Original Research Article

Investigation of hepatoprotective properties of *Trichosanthes dioica* leaves extract in paracetamol induced hepatotoxicity in rats.

Abstract

Traditional plants have been used to treat hepatotoxicity by folk medical practitionars. *Trichosanthes dioica (TD)* has been used in folk medicine to alleviate several diseases. In the present study, ethanolic extract of *Trichosanthes dioica* leaves has been utilized to study its activity on paracetamol induced hepatotoxicity in rats.

Swiss albino mice (25-30g) and SD Rats (140–200g) were used. Acute hepatotoxicity was induced by paracetamol (800mg/kg body weight) administered once daily for one week whereas the test extract was given orally throughout the whole experiment at 250 and 500mg/kg body weight. Silymarin (100mg/kg b.w.) was given orally as standard hepatoprotective drug. The degree of hepatoprotection was determined by the estimation of biochemical parameters like ALT, AST, ALP, bilirubin, total protein and albumin.

The increased plasma levels of hepatic marker enzymes including AST, ALT, ALP and bilirubin found in the paracetamol control group, which may be due to the liver cell destruction or changes in the cell membrane permeability indicating severity of hepatocellular damage induced by paracetamol. Pre-treatment with *TD* as well as standard hepatoprotective agent silymarin recovered the increased plasma levels of these hepatic enzymes to reduced levels.

The results of the study provide evidence that the *Trichosanthes dioica* leaves has shown hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Key words: *Trichosanthes dioica,* hepatoprotective activity, paracetamol induced hepatotoxicity, liver enzymes.

1. INTRODUCTION

The liver is “Great Chemical Factory” of body as involved in regulation, synthesis, storage and secretion of many important proteins, nutrients and chemicals. Liver is also involved in

detoxification of toxic chemicals and xenobiotics. It is exposed to various toxins, changing liver functions and eventually leading to liver ailments like hepatitis, cirrhosis and alcoholic liver disease [1]. During many biochemical processes, free radical reactive oxygen species are generated that cause liver injury [2].

A large number of drugs have been reported to have potentially hepatotoxicity [3]. Paracetamol induces hepatotoxicity which results with several cases of cirrhosis, hepatitis and suicidal attempts [4]. An overdose of the analgesic drug paracetamol can lead to severe liver injury in humans and in experimental animals [5].

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. One of the important and well -documented uses of plant products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent [6]. The biodiversity of flora of Bangladesh is very broad, and several native Bangladeshi medicinal plant species have a long tradition of use with great phytotherapeutic potential [7]. So, research in medicinal plants is a vital sector for the discovery of promising drugs in Bangladesh [8].

Trichosanthes dioica Roxb. L. (family: Cucurbitaceae) is an annual or perennial herb. It is called pointed gourd in English, Potol in Bengali and Patola in Sanskrit, is a dioecious climber distributed in tropical Asia, Polynesia, and Australia [9,10,11]. Early chemical study revealed the presence of the toxic bitter principles cucurbitacins. The main phytochemical groups that were present were alkaloids, glycosides, flavonoids, carbohydrates, fixed oils, steroids, saponins, tannins, and phenols. The various chemical constituents present in *T. dioica* are vitamin A, vitamin C, tannins, and saponin [12,13,14,15]. Various parts of TD have been using in several diseases like as tonic, febrifuge, in alcoholism, jaundice, edema, alopecia; also as antipyretic, diuretic, cardiotonic, hypoglycemic and laxative [16,17,18].

The purpose of this work was to explore the possible hepatoprotective activity of *T. dioica* leaves extract in experimental animals.

2. MATERIALS AND METHODS

2.1 Plant collection and Extraction

The plant *Trichosanthes dioica* (TD) leaves was collected from Savar area and was identified and authenticated by the Dept. of Botany, Jahangirnagar University, Savar, Dhaka. The collected materials were thoroughly washed in water, cut into smaller parts and shed dried at 35º–40ºC and pulverized in electric grinder to get extractable powder. Then powder was extracted in soxhlet apparatus with ethanol (96%).

2.2 Experimental animals

For the experiment, Sprague Dawley rats of either sex, weighing between 140-200g, were collected from the animal research lab in the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. Animals were maintained under standard environmental conditions (temperature: 27.0±1.0°, relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

2.3 Toxicity studies

Toxicity studies of the extracts were carried out in Swiss Albino mice of either sex weighing between 20 and 25g. The extract was found to be safe till 4000 mg/kg p.o. [19].

2.4 Experimental design for the assessment of liver functions

Animal study was performed at Pharmacology Laboratory, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342. The rats were housed in polypropylene cages at room temperature (27±2ºC). The rats were divided into five groups of 6 animals (n = 6) each.

Group I: received water (10 mL/kg p.o.) once daily for 7 days, and served as normal control

Group II: received water (10 mL/kg p.o.) and paracetamol 800 mg/kg, p.o. once daily for 7 days and served as positive control.

Group III: received standard drug silymarin (100mg/ kg p.o.) and paracetamol 800 mg/kg, p.o. once daily for 7 days, serving as STD.

Group IV and V: received *Trichosanthes dioica* (TD) leaves extract (250 and 500 mg/kg respectively) and paracetamol 800 mg/kg p.o. once daily for 7 days.

Rats were anesthetized using ketamine. After sacrifice, blood samples were collected and the serums were separated by centrifugation. Serum samples were subjected to liver function tests of enzymes such as glutamate pyruvate transaminase (GPT/ALT), glutamate-oxaloacetate tranaminase (GOT/AST), alkaline phosphatase (ALP), total bilirubin, total protein, albumin and globulin by standard enzymatic colorimetric method.

2.4 Statistical Analysis

Statistical analysis for animal experiments was carried out using One way ANOVA following Dunnet's post hoc test using SPSS 16.0 for windows. Data were presented as Mean ± SEM. The results obtained were compared with the vehicle control group. p values <0.05, <0.01 and <0.001 were considered to be statistically significant, highly significant and very highly significant respectively.

3. RESULT AND DISCUSSION

Acetaminophen (AAP) is a frequently used analgesic that can cause hepatic necrosis in high doses by the formation of NAPQI which causes the hepatic GSH depletion. At high doses, more NAPQI will bind covalently to cellular macromolecules [20, 21, 22, 23]. Among the macromolecules that leak from the damaged tissues, enzymes, because of tissue specificity and catalytic activity, are released into the bloodstream, [24] and a study of these enzyme

activities in plasma has been found to be of great importance in the assessment of liver damage [25].

The increased levels of AST and ALT are indicative of cellular damage and loss of functional integrity of the cell membrane in the liver [26]. The increase in ALP in liver disease is the result of increased synthesis of the enzyme by cells lining the canaliculi, usually either intra- or extrahepatic, which reflects the pathological alteration in biliary flow [27].

The present study demonstrated the ability of the TD extract to decrease the ALT and AST level significantly ($p<0.05$) at 500 mg/kg dose and also the ALP level at 250 mg/kg ($p<0.01$) and 500 mg/kg dose ($p<0.001$) (table 2, figure 2.1).

Reduction of the enhanced level of serum ALT, AST, ALP and total bilirubin by TD extract seemed to offer protection and maintain the functional integrity of hepatic cells.

An abnormal increase in the levels of bilirubin in plasma indicates hepatobiliary disease and severe disturbance of hepatocellular function [28]. Prior oral administration of *Trichosanthes dioica* (TD) extract exhibited significant protection against AAP-induced hepatotoxicity. It decreased the levels of bilirubin significantly ($p<0.05$) at 500 mg/kg which is an indication of protection against hepatic damage caused by AAP (figure 2.2).

Most proteins found in plasma are produced by the liver, the principle exception being immunoglobulins. Severe liver damage has been associated with decreased production of various proteins resulting in reduced serum levels of total protein, albumin, and/ or globulin [29, 30]. Decreased protein production may render other abnormal test values. e.g. depletion of coagulation factors (all are globulins) may result in prolonged prothrombin or activated partial thromboplastin times [31]. And, increased loss of protein via urine or feces due to renal or gastrointestinal disease will reduce serum protein levels. Inflammation anywhere in the body often results in increased production of specific globulin proteins produced by liver [29].

The results indicate that protein level was slightly decreased at 250 mg/kg dose whereas it was slightly increased at 500 mg/kg dose. But plasma albumin level was decreased at both the doses although it was not significant (table 3, figure 3).

Through this study also the consumption of TD for 7 days was found to reduce the body weight as well as the liver weight of rats (table 1, figure 1.1 and 1.2).

Table 1: Effect of *Trichosanthes dioica* leaves on the body weight and liver weight in paracetamol-induced hepatotoxicity in rats.

Group	**Body Weight (gm)** (Mean±SEM)		**Liver Weight** (gm) (Mean±SEM)
	Initial Day	Final Day	
Normal Control	148.33±2.95	162.17±2.36	4.83±0.23
Paracetamol Control	149.33±3.85	141.0±3.27	6.10±0.25
STD (Sylimarin 100mg/ kg)	150.0±4.66	145.67±4.70	5.25±0.18
TD 250mg/kg	148.33±4.78	141.83±5.01	5.87±0.10
TD 500mg/kg	150.0±2.63	145.83±2.47	5.40±0.14

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant compared to diabetic control. † ($p<0.05$), †† ($p<0.01$) and ††† ($p<0.001$) as compared to normal control group.

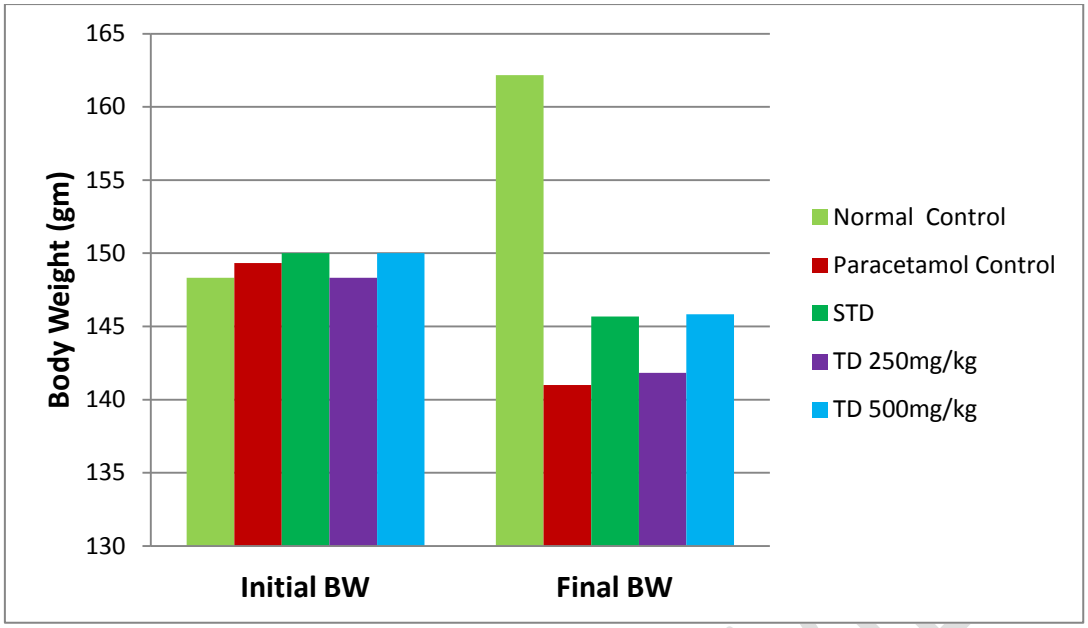

Figure 1.1: Effect of *Trichosanthes dioica* leaves on the body weight in paracetamol-induced hepatotoxicity in rats.

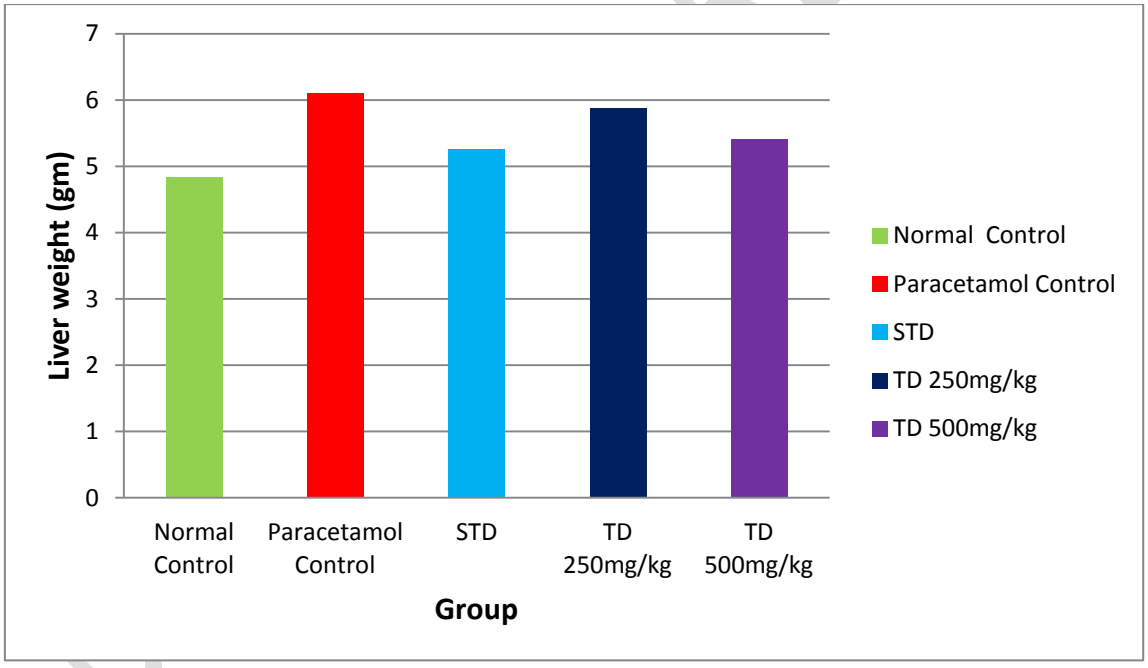

Figure 1.2: Effect of *Trichosanthes dioica* leaves on the liver weight in paracetamol-induced hepatotoxicity in rats.

Table 2: Effect of *Trichosanthes dioica* leaves on plasma ALT, AST, ALP and bilirubin level in paracetamol-induced hepatotoxicity in rats.

Group	**ALT** (IU/L) (Mean±SEM)	**AST** (IU/L) (Mean±SEM)	**ALP** (IU/L) (Mean±SEM)	**Bilirubin** (g/dl) (Mean±SEM)
Normal Control	36.37±6.82	43.33±1.86	167.17±4.89	1.17±0.13
Paracetamol Control	74.67±3.01	76.0±4.91	291.0±11.04	3.02±0.14
STD (Sylimarin 100mg/kg)	44.67±2.22	51.50±2.72	138.67±2.74	1.20±0.18
TD 250mg/kg	63.0±2.08	70.0±1.42	218.83±8.67	2.57±0.17
TD 500mg/kg	51.0±1.63	54.50±1.96	180.17±6.80	1.80±0.22

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant compared to diabetic control. † ($p<0.05$), †† ($p<0.01$) and ††† ($p<0.001$) as compared to normal control group.

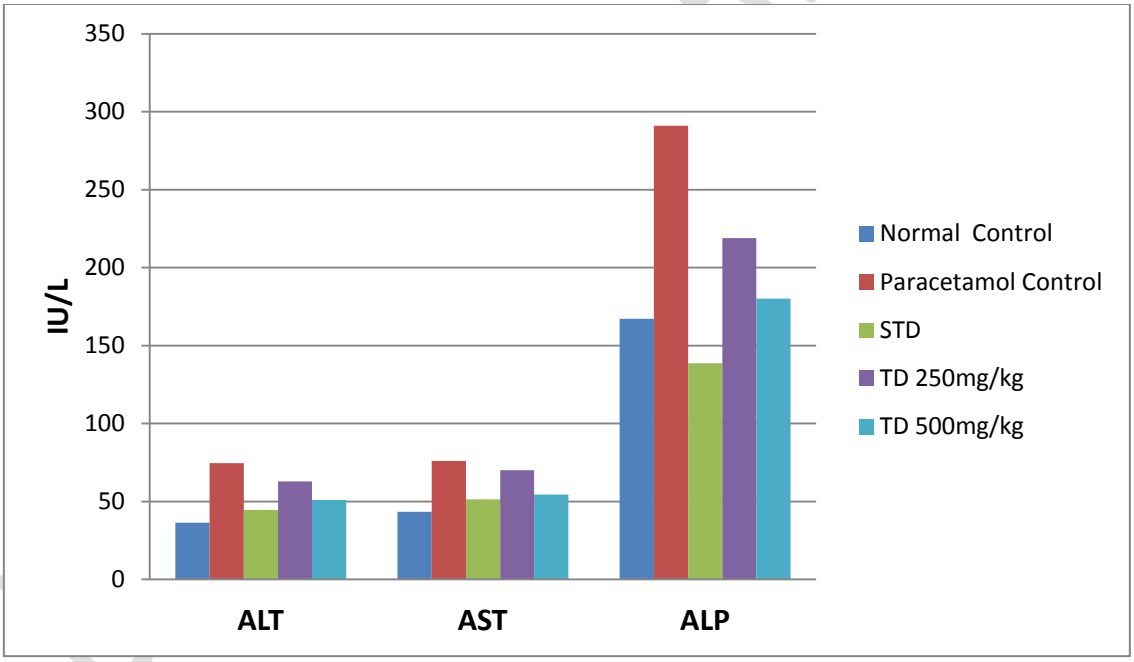

Figure 2.1: Effect of *Trichosanthes dioica* leaves on plasma ALT, AST and ALP level in paracetamol-induced hepatotoxicity in rats.

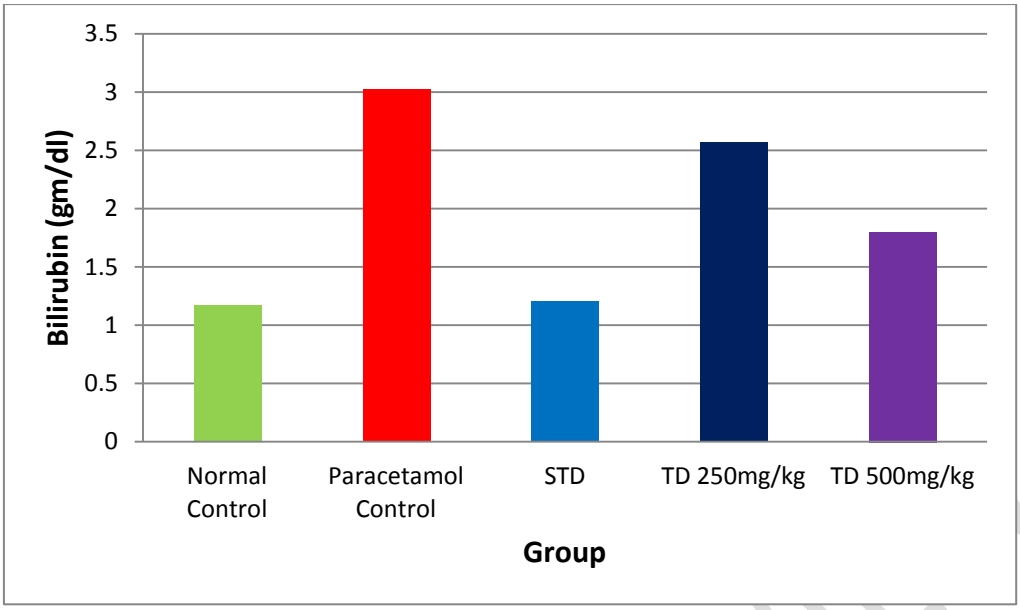

Figure 2.2: Effect of *Trichosanthes dioica* leaves on plasma bilirubin level in paracetamol-induced hepatotoxicity in rats.

Table 3: Effect of *Trichosanthes dioica* leaves on plasma total protein, albumin and globulin in paracetamol-induced hepatotoxicity in rats.

Group	**T. Protein (g/dl)** (Mean±SEM)	**Albumin (g/dl)** (Mean±SEM)	**Globulin (g/dl)** (Mean±SEM)
Normal Control	6.02±0.18	4.05±0.28	1.97±0.35
Paracetamol Control	4.43±0.15	2.40±0.21	2.03±0.32
STD (Sylimarin 100mg/ kg)	5.70±0.17	3.93±0.37	1.77±0.42
TD 250 mg/kg	4.77±0.11	3.02±0.15	1.75±0.24
TD 500 mg/kg	5.68±0.18	3.77±0.16	1.92±0.33

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant compared to diabetic control. † ($p<0.05$), †† ($p<0.01$) and ††† ($p<0.001$) as compared to normal control group.

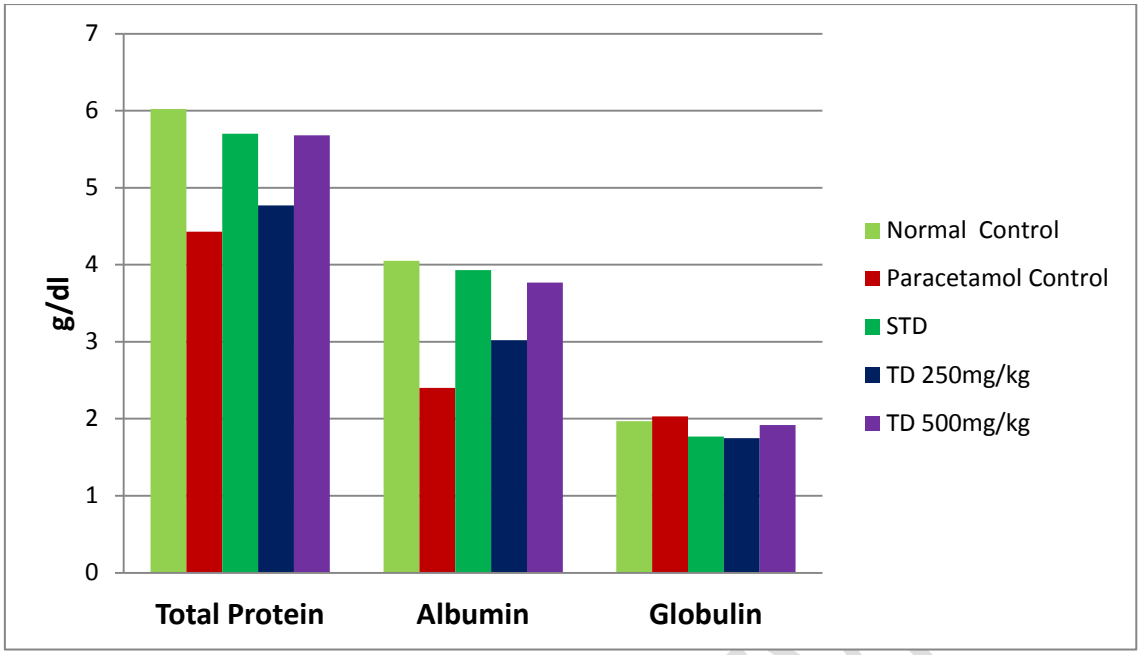

Figure 3: Effect of *Trichosanthes dioica* leaves on plasma total protein, albumin and globulin in paracetamol-induced hepatotoxicity in rats.

4. CONCLUSION

The overall significance of this study was that, the treatment with *Trichosanthes dioica* leaves extract was able to protect the changes induced by AAP. On the basis of the above results it can be concluded that *Trichosanthes dioica* has significant value as hepatoprotective plant against paracetamol induced liver injury model. Further studies are required to determine the possible hepatoprotective mechanism(s) involved and, to isolate and identify the responsible bioactive compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by the institutional animal ethical committee.

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