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Research Article

ACUTE AND SUBACUTE TOXICITY STUDIES OF *INDIGOFERA BARBERI* GAMBLE, AN ENDANGERED MEDICINAL PLANT

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ABSTRACT

Indigofera barberi Gamble, is a shrub used for various therapeutic activities in traditional system of medicine including liver disease. In this paper, we have evaluated the acute and subacute toxicity studies of crude extracts of *Indigofera barberi* Gamble. *Indigofera barberi* was administered in doses from 100 to 2000 mg/kg (single dose) for the acute toxicity study. In subacute toxicity test animals were administered 500 and 1000 mg/kg doses by orally for 28 consecutive days. The subacute toxicity study of *Indigofera barberi* did not reveal alterations in body weight, food and water consumptions. The haematological parameters, relative organ weights, biochemical and lipid profile analyses did not show any significant differences between control and treated groups. No abnormalities or histopathological changes were observed in the liver cells of all treated groups. The study concluded that administration of *Indigofera barberi* in wistar albino rats is nontoxic and is safe for use.

Keywords: Toxicity study, Biochemical parameters, Lipid profile, Lethal dose

INTRODUCTION

In large number of peoples of world population, still herbal medicines are popular remedies for ill health. Asian countries like India, China, Indonesia, and Japan herbal medicines are used to treat and prevention of various diseases. Many traditional system of medicine still used for the treatment such as Ayurveda, Siddha, Chinese and Kampo etc. In all the traditional system of medicine herbal ingredients are most common. In modern society, there is increased attentiveness in use of complementary and alternative medicine; scientific evidence for the safety and efficacy of several herbal medicines for various disorders has been reported[1]. Nearly 60% of Americans reported using various alternate therapies for the treatment of both chronic illness and disease prevention. In 1997, World Health Organization suggested that effective, locally available plants to be used as substitutes or alternate or replacement for drugs. The speedy growth of use of herbal medicines containing herbal preparations, there is restricted evidence available about the effectiveness and toxicity. The pharmacological effects of many herbal medicines have been studied in various laboratories, where are many limitations regarding safety and efficacy of herbal medicines and their herbal preparation. Since there is urgent needs to be done to develop the evidence base for herbals, botanicals and dietary supplements[2]. The main objective of the present study was to evaluate the oral toxicity, safety and tolerability profile in long term treatment of *Indigofera barberi* in rats according to the guidelines established by the Organization for Economic Cooperation [OECD].

MATERIAL AND METHODS

Collection of plant material

The aerial parts of the above plant were collected from Thalakona (Nelakona regions) of Chitoor District of Andhra Pradesh, India in the month of November 2010. Taxonomically the plant was identified by Professor P. Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. The voucher for the herbarium specimen (PARC/2012/1246) has been preserved in our laboratory for further reference. The aerial parts of *Indigofera barberi* were shade dried for about two and half months, then segregated, pulverized by a mechanical grinder and passed through a 22 mesh sieve. The coarse powdered plant materials were kept in an airtight container for further analysis.

Preparation of various extracts from *Indigofera barberi*

The powdered plant materials were successfully extracted with petroleum ether (40-60ºC) by hot continuous extraction method in Soxhlet apparatus for 24 hrs[3]. Then this marc was dried and then subjected to chloroform extraction (60ºC) for 24 hrs, then marc was dried and then it was subjected to ethanol (95%) extraction (80ºC) for 24 hrs. The extracts were filtered and concentrated using rotary flash evaporator and residues were dried in a desiccators over sodium sulphite below 60°C.

The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken using the formula given below.

$$\text{Percentage yield with reference to crude plant} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

Phytochemical screening

Freshly prepared extracts were subjected to phytochemical screening for the detection of various constituents using conventional protocol[4].

Acute toxicity activities of various extracts of *Indigofera barberi*

Determination of acute oral toxicity is usually the initial selection step in the assessment and valuation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals[5].

The acute toxicity studies of various extracts of *Indigofera barberi* were carried as per (OECD) draft guidelines 423 adopted on 17th December 2001 acknowledged from Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA). Depending on the mortality and/or the morbidity status of the animals, an average 2-4 steps may be require allowing decision on the acute toxicity of the substance/extracts. This procedure was reproducible, uses very few animals and was able to rank substances/extracts in a similar manner to the other acute toxicity testing method. The acute toxic class method is based on biometric evaluation with fixed doses, adequately separated to enable a substance to be ranked for classification purpose and hazard assessment[6].

Acclimatization of Animals

Both sex wistar albino rats (130-180g) were maintained under standard laboratory condition at the centre for experimental animals at Annamalai University. The study was approved by the Animal Ethical Committee of Rajah Muthiah Medical College and Hospital [Registration No 160/1999/ (CPCSEA) Annamalai Nagar, Tamil Nadu, India. After seven days of acclimatization, the animals were randomly assigned for the acute toxicity groups. Each group containing three animals and they were housed individually in labelled cages with solid plastic sides and floor with stainless steel grid tops. Animals were allowed free access to standard pellet diet (Ashirwad Industries Ltd; Bengaluru, India) and water ad libitum. They were maintained in controlled laboratory conditions of 12 hours dark/light cycle, 22±2ºC temperatures and 45-60% humidity.

Administration of various extracts of *Indigofera barberi*

Three animals were used for each step of the study. Animals made to fast prior to dosing (food was withdrawn overnight and water is withdrawn 3 hours before drug administration) following the period of fasting. The animals are weighed and the extracts are administered in a single dose, as 1% suspension in gum acacia, by oral intubation food was withheld for further one hour after the administration of drug. The starting dose levels selected for the study with a dose of 5 mg/kg and the dose was increased step by step to 50, 300 and 2000 mg/kg body weight. The mortality of the dosed animals at one step is determining the next step. The procedure flow chart described the procedure followed for each of the starting doses. The time interval between treatment groups was examined by the onset, period and harshness of toxic signs. Treatment of the animals at the subsequent dose should be belated until one becomes confident of survival of the earlier dosed animals.

Observations

The animals were observed individually at every 30 minutes for the first 24 hours thereafter for a total of 14 time at which signs of toxicity appear and disappear are observed systematically and recorded for each animal. Additional signs of toxicity such as changes in bodyweight, skin and fur, eyes and mucus membranes, respiratory system, circulatory system, autonomous system and central nervous system, somatic motor activity and behaviour pattern are also recorded. Attention is given to observe the tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The presence or absence of the given compound related mortality of the dosed animals at one step is determining the next step. Any mortality during the experiment for 14 days is observed and recorded.

Sub-Acute Toxicity Study

Methodology

Sub-acute toxicity was carried out in accordance to the OECD guideline; Test Guide lines 407. Both sex wistar albino rats (weight range 30-180gm) are selected and separated into 7 groups with 3 male and 3 female rats in each. The groups designed for the study is as follows

Table.1: Experimental protocol for evaluation of Sub-acute toxicity

Group	Treatment
Group- I	Rats were treated with 5ml/kg saline, per orally ,Control group
Group- II	Rats were treated with pet. ether extract of *Indigofera barberi* (500mg/kg/body weight) in 0.5% w/v CMC, p.o.
Group- III	Rats were treated with pet. ether extract of *Indigofera barberi* (1000mg/kg/body weight) in 0.5% w/v CMC, p.o.
Group- IV	Rats were treated with chloroform extract of *Indigofera barberi* (500mg/kg/body weight) in 0.5% w/v CMC, p.o.
Group- V	Rats were treated with chloroform extract of *Indigofera barberi* (1000mg/kg/body weight) in 0.5% w/v CMC, p.o.
Group- VI	Rats were treated with ethanolic extract of *Indigofera barberi* (500mg/kg/body weight) in 0.5% w/v CMC, p.o.
Group -VII	Rats were treated with ethanolic extract of *Indigofera barberi* (1000mg/kg/body weight) in 0.5% w/v CMC, p.o.

Choosing Group I as control all the remaining groups (Group II-VII) has been considered as treated groups. For all the above stated groups, selected doses should be provided for duration of 28 days.

Observation

All the treated groups and control group are experiential for mortality and morbidity two times a day, till the end of the research and clinical investigations are made once every day to identify signs of toxic condition. A body weight has been observed as soon as a week and all other findings had been exact same as explained in the acute toxicity study.

Blood Analysis

At the end of the research period, the overnight fasted rats were anesthetized, whole blood sample was collected by cardiac puncture for haematological and biochemical evaluation. Blood parameters such as red blood corpuscles (RBC), haemoglobin (Hb), white blood corpuscles (WBC), Platelets and Eosinophils had been analysed by fully automated analyser[7]. Biochemical parameters like Serum glucose[8], Urea[9], Creatinine[10], Albumin, Total protein[11] AST, ALT[12] and ALP[13] has been analysed. Lipid

profiles such as Total cholesterol, Phospholipids, Triglycerides and free fatty acid[14] has been analysed.

Histopathology

On 28th day all the group of rats immediately right after the blood collection all the rats have been euthanized for gross pathological exams of all major internal organs. Relative organ weights of all the rats have been determined by weighing the liver organs and delivered for histopathological studies. At first histopathological exams should be done for control and high dosage treated groups, if any, unusual changes observed in high dosage treated group are studied. In 10% neutral buffered formalin organs are fixed, trimmed and cells 5µ thickness are ready and stained with haematoxylin and eosin for histopathological studies.

Statistical analysis

The Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Newmann-Keul's multiple range tests. The values are represented as Mean+SEM. Probability value of $P < 0.01$ was determined to be statistically significant.

RESULTS

Table 2: Results of successive solvent extraction - consistency, colour and percentage yield of *Indigofera barberi*

Parameters	Extracts		
	Pet. ether	Chloroform	Ethanol
Consistency	Waxy	Oily	Viscous
Colour (Visible/ Day light)	Greenish black	Green	Reddish black
Percentage Yield (%w/w)*	1.24	3.14	3.28

*Mean ± SEM (n = 3)

Results of phytochemical analysis of various extracts of *Indigofera barberi*

The results of the phytochemical analysis of various extracts from *Indigofera barberi* were carried out for petroleum ether extract; chloroform extract and ethanolic extract separately were given in the following Table 3. The preliminary phytochemical screening showed that PEEIB was rich in glycosides, tannins & phenolic compounds and flavonoids; CEIB was rich in glycosides, flavonoids, tannins & phenolic compounds and carbohydrates and EEIB was rich glycosides, steroids, flavonoids, tannins & phenolic compounds and carbohydrates. Among these three different fractions of plant extracts, chloroform and ethanolic extracts show the presence of major classes of phytoconstituents.

Table 3: Results of phytochemical analysis of various extracts of *Indigofera barberi*

Phytochemical	Pet. ether extract	Chloroform extract	Ethanolic extract
Alkaloids	–	–	–
Glycosides	+	+	+
Steroids	–	–	+
Flavonoids	+	+	+
Tannins & phenolic compounds	+	+	+
Proteins and free amino acids	–	–	–
Carbohydrates	–	+	+
Volatile oils	–	–	–
Saponins	–	–	–

(+): presence (-): absence

Acute toxicity study

The acute toxicity of various extracts of *Indigofera barberi* was carried out as per OECD-423 guidelines for safe dose administration to animals and the study was carried out. From the acute toxicity studies (as per OECD- 423 guidelines), the LD_{50} values of PEEIB, CEIB and EEIB were determined as 2000mg/kg/oral individually. At this dose levels the gross behaviour studies in autonomic and central nervous systems shows normal behaviour pattern of eyes, mucous membranes, respiration, salivation, sense of touch & sound, sleep & urination and further no sign of changes in skin, convulsions, coma, diarrhoea, hind limb paralysis, lethargy, mortality, tremors and writhing was observed. There is no moribund or mortality up to the dose level 2000 mg/kg/body weight and the animals were alive up to the end of the study.

Subacute toxicity study

The subacute toxicity of various extracts of *Indigofera barberi* carried out accordance to the OECD test guidelines 407 (28 days repeated dose by orally)

Table 4: Results of changes in body weight of rats following treatment with different doses of extract of *Indigofera barberi* for 28 days repeated dose oral toxicity study

Groups	Body weight (gm)	
	Initial	After 28 days treatment
Group-I – Control (saline 5ml.kg^{-1})	175 ± 1.3	194 ± 1.5
Group-II - Pet. ether extract (500mg.Kg^{-1})	164 ± 1.5	187 ± 1.6
Group-III - Pet. ether extract (1000mg.Kg^{-1})	160 ± 1.2	180 ± 1.9
Group-IV - Chloroform extract (500mg.Kg^{-1})	163 ± 1.4	193 ± 1.8
Group-V - Chloroform extract (1000mg.Kg^{-1})	174 ± 1.5	193 ± 1.4
Group-VI - Ethanolic extract (500mg.Kg^{-1})	180 ± 1.3	193 ± 1.2
Group-VII - Ethanolic extract (1000mg.Kg^{-1})	170 ± 1.7	193 ± 1.7

The values are expressed as mean± SEM; N = 06 animals in each group.

Effect on body weight

The body weight of rat in control group showed gradual increase during the 28th day's treatment period up to of weight gain. The values are represents in table 4. In contrast administration of three different extract at two different dose 500 and 1000 mg/kg/body weight per day doses showed increase in body weight.

Table 5: Results of relative organ weights of various extracts of *Indigofera barberi* treated rats in 28 days repeated dose oral toxicity study

Groups	Liver (g/100 g)	Heart (g/100 g)	Lungs (g/100 g)	Kidney (g/100 g)	Spleen (g/100 g)
Group-I–Control (saline 5ml.kg^{-1})	4.28 ± 0.12	0.43±0.018	0.87±0.18	0.46 ± 0.04	0.028±0.010
Group-II - Pet. ether extract (500mg.Kg^{-1})	4.26± 0.13^{ns}	0.42±0.020^{ns}	0.84±0.16^{ns}	0.47 ± 0.09^{ns}	0.026 ±0.012^{ns}
Group-III - Pet. ether extract (1000mg.Kg^{-1})	4.28 ± 0.38^{ns}	0.40±0.022^{ns}	0.85±0.17^{ns}	0.46 ± 0.28^{ns}	0.024 ±0.012^{ns}
Group-IV - Chloroform extract (500mg.Kg^{-1})	4.21 ± 0.22^{ns}	0.44±0.038^{ns}	0.87±0.22^{ns}	0.45 ± 0.01^{ns}	0.025 ±0.008^{ns}
Group-V - Chloroform extract (1000mg.Kg^{-1})	4.24± 0.28^{ns}	0.42±0.036^{ns}	0.85±0.28^{ns}	0.47 ± 0.24^{ns}	0.024 ±0.052^{ns}
Group-VI - Ethanolic extract (500mg.Kg^{-1})	4.26 ± 0.52^{ns}	0.43±0.042^{ns}	0.83±0.30^{ns}	0.48 ± 0.68^{ns}	0.025 ±0.056^{ns}
Group-VII - Ethanolic extract (1000mg.Kg^{-1})	4.25 ± 0.46^{ns}	0.46±0.048^{ns}	0.84±0.26^{ns}	0.47 ± 0.81^{ns}	0.026 ± 0.012^{ns}

The values are expressed as mean±SEM; ns - Statistically not significant. The results of group I animals were compared with other groups II, III and IV, V, VI and VII. There is no statistically significant variation found when compare with control group-I.

Effect on relative organ weight

The intact weight of organs was converted to relative weight of 100 g body weight as shown in table: 5. The result showed that different extract in different doses (500 and 1000 mg/kg per day) administered for 28 days has no significant effect on various organ weight compared to control group.

Table 6: Results of haematological profile of various extracts of *Indigofera barberi* treated rats in 28 days repeated dose oral toxicity study

Groups	Hb (g/dL)	RBC (10^6/µl)	Platelets (10^3/µL)	WBC (10^3/µl)	Eosinophils (%)
Group-I – Control (saline 5ml.kg^{-1})	12.34± 1.82	8.6 ± 0.34	1045 ± 13.8	11.38 ±1.34	1.8 ±0.54
Group-II - Pet. ether extract (500mg.Kg^{-1})	12.32± 1.12^{ns}	8.8 ± 0.22^{ns}	1039 ± 14.2^{ns}	11.37±1.32^{ns}	1.7 ±0.53^{ns}
Group-III - Pet. ether extract (1000mg.Kg^{-1})	12.31± 1.65^{ns}	8.5 ± 0.40^{ns}	1038 ±13.9^{ns}	11.36 ± 1.12^{ns}	1.9 ±0.48^{ns}
Group-IV - Chloroform extract (500mg.Kg^{-1})	12.38± 1.02^{ns}	8.9 ± 0.42^{ns}	1047 ± 12.9^{ns}	11.39 ± 1.68^{ns}	1.8 ±0.58^{ns}
Group-V - Chloroform extract (1000mg.Kg^{-1})	12.39± 1.12^{ns}	8.95 ± 0.17^{ns}	1049 ± 12.5^{ns}	11.40 ± 1.12^{ns}	1.9 ±0.35^{ns}
Group-VI - Ethanolic extract (500mg.Kg^{-1})	12.41± 1.65^{ns}	9.10 ± 0.25^{ns}	1051 ± 11.8^{ns}	11.46 ± 1.62^{ns}	2.0 ±0.56^{ns}
Group-VII - Ethanolic extract (1000mg.Kg^{-1})	12.48± 1.02^{ns}	9.4 ± 0.27^{ns}	1057 ± 11.7^{ns}	11.72 ± 1.02^{ns}	2.3 ±0.12^{ns}

The values are expressed as mean±SEM; N = 6 animals in each group, NS-Statistically not significant. The results of group I animals are compared with other groups II, III, IV, V, VI and VII.
(**Hb**: Haemoglobin, **RBC**: Red Blood Corpuscles and **WBC**: White Blood Corpuscles).

The effect of various extracts of *Indigofera barberi* on haematological indices was examined at the end of treatment (Table 6). Treatment for 28 days has nonsignificant effect on Hb, RBC, platelet count, WBC and eosinophil.

Table 7: Results of biochemical parameters of various extracts of *Indigofera barberi* treated rats in 28 days repeated dose oral toxicity study

Groups	Glucose (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)
Group-I – Control (saline 5ml.kg^{-1})	102.7±2.33	16.10±1.73	0.75 ±0.28	6.45 ±0.12	5.85 ±0.76
Group-II - Pet. ether extract (500mg.Kg^{-1})	102.4±1.88^{ns}	16.18±1.65^{ns}	0.75 ±0.18^{ns}	6.40±0.12^{ns}	5.83 ±0.58^{ns}
Group-III - Pet. ether extract (1000mg.Kg^{-1})	102.5±1.65^{ns}	16.22±1.38^{ns}	0.74 ±0.68^{ns}	6.46±0.52^{ns}	5.80 ±0.68^{ns}
Group-IV - Chloroform extract (500mg.Kg^{-1})	101.4±1.34^{ns}	16.08±1.67^{ns}	0.73 ±0.67^{ns}	6.30 ±0.34^{ns}	5.74 ±0.32^{ns}
Group-V - Chloroform extract (1000mg.Kg^{-1})	101.1±0.98^{ns}	15.68±1.23^{ns}	0.72 ±0.52^{ns}	6.23 ±0.18^{ns}	5.68 ±0.43^{ns}
Group-VI - Ethanolic extract (500mg.Kg^{-1})	100.5±1.23^{ns}	15.44±1.87^{ns}	0.74 ±0.54^{ns}	6.18 ±0.58^{ns}	5.56 ±0.54^{ns}
Group-VII - Ethanolic extract (1000mg.Kg^{-1})	98.4±1.34^{ns}	15.28±1.2^{ns}	0.70±0.24^{ns}	6.10±0.44^{ns}	5.54 ±0.42^{ns}

The values are expressed as mean±SEM; N = 6 animals in each group. NS - Statistically not significant. The results of group I animals are compared with other groups such as II, III, IV, V, VI and VII.

Table 8: Results of effect of different extract of *Indigofera barberi* on biochemical parameters in rats

Treatment	AST U/L	ALT U/L	ALP U/L
Group-I – Control (saline 5ml.kg^{-1})	114 ± 2.1	45.8 ± 1.6	186 ± 2.10
Group-II - Pet. ether extract (500mg.Kg^{-1})	120.5 ± 1.6^{ns}	41.0 ± 1.5^{ns}	179 ± 2.5^{ns}
Group-III - Pet. ether extract (1000mg.Kg^{-1})	122.5 ± 1.6^{ns}	46.0 ± 0.7^{ns}	181 ± 1.23^{ns}
Group-IV - Chloroform extract (500mg.Kg^{-1})	119.5 ± 1.8^{ns}	48.0 ± 1.1^{ns}	175 ± 2.0^{ns}
Group-V - Chloroform extract (1000mg.Kg^{-1})	121.5 ± 1.9^{ns}	49.0 ± 1.4^{ns}	171 ± 2.3^{ns}
Group-VI - Ethanolic extract (500mg.Kg^{-1})	122.6 ± 1.4^{ns}	47.0 ± 1.8^{ns}	180 ± 2.7^{ns}
Group-VII - Ethanolic extract (1000mg.Kg^{-1})	119.8 ± 1.7^{ns}	50.5 ± 1.3^{ns}	173 ± 1.9^{ns}

The values are expressed as mean±SEM; N = 6 animals in each group NS = statistically not significant. (AST-Aspartate Aminotransferase; ALT-Alanine transaminase; ALP-Alkaline phosphatase)

Effect on serum biochemical parameters

The effect of various extracts of *Indigofera barberi* for 28 days doesn't showed significant changes in glucose, urea, creatinine, albumin, total protein, Aspartate Aminotransferase, Alanine transaminase and Alkaline phosphatase (table 7 and 8) at doses500 and 1000 mg/kg per day compare to control.

Table 9: Results of lipid profile of various extracts of *Indigofera barberi* treated rats in 28 days repeated dose oral toxicity study

Groups	Total cholesterol (mg/dL)	Phospholipids (mg/dL)	Triglycerides (mg/dL)	Free fatty acid (mg/dL)
Group-I – Control (saline 5ml.kg^{-1})	99.67± 1.48	108.45 ±4.5	62.64 ±2.87	8.73 ±2.55
Group-II - Pet. ether extract (500mg.Kg^{-1})	99.43±0.98^{ns}	110.20 ±2.8^{ns}	58.50 ±1.34^{ns}	8.50 ±1.24^{ns}
Group-III - Pet. ether extract (1000mg.Kg^{-1})	97.22±1.22^{ns}	108.10 ±3.2^{ns}	64.50 ±1.46^{ns}	9.10 ±1.85^{ns}
Group-IV - Chloroform extract (500mg.Kg^{-1})	98.76±1.52^{ns}	107.68±2.52^{ns}	60.54±1.66^{ns}	8.95±1.87^{ns}
Group-V - Chloroform extract (1000mg.Kg^{-1})	96.49±1.32^{ns}	111.78±2.78^{ns}	59.60±1.48^{ns}	9.18±1.54^{ns}
Group-VI - Ethanolic extract (500mg.Kg^{-1})	92.66± 2.2^{ns}	106.89±3.67^{ns}	58.60±1.50^{ns}	7.90±1.13^{ns}
Group-VII - Ethanolic extract (1000mg.Kg^{-1})	94.50±4.7^{ns}	110.76 ±5.8^{ns}	57.85±1.56^{ns}	8.05±1.76^{ns}

The values are expressed as mean±SEM; N=6 animals in each group. NS = statistically not significant. The results of group I animals are compared with other groups such as II, III, IV, V and VI.

Effect on lipid profile

The effect of various extracts of *Indigofera barberi* for 28 days doesn't showed significant changes in total cholesterol, phospholipids, triglycerides and free fatty acid at doses500 and 1000 mg/kg per day compare to control.

Results of histopathological results of PEIB, CEIB and EEIB in rats liver cells Photomicrograph of sections (H&E staining, magnification × 10)

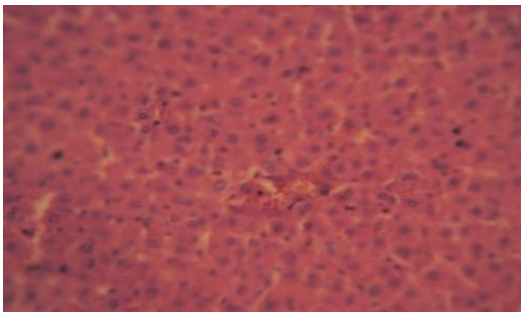

Fig 1: Liver section of normal control rats showing normal liver lobular architecture with well brought out central vein and prominent nucleus and nucleolus (Saline-5ml.Kg^{-1})

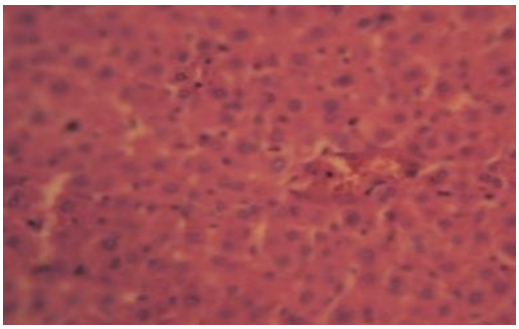

Fig 2: Petroleum ether extracts (500mg.Kg^{-1}) of *Indigofera barberi* treated rat liver section showing normal liver lobular architecture

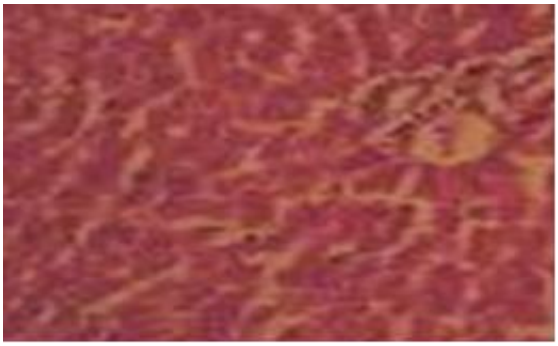

Fig 3: Petroleum ether extracts (1000mg.Kg^{-1}) of *Indigofera barberi* treated rat liver section showing normal liver lobular architecture

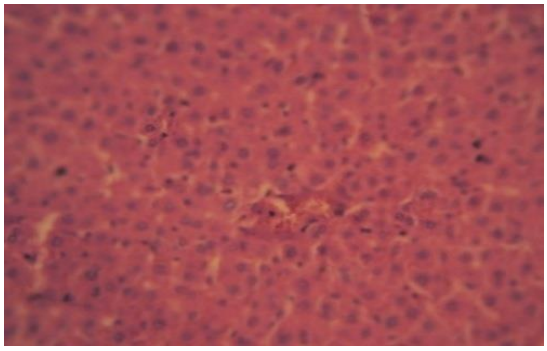

Fig 4: Chloroform extracts (500mg.Kg^{-1}) of *Indigofera barberi* treated rat liver section showing normal liver lobular architecture

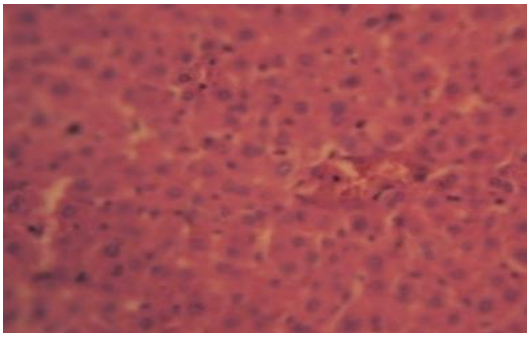

Fig 5: Chloroform extracts (1000mg.Kg^{-1}) of *Indigofera barberi* treated rat liver section showing normal liver lobular architecture

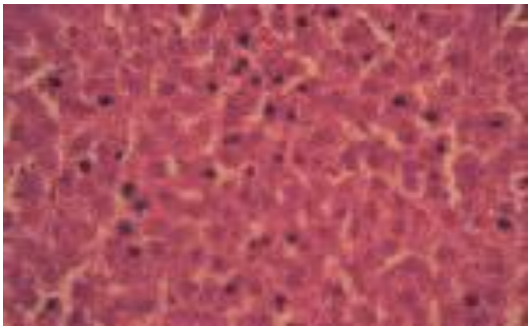

Fig 6:Ethanolic extracts (500mg.Kg^{-1}) of *Indigofera barberi* treated rat liver section showing normal liver lobular architecture

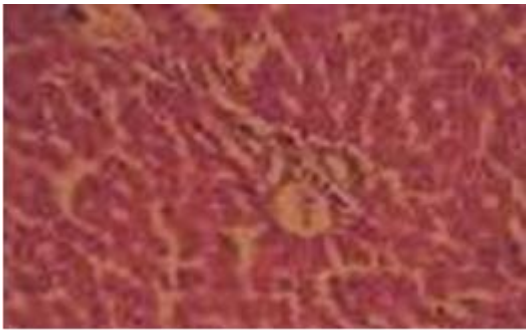

Fig 7: Ethanolic extracts (1000mg.Kg^{-1}) of *Indigofera barberi* treated rat liver section showing normal liver lobular architecture

DISCUSSION

The purpose of this study is to look at the toxicity profile of the *Indigofera barberi*. A 28-days study is considered a subacute study, which is well accepted for eliciting any toxicity on long-term feeding. It gives valuable information on the cumulative toxicity of a substance on target organs or physiological and metabolic effects of the compound at low dose on prolonged exposure. A wide variety of adverse effects can be detected from subacute toxicity studies. The result from such studies can provide information, which will aid in selecting dose level. The long term safety level of a compound can be predicted from acute or shorter than subacute studies. Acutely nontoxic compounds may be toxic on prolonged exposure even at low dose levels due to cumulating, changes in enzyme level and disruption of physiological and biochemical homeostasis. Subacute toxicity studies are generally carried out in few days to three months.

The results of changes in body weight and organ weights of heart, lung, liver, kidney and spleen were recorded relatively between the control and various extracts of *Indigofera barberi* treated groups and the results are shown in the Table 4 & 5. Rats treated with the various doses of the extract (500 and 1000 mg/kg) were not alter in the body weight and no statistically significant differences existed in the absolute and relative weights of all the isolated organs between the treated and the control rats. It was 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[15]. The *Indigofera barberi* did not induce any toxic effect on the kidneys and the other organs going by this indicator, since the absolute and relative weights of the organs are not significantly different from control values.

During the stipulated period of study, behavioural changes, therapy related abnormal signs and death rate was not appeared in rats administered at the doses 500mg and 1000mg/kg body weight of petroleum ether, chloroform and ethanolic extract of *Indigofera barberi*.

All the estimated haematological parameters were resulted within the normal range of rats in the investigated groups. Haematological parameters such as RBC, WBC (Total), Hb, Eosinophils and Platelets, in both control and experimental rats,

were not significantly different for *Indigofera barberi* and the extract treated group. Anaemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cell synthesis by the active constituents of the extract, and decrease in haematological parameters in experimental animals has been associated with anaemia[16].There was no significant change in haematological parameters in the extracts treated animals compared to the control (Table 6), which indicates that there was no lysis of blood cells and/or inhibition in blood cells synthesis by the active constituents of *Indigofera barberi* extract. The above results suggest the non-toxicity of *Indigofera barberi* in rats.

The petroleum ether, chloroform and ethanolic extracts of *Indigofera barberi* (doses 500mg and 1000mg/kg b. w.) has not shown any significant changes in biochemical parameters like such as Glucose, Urea, Creatinine, Albumin, Total protein, AST, ALT and ALP. The results are shown in Table 7 and 8. The petroleum ether and chloroform and ethanolic extracts of *Indigofera barberi* (doses 500mg and 1000mg/kg b.w.) were not shown any significant changes in lipid parameters like total cholesterol, phospholipids, triglyceride and free fatty acids. The results are shown in Table 9. Clinical chemistry parameters were statistically not significant in comparison to control groups. However, there is no alteration shown in histopathological studies of liver tissues which were confirming the absence of adverse effects. No adverse effect has been observed in renal function test also. No mortality is recorded in rats treated with higher dose of 1000 mg/kg. The subacute toxicity studies of PEEIB, CEIB, and EEIB reflected the innocuous nature of this plant extracts on hepatic, renal and haemopoetic system even at high dose.

Microscopic data together with the data of macroscopic evaluation of the animal's organ showed that both test and control groups are practically healthy. According to the data of histological examination, no toxic or allergic effects of *Indigofera barberi* were detected in the test group. No local irritating effects of the drug preparation are observed in the test group during the study period. The photomicrograph slides of rat liver treated with 500 mg/kg/oral and 1000 mg/kg/oral of PEIB, CEIB, and EEIB respectively (Fig 1 to 7) showed distinct hepatic cells with sinusoidal space and central vein having normal architecture. The study also revealed the absence of disarrangement, degeneration of hepatic cells, necrosis, sinusoidal haemorrhages, dilatations, focal steatosis and congestion of central vein and inflammation of portal tract when compared with control animals. Thus, it is concluded that *Indigofera barberi* does not produce any toxic effect in wistar albino rats. From the above results, it is clear that the *Indigofera barberi* did not produced toxic effect in rats.

CONCLUSION

In conclusion, the present results show that petroleum ether, chloroform and ethanolic extract of *Indigofera barberi* Gamble does not cause any apparent in vivo toxicity in an animal model. No death or signs of toxicity were observed in rat treated with extract at doses 500 and 1000 mg/kg body weight, thus its safety in use. The histology examination revealed no changes in the architecture liver of rat, in both control and treated groups. This could be an assurance for the medicinal use *Indigofera barberi* in folk medicine. A detailed experimental analysis of its chronic toxicity is essential for further support of this plant.

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