Research Article

TOXICOLOGICAL EVALUATION OF A MONOTERPENE ESTER, BORNYL PIPERATE FROM *PIPER CHABA* ROOT

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ABSTRACT

Toxicological evaluation of bornyl piperate, a cytotoxic, antifungal and anti tubercular compound isolated from *Piper chaba*, was carried out on Swiss albino mice. After administration of the compound, blood and tissue samples of the mice were examined for haematological, biochemical and histopathological assay. A decrease in serum levels of SGPT, SGOT and ALP was observed when two groups of the experimental mice were given doses of 10 and 20 mg/kg of bornylpiperate, respectively, for 14 days. Other parameters such as RBC, WBC and platelet counts were found normal. Microscopic examination of the heart, liver, lung and kidney tissues showed inflammation, sinusoidal congestion and necrosis only at a dose of 20 mg/kg. Thus the results indicate that the bornylpiperate showed moderate toxicity at higher dose in mice.

Keywords: *Piper chaba*, Bornylpiperate, Toxicity, Biochemical, Histopathological.

INTRODUCTION

The plant *Piper chaba* (Piperaceae) is a climbing, glabrous shrub available in various parts of India and Malaysia¹. In Bangladesh, it grows in plenty in the southern part. The plant has vast folklore uses, astraditional medicine and as spices. The root is alexiteric and useful in asthma, bronchitis and consumption. The fruit is also useful in asthma, bronchitis, fever, inflammation, piles, pain in the abdomen and at the anus¹. Previous phytochemical studies of the stem bark of *Piper chaba* yielded piperine and its analogs^{2,4}, a piperine dimer chabamide⁵, lignan⁶ and sterols⁷. A survey of literature on pharmacological study has shown that the crude extract of the plant increased the oral bioavailability of sulfadiazine and tetracycline hydrochloride⁸. In another study, the amides from *Piper chaba* have been found to significantly inhibit ethanol and indomethacin induced gastric lesions at a dose of 25 mg/kg⁹. Extracts of the plant have been found to have anti-amoebic (IC₅₀ 71.4 µg/mL)¹⁰, antibacterial, antifungal and cytotoxic activities¹¹. Early studies by our group reported the isolation of bornyl piperate from *Piper chaba*¹². The compound showed significant cytotoxic and antifungal properties¹². The compound (isolated from *Piper aff. Pedicellatum*) also exhibited antituberculosis activity against *Mycobacterium tuberculosis* (H (37) Ra strain) with the minimum inhibitor concentration (MIC) of 25 µg/mL¹³. However, no acute or subacute toxicity studies of bornyl piperate have been studied so far. Considering various pharmacological applications of this compound, the aim of this study was to investigate the possible toxic effects of bornyl piperate from *P. chaba* root in Swiss albino mice. Thus this paper describes the biochemical, hematological and histopathological toxicity study of bornyl piperate from *P. chaba* root against swiss albino mice at different doses.

MATERIALS AND METHODS

Plant collection and extraction

Roots of *Piper chaba* were collected from the Bagerhat District, Bangladesh. The plant was botanically identified by Prof. A. T. M. Naderuzzaman, Department of Botany,

University of Rajshahi, Bangladesh. A voucher specimen was deposited in Bangladesh National Herbarium (Voucher No. 30276).

Extraction, isolation and identification of bornylpiperate

After collection and drying the roots (1 kg) were extracted at room temperature with ethanol (5 L). Evaporation of the solvent under reduced pressure afforded a semi-solid mass (40 g) as ethanol extract. The crude ethanol extract was successively partitioned with petroleum ether, chloroform, ethyl acetate and methanol. The solvents of these extracts were concentrated separately under reduced pressure below 50°C, which yielded 10.0, 12.3, 1.2 and 5.6 g of the dried extract, respectively. Column chromatography and preparative thin layer chromatography (PTLC) of the petroleum ether extract (5 g) with *n*-hexane, ethyl acetate and methanol yielded the crystals of bornylpiperate, compound 1 (100 mg) (Figure 1). The structure was determined by comparing spectral data to those previously reported^{12,13}. The compound was kept in a refrigerator at 4°C. The compound was dissolved in normal saline with help of Tween-80 as co-solvent.

Experimental animals

Swiss Albino mice (n = 4 in each group) of either sex, weighing 34 g obtained from the Animal Resource Division, International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR,B), were used to evaluate sub acute toxicity. Four weeks old Swiss Albino mice were kept under constant conditions of temperature (25 ± 2°C), relative humidity (42-54 %), for two weeks during the experiment. Animals were provided with standard rodent pellet diet (ICDDR, B). They were randomly divided into control and two treatment groups (4 mice/group). All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals¹⁴.

Administration of sample

The animals were divided into three groups. Animals of group I was received isotonic vehicle and served as a healthy control group. Animals of group II and III were treated with 10 and 20 mg/kg doses of compound 1.

Assessment of biochemical parameters

After 14 days of continuous treatment with extract and pure compound blood was withdrawn and serum was separated for determination of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP) and serum bilirubin (SB). The enzymatic parameters of serum, SGPT, SGOT and ALP and non-enzymatic parameter, SB were assayed according to standard methods¹⁵⁻¹⁷.

Histopathological studies

Following blood collection, mice were sacrificed by decapitation and liver, heart, lung and kidney of all animals were removed. The specimens obtained from the control and treated groups of animals were fixed in 10 % buffered formalin for 24 h. The formalin- fixed organ samples were stained with haematoxylin-eosin for photo microscopic observations of the histological architecture. The histological damage was expressed using the following score system: -, absent; +, mild; ++, moderate; + + +, severe.

Statistical analysis

Data were analyzed with the help of computer software SPSS version 10 for windows. All data were expressed as mean $\pm$ S.E. The results were analyzed by one-way analysis of variance (ANOVA) followed by multiple comparisons Fisher's protected *t*-test.

Table 1: Hematological values of mice in subacute toxicity of compound 1

Hematological parameters	Group I (Control)	Group II	Group III
RBC (million/mm ³)	5.02 $\pm$ 0.17	5.20 $\pm$ 0.28	4.92 $\pm$ 0.15
WBC (thousand/mm ³)	6.30 $\pm$ 0.25	7.47 $\pm$ 0.15	7.05 $\pm$ 0.19
Diff. WBC Count	Neutrophil	64.50 $\pm$ 2.36	60.5 $\pm$ 1.91
	Lymphocyte	32.00 $\pm$ 1.63	34.0 $\pm$ 0.81
	Monocyte	2.75 $\pm$ 0.95	2.5 $\pm$ 0.57
	Eosinophil	1.0 $\pm$ 1.15	1.0 $\pm$ 1.41
Platelet (no/mm ³)	359750 $\pm$ 16938.60	331250 $\pm$ 11086.78*	300000 $\pm$ 27988.09*
Haemoglobin (%)	58.25 $\pm$ 1.25	60.25 $\pm$ 3.095	62.75 $\pm$ 1.70

Values are expressed as mean $\pm$ S.E.M., n = 4. Group I was given vehicle only. Group II was given 10 mg/kg of compound 1 for 14 days; Group III was given 20 mg/kg of compound 1 for 14 days. Significantly different from control, *p < 0.05

Table 2: Blood chemistry values of mice in subacute toxicity of compound 1

Parameters	Group I	Group II	Group III
SGPT (U/l)	43.00 $\pm$ 2.58	26.25 $\pm$ 2.62**	19.00 $\pm$ 2.58**
SGOT (U/l)	53.75 $\pm$ 2.62	35.00 $\pm$ 4.08**	26.50 $\pm$ 3.41**
ALP (U/l)	182.25 $\pm$ 6.08	154.00 $\pm$ 4.54**	48.50 $\pm$ 3.10**
Bilirubin (mg/dl)	0.27 $\pm$ 0.00	0.25 $\pm$ 0.00	0.25 $\pm$ 0.01
Creatinine	0.59 $\pm$ 0.01	0.57 $\pm$ 0.00	0.59 $\pm$ 0.00
Blood urea	17.75 $\pm$ 0.95	17.50 $\pm$ 0.58	16.75 $\pm$ 0.96

Values are expressed as mean $\pm$ S.E.M., n = 4. Group I (control) was given vehicle only. Group II was given 10 mg/kg of compound 1 for 14 days; Group III was given 20 mg/kg of compound 1 for 14 days. Significantly different from control, *p < 0.05, **p < 0.001

Table 3: Histopathological changes in the liver, kidney, heart, lung given two different doses of compound 1 for 14 days

Microscopic observation		Group I	Group II	Group III
Liver	Accumulation of fat	-	+	++
	Inflammation	-	+	+++
	Congestion of blood vessel	-	+	+++
	Necrosis	-	+	+++
Heart	Inflammation	-	+	++
	Congestion of blood vessel	-	+	+++
	Necrosis	-	++	+++
Kidney	Inflammation	-	-	++
	Congestion of blood vessel	-	+	+++
	Necrosis	-	+	++
Lung	Accumulation of fat	-	-	++
	Inflammation	-	+	++
	Congestion of blood vessel	-	+	++
	Necrosis	-	+	+++

Group I was given vehicle only. Group II was given 10 mg/kg of compound 1 for 14 days; Group III was given 20 mg/kg of compound 1 for 14 days. Histological damage score system: -, absent; +, mild; ++, moderate; + + +, severe

Figure 1: Structure of compound 1

Figure 2: Histopathological studies of various organ with the compound 1 at a dose of 20 mg/kg for 14 days in mice (a), (b), (c) and (d) are the microscopic view ($\times 400$) of liver, heart, lung and kidney tissues of normal or control group and (e), (f), (g) and (h) are the microscopic view ($\times 400$) of liver, heart, lung and kidney tissues of experimental group (group III, received dose 20 mg/kg for 14 days)

RESULTS AND DISCUSSION

Toxicological evaluation of the monoterpene ester, bornylpiperate (Figure 1) was carried out by sub acute toxicity study for 14 days. The tested compound had a good hematological tolerance. Only the number of WBC was slightly increased and platelet counts were decreased in higher dose treated group. In overall, hematological parameters of the compound 1 treated mice were not significantly different from those of the control group ($P > 0.05$) (Table 1). A study of biochemical parameters showed that the compound 1 intake induced a significant decrease of SGPT, SGOT and ALP levels ($p < 0.001$) at two different doses (20 and 10 mg/kg) (Table 2). No significant changes were observed in plasma levels of creatinine and blood urea levels. After 2 weeks of treatment, histopathological analysis of target organs (liver, lung, heart and kidney) of treated animals revealed significant changes in the tissues at the higher dose (20 mg/kg). Histological profile of the control animals showed normal architecture [Figures 2(a), 2(b), 2(c), 2(d)]. But group III animals those who received 20 mg/kg of compound 1 exhibited large areas of necrosis, congestion of blood vessel and inflammation in liver, heart and lung tissues [Table 3, Figures 2(e), 2(f), 2(g)]. At lower dose of 10 mg/kg of the tested compound 1 showed mild toxicity on these tissues (Table 3). The toxicity of compound 1 on kidney tissues was found lower than the heart, lung and liver tissues (Table 3, Figure 2).

CONCLUSION

Hepatoprotection can be assessed in terms of reduction in histological damage, changes in serum enzymes (SGOT, SGPT and ALP), metabolites bilirubin, reduced glutathione (GSH) etc. Present study has shown that the compound, bornylpiperate isolated of *P. chaba* have decreased serum enzymes (SGOT, SGPT and ALP). Therefore this compound can be assessed for hepatoprotective activity study by inducing toxicity in mice and treating with the compound afterward. It is also revealed that the compound bornylpiperate is quiet safe for therapeutic use at low dose but exhibited moderate toxicity at a dose of 20 mg/kg. Although the compound, bornylpiperate showed toxicity at higher dose in histopathological assay, it can be minimized by adjusting dose. However, more extended toxicological study is needed to evaluate its side effect for therapeutic uses.

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