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Sub chronic toxicity potential of the alcoholic extract of Biophytum reinwardtii whole plant

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Abstract
Biophytum reinwardtii Edgew is an important and highly valued sensitive medicinal small herb and whole plant is ethnobotanically used in the treatment of insomnia, convulsion, inflammation, lithiasis, fevers and gonorrhea. The present study was carried out to evaluate acute and sub-chronic toxicity of alcoholic extract of *Biophytum reinwardtii* Edgew *(B. reinwardtii)* whole herb extract in albino mice and rats. The median acute toxicity value (LD_{50}) of the extract of *B. reinwardtii* was determined to be 588.88 mg/kg b.w. *(i.p.)* in mice. In sub-chronic toxicity studies 50, 75 and 100 mg/kg b.w. of the alcoholic extracts of *B. reinwardtii* (AEBR) whole plant were administered orally to the test groups while distilled water was given to the control group. The parameters measured include food and fluid intake, body weight, absolute and relative weight of various organs, haematological parameters [total white blood cell (WBC) and packed cell volume (PCV)], and tests for liver function: Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase and total bilirubin. Rats treated with AEBR in the therapeutic dose level had no progressive increase in body weight or fluid intake. There were no significant changes in both the absolute and relative organ weights between the control and the test groups after 30 days. The liver enzymes and haematological parameters were statistically equal in all the groups. The results of the present study therefore indicated that *B. reinwardtii* whole herb is safe in adult male albino mice demonstrating no noticeable toxicity.
Keywords: *Biophytum reinwardtii,* sub-chronic toxicity, liver function, packed cell volume, absolute weight, relative weight.

1. Introduction

The genus *Biophytum* is a member of the family geraniaceae and distributed in tropical Asia, Africa, America and Philippines. In India nine species are found and out of nine species three species viz. *Biophytum sensitivum* DC. Syn.*Oxalis sensitive* Linn., *Biophytum reinwardtii* Edgew and *Biophytum umbraculum* Welw. Syn. *B. petersianum* Klotzsch are reported to have ethnomedicinal properties. *Biophytum reinwardtii* , a sensitive herb, is distributed from Kumaun to Arunachal Pradesh up to an altitude of 1,800 m and extending into peninsular India (Mitra and Ambasta1988, Kirtikar and Basu 1975). *B. reinwardtii* is a graceful little herb with its crown of pinnate leaves always terminating a slender stem 2.5-10 cm high. Leaves 0.2-0.3 cm long with usually 8-12 pairs of leaflets, which decrease in size very rapidly towards the base, so that the uppermost are 0.4-0.5 cm long and the lowest are often only 0.1 cm long, rachis usually hairy, often pink. Peduncles shorter or longer than the leaves, pubscent; crown of chaffy bracts shorter than the pedicles, which again are longer than the 0.1 cm long sepals. Sepals equal or somewhat longer than the capsule (Haines 1988). Traditionally in Jharkhand (India), the leaves and roots of *B. reinwardtii* are given for the treatment of insomnia and other mental disorders. The decoction of the whole herb is used against fevers (Kirtikar and Basu 1975, Haines 1988). The present study was undertaken to investigate the acute and sub-chronic toxicity studies of the alcoholic extract of *B. reinwardtii* Edgew. in rats.

2. Materials and Methods

2.1 Plant Material

The whole herbs of *B.reinwardtii* Edgew (Geraneaceae) were collected from Khunti, Torpa, Basia,Palkot , Bolba, Bishunpur, Netarhat, Gangaghat and Sisai of Ranchi, Gumla, Simdega and Lohardaga District . The parts were authenticated by Dr. T.K. Ghosh, Taxonomist, Ranchi University, Ranchi, India and Botanical survey of India, Govt. of India, Kolkata(India). The voucher specimen no. (GPI 81/2005) was deposited in the Pharmacognosy laboratory, Govt. Pharmacy Institute, Ranchi (India) for future references.

2.2 Preparation of Extract

The whole herb was dried under shade and then powdered with a mechanical grinder and stored in an airtight

container. The dried and powdered plant material (whole plant) was subjected to hot extraction in a Soxhlet continuous extraction apparatus with 50% alcohol. The extraction time period was 72 hours. The extract was then filtered through whatmann filter paper and the filtrate obtained was lyophilized. The yield of 50% alcoholic extract (AEBR) was 9.28% w/w.

2.3 Phytochemical Screening

The extract was subjected to preliminary phytochemical analysis for major group of phytoconstituents (Evans 1989, Khandelwal 2005, Williamson et al 1996).In each test 10% (w/v) solution of the alcoholic extract was used unless otherwise specified for an individual test. Phytochemical screening of the extracts revealed the presence of terpenoid(s), steroid(s), tannin(s), flavonoid(s) and saponin(s).

2.4 Animals

Studies were carried out using albino rats and albino mice of either sex weighing between 180-200 g & 20 to 25 g respectively. They were obtained from the animal house, Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (14/10 h). Animals were allowed free access to standard pellet (Hindustan Lever, Mumbai, India) food and drinking water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment (Bose et al 2007). All procedures described were reviewed and approved by the Institute animal ethical committees (Reg.No.-621/02/ac/CPCSEA and Protocol No.-PH/IAEC/29/2006).

2.5 Acute toxicity studies

The LD_{50} was determined using the graphical method of Litchfield and Wilcoxon in mice (Litchfield and Wilcoxon 1949). Briefly, geometric doses of AEBR (100-1000 mg/kg) was administered i.p. to 10 groups of mice (n=6). Control group received normal saline (10 ml/kg i.p.) and vehicle control received 10% propylene glycol (PG) (10 ml/kg i.p.). After administration of dose the animals were observed continuously for first 4 h for behavioral changes and signs of toxicity and mortality within 24-72 h were noted. The values thus obtained were plotted against the corresponding log dose. The antilog of log dose corresponding to probit 5 gave the value of LD_{50}.

2.6 Sub-chronic toxicity study

A total of twenty four mature Wister rats were used in this study. They were divided into four groups of six rats each. Three of the groups received 50, 75 and 100 mg/ kg body weight of the AEBR *(p.o.),* respectively, while the control group received distilled water only. Food and water intake were monitored daily. After 30 days of exposure, blood was collected from the animals, by cardiac puncture, for haematological and biochemical analysis. Thereafter, the animals were sacrificed and the following organs isolated and weighed: heart, lungs, kidney, liver, spleen and pancreas. Relative weight of the respective organs was calculated from each organ's wet weight and the animal's body weight.

2.6.1 Effect of extract on Liver function

About 5 ml of whole blood collected into a plain tube was centrifuged at 3500 rpm for 5 min using table centrifuge (Remi, India) and the serum separated and analyzed for the liver enzymes. Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) were assayed using the methods of Reitman and Frankel, alkaline phosphatase (ALP) was analysed by the method of King and Armstrong, while total bilirubin level was determined by the method of Malloy and Evelyn. All assay methods employed were as reported by Varley (Varley et al 1991).

2.6.2 Haematological assay

EDTA-anticoagulated tubes were used to collect whole blood for these investigations. Packed cell volume (PCV) was determined by the microhaematocrit method, while total WBC was determined by visual method (Dacie and Lewis 1991).

2.7 Statistical analysis

Data were analyzed using Student's t-test and expressed as mean ± standard error of mean (SEM).

3. Results

3.1 Effect of extract on fluid and food intake

The effect of the extract on weekly consumption of food and fluid intake of experimental animals treated with AEBR is shown in Table 1 and Table 2. Alcoholic extract of whole herb of *Biophytum reinwardtii* did not increase the food or water intake of the experimental animals compared to control throughout the three weeks of exposure.

3.2 Effect of extract on different organ weight

Rat treated with the various therapeutic doses of alcoholic extract of *Biophytum reinwardtii* (50, 75 and

100mg/kg) had no significant change in body weight. No statistically significant differences existed in the absolute and relative weight of all the isolated organs between the treated and the control rats (Table 3).

3.3 Effect of extract on SGOT, SGPT and ALP

Subchronic administration of ethanolic extract of *B. reinwardtii* did not exert any significant effect on the level of SGOT, SGPT and ALP of the extract treated rats. There was no statistical significant difference between the means of treated groups and that of the control (Table 4).

3.4 Effect of extract on hematological parameters

The results of the effect of subchronic administration of alcoholic whole plant extract of *B. reinwardtii* on haematological parameters of rats is shown in Table 5. There was no significant change in haematological parameters in the extract treated animals compared to the control.

4. Conclusion

The present study was aimed to investigate the possible toxic effects of the alcoholic extract of *B. reinwardtii* whole herb in adult swiss albino rats and mice. In results of acute toxicity study revealed that AEBR may be safe in swiss albino mice. Various physical, chemical and haematological parameters were thoroughly studied in the sub-chronic toxicity study.

The body weights, food and water intakes were found to be unaltered during the 30 days treatment period when compared to control group. Similarly there were no significant changes in different organ weights also. No mortality was observed during this period. Kluwe documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxicants. An increase in kidney weight (either absolute or relative) indicates nephrotoxicity (Kluwe 1981). AEBR did not induce any toxic effect on the kidney and the other organs.

Biochemical parameters related to hepatic vital functions viz. SGPT, SGOT, ALP and bilirubin contents exhibited no significant alteration as compared with the normal control mice. Certain drugs and other substances are known to affect and influence circulating bilirubin levels and elevation in bilirubin levels suggests increase in haemolysis (Kelly 1977). AEBR, however, did not alter significantly the bilirubin levels of the exposed rats as well as other liver enzymes compared to the control. The extract was observed to cause no significant change in PCV% and WBC count in the extract treated animals compared to the control. According to Onyenyili and co-workers, anemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cells synthesis by the active constituents of the extract, and decrease in haematological parameters in an experimental animals has been associated with anaemia (Onyeyilli et al 1998). Study indicates that there is no lysis of blood cells and/or inhibition in blood cells synthesis by the active constituents of AEBR.

From the present investigation it can be concluded that AEBR exhibited an excellent safety profile in acute and sub-chronic toxicity studies. The present study establishes the reliable safety profile of AEBR in adult swiss albino mice offering no obvious toxicity.

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Table 1. Effect of AEBR on weekly Food (g) intake in Rats

Treatments	Weeks		
	1	2	3
Control	223.66±1.11	228.16±0.94	226.66±0.88
50 mg/kg	281.33±0.66	287.66±1.25	287.66±0.76
75 mg/kg	302.00±0.96	297.33±0.80	291.66±0.49
100 mg/kg	249.83±1.01	252.83±0.94	258.83±0.60

Each value is mean ± S.E.M (n = 6)

Table 2. Effect of AEBR on weekly Fluid (ml) intake in Rats

Treatments	Weeks		
	1	2	3
Control	806.00±0.85	813.50±0.76	810.83±0.30
50 mg/kg	815.16±1.42	822.16±0.94	822.66±0.42
75 mg/kg	811.33±0.88	805.66±1.05	802.66±0.33
100 mg/kg	812.33±0.75	809.16±0.60	808.00±0.25

Each value is mean ± S.E.M (n = 6)

Table 3. Effect of Various Doses of AEBR on the Relative (%) and Absolute Weights* (g) of organs

Organ	Treatment			
	Control	50 mg/kg	75 mg/kg	100 mg/kg
Heart	0.48 ± 0.008 (0.78 ± 0.008)	0.47 ± 0.011 (0.77 ± 0.009)	0.46 ± 0.013 (0.78 ± 0.010)	0.48 ± 0.009 (0.78 ± 0.011)
Lung	0.88 ± 0.005 (1.60 ± 0.007)	0.86 ± 0.008 (1.58 ± 0.007)	0.86 ± 0.010 (1.59 ± 0.004)	0.85 ± 0.008 (1.57 ± 0.010)
Kidney	0.81 ± 0.004 (1.51 ± 0.004)	0.82 ± 0.006 (1.53 ± 0.006)	0.80 ± 0.004 (1.52 ± 0.005)	0.80 ± 0.005 (1.51 ± 0.004)
Liver	4.11 ± 0.006 (8.20 ± 0.004)	4.10 ± 0.002 (8.21 ± 0.005)	4.13 ± 0.007 (8.78 ± 0.004)	4.04 ± 0.004 (8.31 ± 0.003)
Pancreas	0.30 ± 0.004 (0.58 ± 0.002)	0.32 ± 0.004 (0.60 ± 0.006)	0.31 ± 0.004 (0.59 ± 0.004)	0.31 ± 0.005 (0.58 ± 0.008)
Spleen	0.50 ± 0.004 (0.91 ± 0.005)	0.51 ± 0.004 (0.90 ± 0.006)	0.50 ± 0.005 (0.91 ± 0.005)	0.50 ± 0.002 (0.90 ± 0.004)

* Values in parenthesis indicate absolute weight. Values are expressed as mean ± S.E.M (n = 6)

Table 4. Dose Effect Relationship of AEBR on the Liver Function of Rats

Treatment	Analyte			
	SGOT (iu/l)	SGPT (iu/l)	ALP (iu/l)	Total Bilirubin (mg/dl)
Control	53.54 ± 0.56	20.00 ± 0.46	174.55 ± 0.48	0.07 ± 0.0002
50 mg/ kg	52.58 ± 0.75	20.75 ± 0.28	175.06 ± 0.07	0.08 ± 0.0002
75 mg/kg	52.78 ± 0.77	20.54 ± 0.24	175.05 ± 0.02	0.07 ± 0.0004
100 mg/kg	52.35 ± 0.37	20.84 ± 0.27	175.07 ± 0.05	0.08 ± 0.0003

Values are expressed as mean ± S.E.M (n = 6) (SGOT = Serum Glutamic Oxaloacetic Transaminase, SGPT = Serum Glutamic Pyruvic Transaminase, ALP = Alkaline Phosphatase)

Table 5. Dose Effect Relationship of AEBR on the Haematological Parameters of Rats

Treatment	PCV (%)	WBC (cells/mm3)
Control	56.51 ± 0.31	7102.09 ± 20.90
50 mg/kg	54.14 ± 0.13	7136.12 ± 14.88
75 mg/kg	56.23 ± 0.24	7036.94 ± 12.19
100 mg/kg	56.04 ± 0.17	7154.87 ± 19.93

Values are expressed as mean ± S.E.M (n = 6)