treating with the TAA were significantly restored by treating with 125, 250 and 500 mg/kg body weight doses of *B. roxburghii* extracts in the different groups. The results of percentage protection of the extracts are well comparable with the standard drug, silymarin, and the tested extracts also showed the significant antioxidant activity on different free radicals[16,21,22]. It could be concluded by the above results that *B. roxburghii* plant extracts have the capacity in reduce the formation of the ROS by TAA, cellular apoptosis, lipid peroxidation by reducing the bonding of the radicals to membarane proteins. All these conditions protect the liver cells from the loss of cellular metabolites and maintain the normal function of the mitochondria. The hydroalcoholic extract of *B. roxburghii* showed more hepatoprotective and antioxidant activities because of the lack of solubility of the bioactive molecules in the other extracted solvents. The observed activities may be due to the presence of different phytocompounds. Further studies are needed to isolate and characterize the active entities presenting in *B. roxburghii* extracts and complete the explanation of their mechanism of action.

Conflict of interest statement

The authors report no conflict of interest.

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