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Original Article

Effect of Aqueous Extract of *Passiflora edulis* on Biochemical and Hematological Parameters of Wistar Albino Rats

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ABSTRACT

Passiflora edulis is traditionally used in folk lore medicine for the treatment of various ailments. To validate its use in traditional medicine, it is important to evaluate its toxicity in the animal system. Therefore, this study aimed to evaluate the toxicological effects of oral administration of aqueous leaf extract of *P. edulis* in Wistar albino rats. Acute toxicity tests were conducted by the oral administration of 200, 500, 1000 and 2000 mg/kg body weight of the animal. In subacute study, they were administered with various doses of aqueous extract of *P. edulis* (100, 200, 300, and 400 mg/kg body weight) to evaluate its toxicity for a period of 7 days. The effect of aqueous extract of *P. edulis* on organ weight, hematological, renal, and hepatic markers were analyzed. In acute toxicity study, no mortality was seen with in 24 h of the administration of *P. edulis* extract. No signs of neurological and behavioral changes were noticed with in 72 h. In the subacute study, the extract intake has not changed the hematological parameters such as RBC, WBC, and platelets and it was also found that the plasma level of amino transferases, ALP, urea, uric acid and, creatinine were also not altered by the administration of *P. edulis* extract throughout the study. The weight of organ was found to be unaltered in all the doses selected. The acute toxicity study reveals that the oral administration of the extract was found to be safe up to the dose level of 2000 mg/kg. The subacute study indicates that the extract is safe on the bone marrow function and it is neither hepatotoxic nor nephrotoxic. This supports the safety use of the aqueous extract of *P. edulis* in pharmacological studies.

Key words: Acute and subacute toxicity studies, hepatotoxic, nephrotoxic, *P. edulis*

INTRODUCTION

Nature has been considered as a source of medicinal agents from the ancient time itself. An impressive number of modern drugs have been isolated from natural sources and the basis for the isolation is mainly based on the traditional use of these plants in treatment of disease. This plant-based, traditional medicine system still acquire an important place in the health care system.[1] Today, medicinal plants are increasingly being used in most parts of the world as hypolipidemic,[2] contraceptive, abortifacients, emmenagogues, or oxytocic, antihypertensive,[3] treatment for skin diseases, wound healers, antimicrobial, and hypoglycemic.[4] Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes.[5] Hence, a large number of medicinal plants are available, it is very important to do the scientific validation of the drug to know their side effects in treatment. *P. edulis* is a medicinal plant distributed in warm temperatures and tropical regions. The pulp of the fruit acts as stimulant and tonic. The fruit has anticarcinogenic effect. The flower extract of *P. edulis* has sedative and hypnotic

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effect. The dried aerial parts of passion flower (*Passiflora incarnata*) have historically been used for treating insomnia and gastrointestinal complaints. The leaves and stems of *P. edulis* have shown antiinflammatory, antianxiety, anti-tumor, antimicrobial, and antioxidant activity.[6] Since this plant is used for a number of treatments, in this study an attempt was made in *P. edulis* to evaluate its toxicity in the animal system of rat.

MATERIALS AND METHODS

Plant material collection and extraction

The leaves of *P. edulis* Sims (Passifloraceae) were collected in the month of September from Pollachi, Coimbatore district, Tamilnadu, India. The plant material was authenticated by the Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore. A voucher specimen of *P. edulis* (Voucher no: BSI/SRC/5/23/09-10/Tech.-723) was deposited in the departmental herbarium for future reference.

In extraction, the *P. edulis* leaves were washed well with water, shade dried for 15 days in the absence of sunlight, and then powdered. Three-hundred gram of powdered plant material was taken, mixed with 1500 ml of 70°C hot water and kept for 2 days in an orbital shaker. The mixture was then centrifuged at 2500 × *g* and the supernatant collected was concentrated under the reduced pressure in a rotary evaporator. The concentrated extract was then lyophilized. The residue obtained was used for the study, and the remaining residue was kept at −20°C for future use.

Animals

Wister strain of albino rats weighing about 150–200 g were obtained from the animal house of Karpagam University, Coimbatore and used for the study. Rats were housed at constant temperature of 21+ 2°C, humidity (55 + 10%) with a 12-h light, 12-h dark cycle and fed on standard pellets with free access to distilled water. The study was approved by Institutional Animal Ethical Committee constituted for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute toxicity studies

The rats weighing between 150–180 g were divided into four groups of five animal each and treated with various doses of the aqueous extract of *P. edulis* corresponding to 250, 500, 1000, and 2000 mg/kg body weight were administered orally to four groups. Another group of five rats served as control and this received 1 ml of physiological saline. They were all placed under observation immediately for 2 h for any behavioral, neurological changes and then mortality for the next 72 h.

Subacute toxicity studies

The rats weighing between 150–180 g were used for the study. The animals were divided into five groups of four animals each. Different doses of aqueous extract of *P. edulis* corresponding to 100, 200, 300, 400 mg/kg were given orally in an intra gastric tube to four groups of five animals each. Another group of five rats that received 1 ml of physiological saline served as control.

Extract administration

The sample was found to dissolve well in water without leaving any residue. This completely dissolved sample was administered to the rats orally through an intragastric tube continuously for 7 days. At the end of experimental period, all the rats from each group were sacrificed under mild chloroform anesthesia. Through cervical decapitation, 5 ml of blood was collected from each rat, out of which 1 ml mixed with dipotassium ethylene diamine tetra acetic acid was used for hematological studies. The remaining 4 ml blood collected was allowed to clot and the serum separated was used for the evaluation of biochemical parameters. The organs such as liver, kidney, pancreas, spleen, and heart were carefully dissected out and the blood was wiped with filter paper and weighed for the determination of relative organ weight.

Parameters investigated

Relative organ weight

Relative organ weight was determined as follows:[7]

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Hematological parameters

The blood with EDTA was used for the count of RBC, total and differential count of WBC and platelet by standard procedures.[8]

Biochemical estimations

Serum urea, uric acid, creatinine, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were determined by standard procedures in an auto analyzer using Ecoline kits (E.Merck, Mumbai, India).

Statistical analysis

Results are expressed as the Mean ± SD. Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 10.0. and the individual comparisons were obtained by the Duncan multiple range test (DMRT).[9] A value of $P<0.05$ was considered to indicate a significant difference between groups.

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RESULTS

Acute toxicity studies

After the administration of heavy doses of drug, the rats were monitored for the signs of toxicity. The rat that receives high doses of drug seems to be hyper active which persisted for first 11/2 h and then they resume their normal activity without any change in any of their activities. Along with high loco motor activity, they also found to scratch around the mouth for about 10 min. In this study, no mortality was observed till the end of the study.

Subacute toxicity studies

Table 1 illustrates the effect of aqueous extract of *P. edulis* leaf extract on the organ–body weight ratios of rats. The organ body weight ratio of rat liver, kidney, pancreas, spleen, and heart were compared favorably with those of the controls and no significant change was observed at all the doses of the extracts investigated.

The effects of *P. edulis* leaf extract on the hematological parameters of rats were shown in the Table 2. No significant change was observed in RBC, WBC count of both extracts but they significantly vary in neutrophil, lymphocyte, and platelet count. Neutrophil count was increased with a higher dose level, in contrast neutrophil with 400 mg/kg treated rats shown a significant decrease in lymphocyte count, where as in other doses there was a significant increase in lymphocyte number. Similar to lymphocyte, the platelet count also increased in its higher concentration.

Significant reduction in plasma levels of urea, uric acid and creatinine were noted mainly in higher doses [Table 3] of *P. edulis* treatment. Individual variation in the level of SGPT, SGOT, and ALP was noticed among the study groups. Plasma SGOT and SGPT levels in the serum were got reduced by the intake of *P. edulis* in some selected dose level.

DISCUSSION

Acute toxicity studies

The nontoxic nature of the aqueous extract of *P. edulis* and its oral safety at selected dose level was confirmed where the administration of the drug does not lead to mortality of the rats. According to Clarke and Clarke,[10] any compound or drug with the oral LD_{50} estimate greater than 1000 mg/kg

Table 1: Effect of *P. edulis* leaf extract on the organ–body weight ratios of control and experimental groups

Organs	Control	(100 mg/kg)	(200 mg/kg)	(300 mg/kg)	(400 mg/kg)
Liver	4.196 ± 0.003^a	4.198 ± 0.003^a	4.197 ± 0.005^a	4.195 ± 0.004^a	4.194 ± 0.005^a
Kidney	1.433 ± 0.003^a	1.430 ± 0.001^a	1.434 ± 0.003^a	1.432 ± 0.001^a	1.435 ± 0.002^a
Pancreas	0.723 ± 0.002^a	0.720 ± 0.002^a	0.722 ± 0.00^a	0.721 ± 0.00^a	0.720 ± 0.002^a
Spleen	0.861 ± 0.001^a	0.863 ± 0.001^a	0.864 ± 0.001^a	0.862 ± 0.001^a	0.861 ± 0.001^a
Heart	0.669 ± 0.001^a	0.673 ± 0.001^a	0.672 ± 0.002^a	0.670 ± 0.002^a	0.672 ± 0.001^a

Values are expressed as Mean ± S.D of five individual experiments. Values not sharing a common superscript letter differ significantly at $P<0.05$, (DMRT)

Table 2: Effect of *P.edulis* leaf extract on hematological parameters of control and experimental groups

Parametres	Control	(100 mg/kg)	(200 mg/kg)	(300 mg/kg)	(400 mg/kg)
RBC (million/cmm)	5.72 ± 0.20^a	5.71 ± 0.38^a	5.74 ± 0.22^a	5.73 ± 0.14^a	5.70 ± 0.15^a
WBC (million/cmm)	7789 ± 28.94^a	7761 ± 18.96^a	7729 ± 27.73^a	7762 ± 5.08^a	7751 ± 12.53^a
NEUTRO (%)	42.11 ± 0.85^a	44.23 ± 0.25^b	43.21 ± 0.65^{ab}	45.77 ± 0.46^c	46.07 ± 0.63^c
LYMPH (%)	38.47 ± 0.27^a	34.21 ± 0.36^b	35.33 ± 0.45^c	36.03 ± 0.29^{cd}	34.93 ± 0.37^d
PLATELET (cells/cmm)	245776 ± 16.11^a	245746 ± 9.56^a	249669 ± 11.81^b	247565 ± 17.92^b	287570 ± 9.08^c

Values are expressed as Mean ± S.D of five individual experiments. Values not sharing a common superscript letter differ significantly at $P<0.05$, (DMRT)

Table 3: Effect of *P. edulis* leaf extract on hepatic and renal markers in serum of control and experimental groups

Markers	Control	(100 mg/kg)	(200 mg/kg)	(300 mg/kg)	(400 mg/kg)
SGOT (U/l)	55.57 ± 0.14^a	54.86 ± 0.05^b	55.57 ± 0.05^a	54.73 ± 0.10^b	55.43 ± 0.28^a
SGPT (U/l)	55.57 ± 0.14^a	27.3 ± 0.09^a	26.77 ± 0.05^b	27.36 ± 0.09^a	27.42 ± 0.09^a
ALP (U/l)	71.67 ± 0.19^a	71.5 ± 0.09^a	70.77 ± 0.15^b	70.74 ± 0.20^b	71.73 ± 0.21^a
Urea (mg/dl)	24.33 ± 0.07^a	24.5 ± 0.09^a	24.90 ± 0.05^a	23.70 ± 0.09^b	23.57 ± 0.09^b
Uricacid (mg/dl)	2.72 ± 0.12^a	2.67 ± 0.09^b	2.69 ± 0.05^{ab}	2.71 ± 0.09^a	2.70 ± 0.09^{ab}
Creatinine (mg/dl)	0.73 ± 0.03^a	0.69 ± 0.01^b	0.70 ± 0.01^b	0.73 ± 0.02^a	0.71 ± 0.01^{ab}

Values are expressed as Mean ± S.D of five individual experiments. Values not sharing a common superscript letter differ significantly at $P<0.05$ (DMRT)

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could be considered of low toxicity and safe. This supports that the aqueous extract of *P. edulis* was found to be safe up to the dose of level 2000 mg/kg body weight. However LD_{50} has not been considered as a biological constant because many variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions, and time of the day can all affect the $LD_{50\ value}$ obtained. Hence, there are considerable uncertainties in extrapolating the $LD_{50\ value}$ obtained for a species to other species.[11]

Subacute toxicity studies

Effect on organ–body weight ratios

Unfavorable interaction of the plant extract with the major organs would cause cellular constriction and inflammation, which was reflected in the organ/body ratio. In this study, no such alterations were found and this supports the nontoxic nature of *P. edulis*.[12]

Effect on hematological parameters

Altered neutrophil, lymphocyte, and platelet count was explained that the plant extract would have some mild effect on the hematological parameter. Other reasons include the dose level and individual variation of rats. Variation in total count were also observed in a study by the administration of *Hermania incana* leaves that produced mild changes in hematological index.[13] Since blood cells are originated from bone marrow the inertness of the extract on this organ is evident and its use in pharmacological evaluation is again certified. In addition there were no inclusions in the red cells or white cells were observed from the cell morphology[14] again supports the safety nature of the plant extract.

Effect on hepatic and renal markers

Liver and Kidney damage are prominent side effects in severe diabetic patients as well as in severe alloxan-induced diabetes.[15] In diabetic nephropathy, there will be a progressive damage like renal vein thrombosis and fibrin degradation. Destruction of glomeruli causes significant decrease in the glomerular filtration rate (GFR) and increases the blood urea and creatinine and this end up with chronic renal failure.[16] Since the urea and creatinine are markers of kidney function,[14] it is an indication that the extracts were not nephrotoxic.

There are many enzymes found in the serum that did not originally originate from the serum. During tissue damage, some of these enzymes find their way into the serum, probably by leakage.[17] Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. The increase in serum alkaline phosphatase activity may indicate hepatic damage probably by the altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum.[13,18] The results indicate that there were no significant increase in the ALP level, instead in some selected dose level it got reduced.

Alanine and aspartate amino transaminases are considered to be sensitive indicators of hepatocellular damage and within limit can provide a quantitative evaluation of the degree of damage to the liver.[19] The plasma SGOT and SGPT levels in the serum were not increased by the intake of *P. edulis* extract and this indicates that the activity of the liver was preserved.

Our earlier studies of phytochemical screening of *P. edulis* confirms the presence of saponin, terpenoid flavonoid, steroid, and cardiac glycosides in its aqueous extract.[20] These phytochemicals were widely reported as quencher of free radicals in the biological system and amelioration of various diseases associated with free radicals. Flavonoids exhibits a wide range of biological activities such as antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiallergic effects, cytostatic, and antioxidant properties.[21] Aliyu *et al*.[22] reported that phenolic compounds are the major group of compounds that acts as primary antioxidant because it can reacts with oxygen free radicals such as hydroxyl, superoxide anion radicals and lipid peroxyl radicals. There is high correlation between antioxidant activity and phenolic compounds.[23] Oliver[24] listed glycosides, flavonoids, and tannins as active hypoglycemic compounds. Therefore, the beneficial effect observed by the administration of *P. edulis* aqueous extract to rats was because of these phytochemicals.

Alteration in the biochemical indices will lead to impairment of normal functioning of the organs.[25] Since no such major alterations were found in the organ weight, hematological and biochemical parameters, the results of this study have established that the oral administration of *P. edulis* was safe up to 2000 mg/kg, and the intake of different concentrations 100, 200, 300, 400 mg/kg of extract of *P. edulis* was found to be safe and has no adverse effect on the functions of the liver, kidney and bone marrow in albino rats.

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