RESEARCH ARTICLE

Assessment of Dermal Toxicity Profile of Root Bark Extract of *Berberis aristata*

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ABSTRACT

The purpose of this study is to determine the cutaneous safety dosage range of root-bark extract of *Berberis aristata*. The Organisation for economic co-operation and development (OECD) guidelines 402 and 410 were used to assess the acute and subacute dermal toxicity studies. The extract dosages (2000 and 5000 mg/kg) were topically applied in single doses in the acute dermal toxicity investigation. Up to 72 hours, the general behavior, unfavorable effects, and death were identified. After that, the different concentration of the extract was topically applied at dosages of 500, 1000, and 2000 mg/kg for 28 days in a sub-acute dermal investigation, and any changes were noted. Only slight sedation and lethargy were detected in the two groups, *i.e.,* 2000 and 5000 mg/kg extract treatment groups, which showed no mortality or substantial changes in behaviors, sleepiness, or drowsiness. Overall, no signs or symptoms of poisoning were identified. The liver function parameters were somewhat improved when extracts (2000 mg/kg) were given. It was found that extract application had no substantial harmful impact on animals and that it was safe to use as topical formulations.

Keywords: Acute toxicity study, *Berberis aristata*, Ethanolic extract of *Berberis aristata* (EEBA), Sub-acute toxicity study.

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INTRODUCTION

Plants are utilized as alternative medicines to treat a range of ailments due to their wide acceptance, efficacy, affordability, safety, and low cost.[1] Due to the public's strong opinion that herbal formulations are natural and hence safe for the treatment of a variety of ailments, there is also a growing trend in the usage of these products.[2] However, due to their manufacturing process or the inclusion of metals (such as cadmium from the soil), herbal remedies may contain contaminants such as heavy metals, aflatoxins, and pathogenic microbes.[3,4] Since they are derived from nature, the herbal remedies are free of the harmful or adverse side effects sometimes associated with synthetic pharmaceuticals used in conventional treatment.[5] Just as it is for conventional orthodox medicines that have been sufficiently investigated and established, the toxicity study for proper and documented traditional herbal remedies should be conducted. Typically, the toxicity of traditional herbal remedies is not evaluated.[6] As a result, users of herbal medicine frequently concentrate on the drugs' therapeutic advantages while ignoring their adverse effects on other organs, although toxicity research should also be highlighted.

The bark of the *Berberis* root contains several flavonoids, including berberine, isoberberine, palmatine, tetrahydro-palmatine, quercetin, rutin, and isoquercetin. According to the results of various studies, the chemicals berberine and palmatine are important.[7] For each herb, the toxicity parameter is important since the available phytoconstituents are essential and responsible for a variety of functions. The material that could harm or damage the exposed organism is referred to as the test extract. The end consequence is the effect of suspected toxicity on an entire organism, such as an animal, bacteria, or plant. It includes impacts on the organism's substructure, such as skin (dermato-toxicity), cells (cytotoxicity), and organs (genotoxicity), such as the liver (hepatotoxicity). These interactions may differ depending on the cell membrane and the toxicants' molecular characteristics since they might occur on the cell surface, inside the cell body, beneath tissues, and in the extracellular matrix.[8] Before toxicants bind to significant organs like the kidneys, liver, or spleen, toxic consequences must also be seen. Determining a drug's toxicity is crucial for protecting the public's health because chemical exposure is risky and can have harmful consequences on the human

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body. The topic of medicinal plants is one that many healthcare systems are closely monitoring. Additionally, research on the dose toxicity of medicinal herbs is still lacking. However, the historic usage of any plant for biological purposes should be emphasized as it in no way, shape, or form guarantees plant security.[9]

Indian culture is fundamentally based on traditional medicine, predating western medicine's development by thousands of years. The "The Way of Ayurvedic Plants" and "Charaka Samhita" books provide a thorough overview of Indian traditional medicinal herbs. This book provided a starting point for phytochemists and ethnobotanists who desired to investigate and research medicinal plants. These research all contribute to the corpus of knowledge on Indian medicinal plants.[10] India has long recognized the *Asteraceae* family of plants as a rich source of bioactive compounds that are useful in the treatment of a wide range of illnesses. After a thorough review of the literature, we came to the conclusion that acute and subacute dermal toxicity tests on laboratory animals have not yielded any conclusive findings about the safe dose range of an ethanolic extract from the root bark of *Berberis aristata*.

MATERIAL AND METHODS

Materials

A rare herbal plant called *Berberis aristata* was discovered in the mountainous region of India. In March 2022, plant material was gathered in Uttarakhand, India's Garhwal region. The root bark was washed, then dried outside. The Scientist in Charge, BSI, Allahabad (Central region), India, certified the plant's root bark. A voucher sample was delivered to the department (Accession No.: 104540).

Preparation of Root-bark Extract

The dried root bark was ground into a coarse powder and passed through mesh no. 18. Powdered root bark was extracted with petroleum ether, diethyl ether, and ethanol using the soxhlet equipment. The extract was compressed to dryness in a vacuum oven. In the refrigerator, the extracts were stored at a temperature of 2 to 8°C. Following the subsequent extract, the ethanolic extract was chosen for further investigation because, according to phytochemical screening, it contains a variety of phytochemicals.

Animals

Healthy adult (either sex) wistar rats weighing 250–300 g were procured from the IFTM University's animal house in Moradabad. The animals were kept in polypropylene cages at a temperature of 28°C, with a relative humidity of 60–70% and a 12:12 hours dark/light cycle. Throughout the trial, the animals had free access to normal pellet meals. Mineral water was available to the animal for a price. The institute animal ethics committee (IAEC), IFTM University, Moradabad, authorized the pharmacology and acute toxicity procedures with reference number CPCSEA (2017/837ac).

Skin Preparation for Dermal Toxicity Study

As per the OCED guidelines no 402 and 410, at least 10% of surface areas of the rat's skin were clear around the dorsal thoracic region with the help of hair removing cream and then manually shaved with a razor blade. The whole process was done under anesthetic condition, ketamine (50 m/kg) and xylene (5 mg/kg) was used as anesthesia. The root-bark extract was applied topically to the dorsal regions of rats (Figure 1).[11]

Acute Dermal Toxicity Study

The acute dermal toxicity research was evaluated in accordance with OECD Guideline 402. All of the rats in that study were fasted overnight and given free access to water prior to the experiment. All the animals were divided into four groups, each with six either nulliparous or not pregnant rats. According to the OCED recommendations, the acute dermal toxicity study used a limit dose of 2000 mg/kg b.w.; this dose can be regarded as a lower dose. Another dose, 5000 mg/kg, which is to be considered a larger dose, is also included for a better understanding of the toxicity. 5000 mg/kg of white soft paraffin (10% w/w) was given to the positive control group in contrast. All dosages were topically administered once on the first day of the trial. The dose was applied to the rat's dorsal side. All rats were observed for the first 24 hours, with the initial 6 hours receiving particular focus. All rats were meticulously weighed and evaluated after 14 days using biochemical, haematological, and other criteria in accordance with OCED recommendations.[12]

Grouping of Animals in the Acute Dermal Toxicity Study

The animals for acute dermal toxicity were grouped as shown in Table 1.

Sub-acute Dermal Toxicity Study

Subacute dermal toxicity was assessed in accordance with OCED recommendations 410. All of the rats were placed into five groups for this investigation, each with five male and five female rats. The energy and environmental building alliance (EEBA) was topically given once daily for 28 days throughout this investigation, and all of the animals were observed and watched over by a webcam. According to OECD rules, every procedure and observation was carried out 410 (Table 2).[13]

Table 1: Grouping of animals in the acute dermal toxicity study

Group Name	*Treatment*	*Dose*
Group I	No treatment	None
Group II	Paraffin treatment	5000 mg/kg
Group III	EEBA	2000mg/kg
Group IV	EEBA	5000mg/kg

Table 2: Sub-acute dermal toxicity study

Group Name	*Treatment*	*Dose*
Group I	No treatment	None
Group II	Paraffin treatment	2000 mg/kg
Group III	EEBA	500 mg/kg
Group IV	EEBA	1000 mg/kg
Group V	EEBA	2000 mg/kg

Table 3: General behaviors during acute dermal toxicity study

Observation	*Control Group*	*Paraffin treated*	*2000 mg/kg EEBA*	*5000 mg/kg EEBA*
Body Weight	Not change	Not change	Not change	Not change
Temperature	No effect	No effect	No effect	No effect
Food intake	Normally	Normally	Normally	Normally
Urination	Normally	Normally	Normally	Normally
Rate of respiration	Normally	Normally	Normally	Normally
Change in skin	No effect	No effect	No effect	No effect
Drowsiness	No	No	No	slight drowsiness
Sedation	No	No	No	No
Eye colour	Normal	Normal	Normal	Normal
Diarrhoea	None	None	None	None
General physique	Normal	Normal	Normal	Normal
Coma	No	No	No	No

Grouping of Animals in the Sub-acute Dermal Toxicity Study

The animals for sub-acute dermal toxicity were grouped as follows:

Termination of the Experiment

All of the animals fasted for a whole night following the sub-acute dermal toxicity investigation. Ketamine HCl was used in high doses for euthanasia (80 mg/kg i.p.). A heart puncture was used to obtain the blood sample, which was then placed in heparinized and non-heparinized bottles for the assessment of various haematological and biochemical parameters. A sample of the skin, liver, and kidney was also obtained for histological analysis. In the end, all of the rats' organs were removed to determine the average organ weight.[14,15]

Statistical Analysis

The results of the biochemical, hematological and other studies were presented as mean ± SEM. All the data of the results were expressed as mean ± SEM (n=6) and analysis was done by one-way ANOVA followed by Tukey's Test.

RESULT

Acute Dermal Toxicity Study

Following topical application of EEBA, there were no indications or symptoms of dermal toxicity in the acute dermal toxicity trial at doses of 2000 and 5000 mg/kg b.w. The general behavior seen throughout the first 4 hours is depicted in Table 3.

As a result, the EEBA appeared safe at a dosage of 5000 mg/kg, and the LD_{50} was estimated to be > 5000 mg/kg. In comparison to the control group, there were signs of little sleepiness following the administration of EEBA at a dosage of 5000 mg/kg. The parameters reported in the acute cutaneous toxicity investigation following administration of the test plant extract were compared to those observed in the normal (Gr. I) and paraffin-treated (Gr. II) groups (Tables 3 and 4).

Sub-acute Toxicity Study

Using a limit test dosage of 1000 mg/kg, the OECD guideline 410 calculated the sub-acute dermal toxic effect of EEBA. Following dermal application of the EEBA at doses of 500, 1000, and 2000 mg/kg b.w., no treatment-related adverse symptom or mortality was found even after 28 days. According to the study, there are no differences between the extract-treated groups and the control group in terms of any clinical effects or adverse effects.

Effect of EEBA on Relative Organ Body Weight

In that investigation, no appreciable changes in the average organ weight of the animal subjects in the plant-treated groups were seen. The impact of EEBA on the weight of essential organs relative to body weight is shown in Table 5. The results also demonstrated that essential organs such as the kidney, liver, heart, and lungs do not significantly differ.

Effect of EEBA on Hematological Parameters

The outcomes of the hematological test parameters are shown in Table 6. All the hematological measures were determined to be within normal ranges compared to the control group. When comparing the EEBA group data to the control groups, there were no overt toxicity indications and no significant changes in the hematological markers.

Effect of EEBA on Biochemical Parameters

The outcomes of the main biochemical test parameters are shown in Table 7. All of some biochemical parameters, such as total protein (TP), total bilirubin (TB), albumin, globulin, urea, creatinine, and uric Acid, were found to be within normal ranges when compared to the control group, while other parameters, such as Serum glutamic oxaloacetic transaminase (SGOT), and Serum glutamic pyruvic transaminase (SGPT), were marginally significantly different when compared to

Table 4: Mortality rate of rats

Groups	*Drug*	*Drug*	*Mortality rate (%)*
Group I	None	None	0
Group II	Paraffin	2000mg/kg	0
Group III	EEBA	2000mg/kg	0
Group IV	EEBA	5000mg/kg	0

Table 5: Effect of dermal application of EEBA on average organ weight (g) of rats

Organ	*Average organ weight*				
	Control	*Paraffin*	*EEBA (500 mg/kg)*	*EEBA (1000 mg/kg)*	*EEBA (2000 mg/kg)*
Liver	3.125 ± 0.012	2.925 ± 0.011	3.115 ± 0.011	3.128 ± 0.010	3.125 ± 0.016
Kidney	1.220 ± 0.007	1.123 ± 0.008	1.221 ± 0.008	1.230 ± 0.007	1.198 ± 0.008
Heart	0.652 ± 0.004	0.552 ± 0.007	0.671 ± 0.007	0.661 ± 0.006	0.671 ± 0.006
Lungs	4.121 ± 0.021	3.921 ± 0.022	4.111 ± 0.020	4.124 ± 0.022	4.114 ± 0.021
Spleen	0.202 ± 0.006	0.219 ± 0.004	0.201 ± 0.005	0.203 ± 0.003	0.207 ± 0.004

Table 6: Effect of dermal application of EEBA on Hematological parameters of rats

Parameters	*Effect of dermal B. aristata extracts on haematological parameters.*				
	Control	*Paraffin treated*	*EEBA (500 mg/kg)*	*EEBA (1000 mg/kg)*	*EEBA (2000 mg/kg)*
Hb	13.950 ± 0.460	11.233 ± 0.276	12.200 ± 0.273	12.500 ± 0.195	13.700 ± 0.195
Lymphocyte	56.968 ± 1.005	50.272 ± 0.867*	56.775 ± 0.469	56.233 ± 1.041	55.300 ± 0.861
MCH	28.783 ± 0.625	25.392 ± 0.309	29.108 ± 0.411	28.125 ± 0.291	28.658 ± 0.395
MCHC	35.533 ± 0.519	31.983 ± 0.519	35.523 ± 0.485	35.400 ± 0.465	36.300 ± 0.229
MCV	89.600 ± 1.472	79.067 ± 1.066*	83.217 ± 0.572	84.600 ± 1.255	84.733 ± 0.883
Monocyte	4.167 ± 0.307	4.033 ± 0.247	4.400 ± 0.208	4.717 ± 0.192	4.767 ± 0.254
Neutrophils	28.855 ± 0.313	23.052 ± 0.229	28.300 ± 0.255	27.680 ± 0.368	27.422 ± 0.280
PCV	43.233 ± 1.316	39.423 ± 0.451	41.783 ± 0.408	42.852 ± 0.627	42.722 ± 0.411
RBC	7.988 ± 0.701	7.050 ± 0.551	7.847 ± 0.698	7.633 ± 0.782	8.093 ± 0.555
WBC	12.138 ± 0.244	11.725 ± 0.253	11.898 ± 0.128	12.533 ± 0.168	11.822 ± 0.714

Table 7: Effect of dermal application of EEBA on Biochemical parameters of rats

Parameters	*Effect of dermal B. aristata extracts on biochemical parameters.*				
	Control	*Paraffin treated*	*EEBA (500 mg/kg)*	*EEBA (1000 mg/kg)*	*EEBA (2000 mg/kg)*
A/G ratio	1.800 ± 0.100	1.121 ± 0.058	1.617 ± 0.600	1.733 ± 0.057	1.833 ± 0.021
Albumin	4.250 ± 0.118	3.067 ± 0.049*	4.233 ± 0.056	4.233 ± 0.138	4.683 ± 0.060
ALP	122.667 ± 0.615	111.000 ± 0.568	129.333 ± 0.715	136.000 ± 1.033	139.000 ± 2.160
Bilirubin (TB)	0.633 ± 0.123	0.350 ± 0.062	0.650 ± 0.085	0.667 ± 0.076	0.683 ± 0.031
Creatinine	0.412 ± 0.034	0.282 ± 0.021*	0.365 ± 0.020	0.370 ± 0.019	0.355 ± 0.022
SGOT	30.050 ± 0.340	25.300 ± 0.301	28.333 ± 0.422	33.500 ± 1.432*	38.367 ± 2.050*
SGPT	65.750 ± 0.998	59.350 ± 0.728	71.083 ± 0.623	82.598 ± 0.684*	87.367 ± 1.495*
Total cholesterol (TC)	118.500 ± 2.247	106.000 ± 1.317	18.500 ± 1.432	122.000 ± 0.856	121.667 ± 0.803
Total protein (TB)	6.083 ± 0.087	4.950 ± 0.043	5.917 ± 0.048	5.917 ± 0.048	6.056 ± 0.085
Urea	32.858 ± 0.415	29.577 ± 0.435	32.730 ± 0.430	32.167 ± 0.560	32.327 ± 0.521
Uric acid	0.548 ± 0.007	0.245 ± 0.006*	0.540 ± 0.009	0.545 ± 0.006	0.547 ± 0.043

control at doses of 1000 and 2000 mg/kg. According to the findings, there was no significant toxicity.

Effect of EEBA on Histopathological Parameters

Figure 1 shows the findings of histological analysis of skin sections in rats treated with normal base and ethanolic extracts (Figure 1A). The histological assessment of all the instances in the control and treatment groups was done in the current intense, and sub-intense dermal toxicity ponders. In the rats who received the ethanolic meals produced from the ground removal in general, no minor damage linked to the therapy was found. The most striking discovery is that no treatment-related changes were seen in any of the measurements, and histological tests revealed no signs of toxicity on the skin tissue. Figure 1B shows the results of a histological examination of a kidney slice treated with a normal base and EEBA. The intact glameli and tubules were found in the kidneys of rats. Tubular necrosis was absent in rats given 500 mg/kg body weight of EEBA, whereas tubular necrosis and lymphocytic infiltration were minimal in rats given 1000 and 2000 mg/kg body weight of ethanolic extract of *B. aristata*. In rats given 2000 mg/kg b.w. of EEBA, cortical necrosis, and tubular edema were not present. Figure 1C shows the results of histological analysis of liver sections in rats treated with normal base and EEBA. Normal hepatic plates and portal veins were seen in the livers of rats. There was no hepatic necrosis in rats given 500 and 1000 mg/kg b.w.

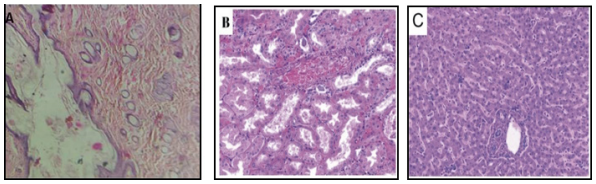

Figure 1: Photomicrographs of Histopathological slides of vital organs(A) Skin section (B) Kidney (C) Liver.

EEBA, however, there was very little hepatocyte degeneration in rats given 2000 mg/kg b.w. EEBA.

DISCUSSION

Because they have few side effects, therapies made from plants are regarded as safe and are now frequently employed as a source of pharmaceuticals in the medical field. Because bioactive compounds derived from herbal plants are believed to be secure and to have no detrimental impact on health, they are commonly used.[16] Researchers prefer plant-based therapies as an alternative to allopathic pharmaceutical drugs in the present era since they are acknowledged to play a significant role in managing a number of chronic illnesses.[17] The toxicity and adverse effects of this therapy, however, have not been the subject of any scientific research. As a result, the objective of this study was to evaluate the EEBA for investigations of acute and subacute toxicity as well as to identify the dose range that might be used as an appropriate reference for subsequent studies. On laboratory animals, the cutaneous acute toxicity research of the evaluated plant extract was done at single doses of 2000 and 5000 mg/kg b.w. for the first 4 hours, then for 72 hours for any adverse effects that might have occurred beyond the treatment period. There were no discernible behavioural or mortality effects in any of the groups. Over 5000 mg/kg is thought to be the LD_{50}. A prospective safe and low toxic substance is any pharmaceutical drug or substance with an oral LD_{50} more than 1000 mg/kg.[18] This suggests that 5000 mg/kg b.w. of EEBA administered once is not hazardous. A sub-acute toxicity study was conducted utilising dosages of 500, 1000, and 2000 mg/kg of extract in accordance with OECD recommendations. Both the control and treatment groups had normal relative weights of vital organs like the liver, kidney, heart, pancreas, and spleen, showing no negative effects and being statistically non-significant ($p > 0.05$). After 28 days of treatment with plant extracts, there was no significant difference between the hematological parameters of the treatment group and the control group ($p > 0.05$). In contrast to the control group, the estimation of blood biochemical parameters in treated animals demonstrated non-significance ($p > 0.05$). However, when compared to the matching control group for extract doses of 500, 1000, and 2000 mg/kg, the transaminase enzymes SGOT (AST) and SGPT (ALT) were shown to be positive, with a substantial increase ($p > 0.001$) in extract-treated animals. Numerous studies have linked liver injury to elevated blood levels of hepatic enzymes and transaminases (SGPT and SGOT). As a result, the increase in liver hepatic enzyme (SGPT and SGOT) levels after administration of the ethanolic extract may be attributable to a phytochemical compound that, at higher dosages, has the potential to cause liver damage. However, these alterations might not be significant from a toxicological standpoint. Future *in-vivo* and clinical research of *B. aristata* will benefit from the crucial information provided in this work regarding the acute and sub-acute skin toxicity profile of this plant. *B. aristata* extract was shown to be safe when evaluated for cutaneous subacute toxicity in lab animals.

CONCLUSIONS

The results of this study show that when *Berberis aristata* extract is treated topically for 28 days at various doses of 500, 1000, and 2000 mg/kg body weight, it demonstrates nontoxic behaviour, as well as 2000 and 5000 mg kg. In the future, a greater dose of ethanolic extract of *B. aristata* will be employed, as in the current study, implying that little modifications are created and nontoxic behaviors are noticed. As a result, *Berberis aristata* extract might be regarded safe for topical usage and other cosmetic purposes. The plant may be used to develop a formulation that may further be subjected to a clinical trial study.

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