

ACUTE AND SUB ACUTE TOXICITY OF METHANOL EXTRACT OF *ELYTRARIA ACAULIS* LINDAU. IN RATS

Ruby.K Koshy^{1,2*}, Raj Kapoor. B¹, Mohammad Azmathulla²

¹ Department of Pharmacology,
Karpagam University, Coimbatore,
Tamil Nadu

²The Oxford college of Pharmacy,
6/9, Begur Road, Hongasandra,
Bangalore-560 068

***Author for Correspondence**

Mrs. Ruby. K Koshy
The Oxford college of Pharmacy,
6/9, Begur Road, Hongasandra,
Bangalore-560 068, INDIA
Phone: +91-80-30219821
E-mail: koshy.ruby@gmail.com

Summary

Elytraria acaulis is a shrub and traditionally used in many countries for the treatment of various diseases and disorders. The aim of the present study was to evaluate the safety of the methanol extract of *Elytraria acaulis* shrub through acute and sub acute toxicity study in rats. For acute toxicity study 50-2000 mg/kg methanol extract of *Elytraria acaulis* were administered orally and obvious toxic symptoms and mortality was studied up to 72 hrs. In sub acute study, effect of multiple weekly dosing of 400 mg/kg (one-fifth of the maximum tolerated dose) of methanol extract of *Elytraria acaulis* was investigated in rats for six weeks and the evaluation was done by the studies of haematological parameters, biochemical estimations of hepatorenal parameters, histological observations of the tissue. The extract was found to be well tolerated up to 2g/kg in acute toxicity study. In sub acute toxicity studies it showed no significant alteration on any of the parameters, which was evident by the histological studies. Hence the results suggest that methanol extract of *Elytraria acaulis* whole plant is quite safe and can be used in the treatment of the chronic diseases without any toxicity.

Key Words: *Elytraria acaulis* haematological parameters, hepatorenal, histological studies, mortality

Introduction

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern medicines, in a number of studies. In developing countries, all over the world, 80% of population continues to use traditional medicine in primary medical problems [1]. Research carried out in last few decades has validated several such claims of use of traditional medicinal plants. Human beings has recognised the need for better control of the present use and the future development of chemicals which should chemically tested and retested before reaching of chemicals primary and cumulative toxicity and its mutagenic, teratogenic and carcinogenic potential which can be obtained from animal studies.

Toxicology is defined as any harmful effect of a chemical or drug on a target organism. Acute and sub acute toxicity has been defined by various experts. Toxicity can be acute, sub chronic, or chronic: Acute toxicity involves harmful effects in an organism through a single or short-term exposure. Sub acute toxicity is the ability of a toxic substance to cause effects for more than one year but less than the lifetime of the exposed organism. Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism [2].

The purpose of toxicity testing is to provide adequate database to make decision concerning the toxicology properties of chemicals and commercial products and to decide whether a drug or chemicals will be safe or not.

Elytraria acaulis is one such plant that is frequently being used, the leaves decoction of this plant is prescribed in fever, venereal diseases and root is used in mammary tumor, abscesses, pneumonia, and infantile diarrhea as well as traditional medicine for long days [3]. Leaves used for treating wounds infected with worms [4]. Locally in Tamilnadu (Tirunelveli Dist) it is used as antidiabetic.

Elytraria acaulis belongs to the family Acanthaceae is a small shrub, which grows in shady dry places. The whole shrub is used for medicinal purposes [5] Despite of the popular use and extensive phytopharmacological studies, the toxicity profile, especially on its chronic use, has not been yet explored. The present investigation was therefore carried out to study the acute and Sub acute toxicity of the methanol extract of *Elytraria acaulis* *in* rats.

Materials and Methods

Plant material

The whole shrub of *Elytraria acaulis* was collected in Tamilnadu from (Tirunelveli Dist), India and identified by the Dr. Chelladurai, Rd. Research officer, CCRSC, Govt of India, Tamilnadu and retained in our laboratory for further reference.

Preparation of plant extract

The whole shrub of *Elytraria acaulis* were dried and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. This extract was filtered and concentrated in vacuum evaporator and kept in a vacuum dessicator for complete removal of solvent. The yield was 150g with respect to 2 Kg of dried powder and used for oral administration.

Animals

Adult female wister rats of weight 125 – 150g were used for acute toxicity study and adult male rats of weight 150 – 175g were used for sub acute toxicity. The animals were kept in polypropylene cages with Husk bedding and maintained under standard laboratory

conditions. Standard pellet diet and water were given *ad libitum*. The rats were acclimatized to laboratory condition for one week before commencement of experiment. The toxicity studies conducted as per internationally accepted protocol drawn under OECD No 420 guidelines [6].

Phytochemical analysis

Preliminary phytochemical screening of the extract was carried out using standard methods.

Acute toxicity study

Healthy adult female wister rats were starved overnight and were divided into five groups (n=3). Group I-IV animals were orally fed with methanol extract of *Elytraria acaulis* in increasing dose levels of 50, 500, 1000, 2000 mg/kg respectively, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioural, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and next 24 h for any lethality or death.

Sub acute toxicity study

Adult male wister rats were starved overnight and were divided in to four groups (n=6). Group I- III animals were orally fed with methanol extract of *Elytraria acaulis* in increasing dose level of 100, 400, 750mg/kg respectively, while group IV (untreated) served as control for 28 days. The animals were observed for sign and symptoms, behaviour alteration, food and water intake and body weight changes. All animals were observed twice daily for mortality during the 28 days period study. The group mean body weights were calculated [7]. At the end of the experiment, after 24 h of the last dose and 18 hours fasting, animals were sacrificed and blood was collected from orbital sinus and taken into heparinised tube for haematological studies and non-heparinised centrifuge tube for biochemical estimations. Liver tissue was collected and taken for the histological studies.

Haematological studies

Red blood cells (RBC), white blood cells (WBC), Haemoglobin (Hb), Erythrocyte sedimentation rate (ESR), Platelet, clotting time and packed cellular volume (PCV) were performed using routine method⁶.

Biochemical estimation

The effect of Methanol extract of *Elytraria acaulis* treatment on the biochemical parameters of the experimental rats were evaluated by the estimation of blood glucose [8], Cholesterol [9], Serum glutamate Pyruvate (GPT) [10], Glutamate oxaloacetate transaminase (GOT) [10], Alkaline Phosphatase [11], Total Bilirubin [11], Urea and BUN [12], Total Protein and albumin [13] and Creatinine [14] were estimated in serum. Urine samples were also collected at the end of the study period and analysed for Specific Gravity, pH, glucose, Proteins Ketones and Occult Blood were performed.

Histological studies

At the end of 28 days, Rats were sacrificed and vital organs Viz. Liver, Kidney, Spleen, Lung, Heart and Brain were remove and subject to Histological examination. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24 h, dehydrated with alcohol, and embedded in paraffin, cut into 4-5µm thick sections and stained with hematoxylin-eosin dye for microscopic observation [15].

Statistical analysis

The values were expressed as mean \pm SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

Results

Preliminary phytochemical screening of *Elytraria acaulis* revealed the presence of Alkaloids, steroids, flavonoids, glycosides, Proteins and carbohydrates.

Acute toxicity study

In acute toxicity study, methanol extract of *Elytraria acaulis* did not show any mortality or toxic effect up to the dose of 2 g/kg during the observational period of 24 hours. It did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal effects. These results showed that in single dose, there are no adverse effects of Methanol extract of *Elytraria acaulis* indicating that the medium lethal dose (LD₅₀) is higher than 2000 mg/kg in rats. Accordingly one-fifth of the maximum tolerated dose ie, 500 mg/kg was considered as the high dose of Methanol extract of *Elytraria acaulis* and used for the sub acute toxicity study in the present investigation.

Sub acute toxicity study

In sub acute toxicity study, Methanol extract of *Elytraria acaulis* administration did not show any significant changes in behaviour or locomotor activity, No ataxia and No sign of intoxication were observed during the 28 days period. No difference in growth between the control group and treated groups. No change in fur coating, eyes and respiratory function. There was no significance difference in the food and water consumption between the treated and control group. (**Table: 1**)

Effect of Methanol extract of *Elytraria acaulis* on haematological parameters has been presented. RBC, WBC, Hb, ESR and PCV were not significantly different in both control and Methanol extract of *Elytraria acaulis* treated animals ($P > 0.05$). Platelet and clotting time shows significant difference by statically when compare with control ($P < 0.01$; $P < 0.05$). All the values were found to be within the normal range and there was no difference between the groups (**Table.2**).

The level of serum analyses, such as glucose, cholesterol, GPT, GOT, Alkaline phosphatase, Total Bilirubin, Urea, BUN, Total Protein, Albumin and creatinine were not significantly different between control and the treated animals ($P > 0.05$). (**Table: 3 and Table: 4**). Analysis of urinary metabolites levels showed traced or no presence of these in both control and treated animals (**Table: 5**). No abnormal changes were observed in organ mass with respect to body mass of extract in comparison with control. (**Table: 6**).

On observation of gross pathology immediately after dissection, all groups were found to be uniform healthy, lacking in any apparent pathological abnormalities.

Histological observation of the Liver tissue and Kidney Tissue in both control as well as extract treated rats showed no difference indicating that feeding these *Elytraria acaulis* extract to rats did not result in any adverse effect on this organ. (Fig.1.A and. B)

Discussion

The use of herbal medicines has received a great attention as alternatives to synthetic pharmaceutical products in recent times leading to the increase in their demand. Experimental screening method including a thorough toxicity study is therefore important to ascertain the safety and efficacy of these herbal drugs.

The purpose of the study was to look at the toxicity profile of the *Elytraria acaulis* a 28 days study is considers a sub acute toxicity 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 the target organs or prolonged exposure. A wide verity of adverse effect can be detected from sub acute toxicity studies. The results from such studies can provide information, which will aid in selecting dose level.

Acute toxicity studies showed the lack of mortality and toxicity up to oral treatment of 2000 mg extract/kg body weight which suggests that the methanol extract of *Elytraria acaulis* is practically nontoxic at single dose. However in case of subsequent use in the treatment of the chronic diseases like diabetes or cancer, whether it will be safe that can be clear from its sub-acute toxicity study.

According to onyenyili a co workers [16], anaemia following administration of agent can be a result of lyses of blood cells and inhibition of blood cell thesis by the active constituents of the extract and decrease in haematological parameters in experimental animals has been associated with anaemia¹⁶. There is no significant changes in haematological parameters like Haemoglobin, RBC, WBC, ESR, Platelets, Clotting time and PCV in the extract treated animals compare with control (**Table:2**), which indicates that there is no lyses of blood cells and inhibition in blood cell thesis by the active constituent of *Elytraria acaulis* extract. The above results suggest the non toxicity of *Elytraria acaulis* in rats.

Clinical parameters were statically not significant in compression to control groups. They conforming the absence of adverse effect. No adverse effect has been observed in renal function test. No mortality was recorded in rats treated with higher dose of 750 mg/kg. The toxicity studies of the drug reflects the innocuous nature of this plant extract on Hepatic, Renal and Haemopotic system even at high dose. Rats treated with various doses of the extract have no significant change in bodyweight. (Table: 1)

An increase in kidney weight indicates nephrotoxicity [17]. The *Elytraria acaulis* did not induce any adverse effect on kidney and the other organs such as Liver Heart, Lung, Spleen and Brain, since absolute and relative weight of the organs were not significantly different from control value.(Table: 6)

Microscopic data together with the data of macroscopic evaluation of the animal's organ showed that both treated and control groups were practically healthy. According to the data of histological examination, no toxic or allergic effect of *Elytraria acaulis* was detected. No local irritating effect of the drug preparation was observed during the study period. Hence, *Elytraria acaulis* is safe.

In histopathological studies, liver of treated animals showed normal histological features at 100, 400 and 750 mg/kg. No degeneration of hepatocyte, focal steatosis, congestion of central vein and inflammation of portal tract when compared with control animals. The kidney of the treated animals showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney. Gross examination of liver and kidney on histology did not reveal any abnormalities. Thus, it was concluded that *Elytraria acaulis* did not produce any toxicity effect in rats. (Figure: 1, 2, 3, 4)

Increase in the level of SGPT, SGOT, Glucose, cholesterol and ALP reflects the structural and functional dysfunction of hepatocellular membrane or cell rupture, and thereby indicates liver damage. The normal value of the hepatic biochemical parameters reveals the safety profile of the extract on liver function even on its use. (Table: 3)

The normal values of the renal biochemical parameters, including urea and creatinine suggest that the extract does not produce any sort of disturbance in the renal function, as has been found in case of various plant extracts and hence is safe on its use in various diseases. (Table: 4)

Histological observations correlate the other results showing the normal cellular architectures in the treated group of animals, without any necrosis or fatty infiltration, which can substantiate the safety profile of the extract clearly.

Treatment	Dose (mg/kg)	Body weight (gm)	
		Initial	After 28 days treatment
Control	-	146 ± 1.2	158 ± 1.6
Methanol extract of <i>Elytraria acaulis</i>	100	143 ± 1.4	160 ± 1.8
	400	148 ± 0.7	164 ± 2.1
	750	140 ± 1.1	162 ± 1.3

Table 1: Changes in growth and body weight of rats following treatment with different doses of methanol extract of *Elytraria acaulis*

N = 6 animals in each group; Values are expressed as mean ± SEM

NS = statistically not significant. Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

The present investigation thus provides evidence for the total safety profile of the methanol extract of *Elytraria acaulis* suggesting its safe use in single dose treatment as well as for long term therapeutic application in case of various chronic diseases, without producing any toxic effects. Hence further phyto pharmacological studies on the basis of its ethno botanical use can help to explore and establish the bioactive constituents of the extract which can be used safely for the treatment of various diseases and disorders in future.

CONCLUSION

Acute toxicity studies showed the lack of mortality and toxicity up to oral treatment of 2000 mg extract/kg body weight which suggests that the methanol extract of *Elytraria acaulis* is practically nontoxic at single dose, thus provides evidence for the total safety profile of the methanol extract of *Elytraria acaulis* suggesting its safe use in single dose treatment as well as for long term therapeutic application in case of various chronic diseases, without producing any toxic effects.

Table 2: Effect of methanol extract of *Elytraria acaulis* on Haematological changes in rats

Treatment	Dose (mg/kg)	Hb gm %	RBC 10 ⁶ /cumm	Total WBC 10 ³ /cu.mm	ESR mm/1 st hr	Platelets (K/ μ L)	Clotting time (Sec)	PCV %
Control	-	13.18 \pm 0.56	4.76 \pm 0.46	8.10 \pm 0.24	3.05 \pm 0.16	510 \pm 17.18	130.5 \pm 1.49	48 \pm 1.83
Methanol extract of <i>Elytraria acaulis</i>	100	12.17 \pm 0.35	4.56 \pm 0.25	8.94 \pm 0.17	3.12 \pm 0.18	526 \pm 15.24	127.83 \pm 1.42	50 \pm 1.36
	400	13.82 \pm 0.52	4.06 \pm 0.38	8.46 \pm 0.26	3.40 \pm 0.10	605 \pm 21.58 ^a	124.13 \pm 1.78 ^b	51 \pm 2.06
	750	14.23 \pm 0.54	4.69 \pm 0.65	7.93 \pm 0.14	3.52 \pm 0.28	598 \pm 19.25 ^b	125.16 \pm 1.10	48 \pm 2.17

N= 6 animals in each group; Values are expressed as mean \pm SEM ^aP<0.01; ^bP<0.05 Vs control

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Hb: Haemoglobin

RBC: Red Blood Cell

WBC: White Blood Cell

ESR: Erythrocyte Sedimentation Rate

PCV: Packed Cellular Volume

Table 3: Effect of methanol extract of *Elytraria acaulis* on Biochemical parameters in rats

Treatment	Dose (mg/kg)	Glucose mg%	Cholesterol mg%	SGPT U/L	SGOT U/L	ALP U/L	Total Bilirubin mg%
Control	-	96 ± 1.7	126 ± 1.70	110 ± 1.9	47.5 ± 1.3	190 ± 2.10	0.80 ± 0.04
Methanol Extract of <i>Elytraria acaulis</i>	100	102 ± 2.0	110 ± 1.30	112.6 ± 2.7	52.0 ± 1.7	198 ± 2.36	0.79 ± 0.05
	400	98 ± 1.6	118 ± 1.95	118.5 ± 2.6	49.0 ± 2.4	188 ± 2.50	0.86 ± 0.02
	750	90 ± 2.14	123 ± 2.86	121.0 ± 2.1	51 ± 2.0	192 ± 2.0	0.78 ± 0.06

N=6 animals in each group; Values are expressed as mean ± SEM.

NS=statistically not significant Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

SGPT: Serum Glutamic Pyruvic Transaminase

SGOT: Serum Glutamic Oxaloacetic Transaminase

ALP: Alkaline Phosphatase

Table 4: Effect of methanol extract of *Elytraria acaulis* on biochemical parameters in rats

<u>Treatment</u>	Dose (mg/kg)	Urea mg %	BUN mg%	Creatinine mg %	Total Protein gm%	Albumin gm%
Control	-	36.5 ± 1.14	28.65 ± 2.13	0.9 ± 0.10	8.3 ± 0.96	4.07 ± 0.54
Methanol extract of <i>Elytraria acaulis</i>	100	38.7 ± 1.43	25.79 ± 1.17	0.8 ± 0.02	7.9 ± 0.65	4.13 ± 0.29
	400	37.5 ± 1.57	27.24 ± 1.78	0.9 ± 0.07	8.5 ± 0.72	4.27 ± 0.24
	750	40.2 ± 2.0	30.63 ± 2.34	1.0 ± 0.09	7.8 ± 0.49	4.33 ± 0.73

N=6 animal in each group; Values are expressed as mean ± SEM.

NS=statistically not significant Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

BUN: Blood Urine Nitrogen

Table 5: Effect of methanol extract of *Elytraria acaulis* on urine analysis in rats

Treatment	Dose (mg/kg)	Specific gravity	pH	Glucose	Protein	Ketone bodies	Blood cells
Control	-	1.02 ± 0.04	6.72 ± 0.19	NIL	NIL	NIL	NIL
Methanol extract of <i>Elytraria acaulis</i>	100	1.03 ± 0.06	6.58 ± 0.18	NIL	NIL	NIL	NIL
	400	1.04 ± 0.03	6.09 ± 0.16	NIL	NIL	NIL	NIL
	750	1.06 ± 0.02	6.25 ± 0.24	NIL	NIL	NIL	NIL

N=6; Values were expressed as Mean ± SEM

NS- Not significant Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Table 6: Effect of methanol extract of *Elytraria acaulis* on weight (gm) of vital organs of rats

Treatment	Dose (mg/ kg)	Liver	Kidney	Heart	Lungs	Spleen	Brain
Control	-	3.46 ± 0.12	1.17 ± 0.19	0.68 ± 0.03	1.25 ± 0.14	0.95 ± 0.07	1.65 ± 0.58
Methanol extract of <i>Elytraria acaulis</i>	100	3.75 ± 0.10	1.13 ± 0.17	0.76 ± 0.04	1.15 ± 0.14	0.90 ± 0.08	1.59 ± 0.36
	400	3.52 ± 0.21	1.27 ± 0.23	0.69 ± 0.02	1.28 ± 0.13	1.06 ± 0.06	1.69 ± 0.24
	750	3.70 ± 0.13	1.10 ± 0.20	0.70 ± 0.05	1.30 ± 0.16	1.15 ± 0.18	1.75 ± 0.38

N=6 animal in each group; Values are expressed as mean ± SEM

NS=statistically not significant. Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Fig A. Effect of methanol extract of *Elytraria acaulis* on Histopathological Studies of Liver in Sub acute Toxicity Studies

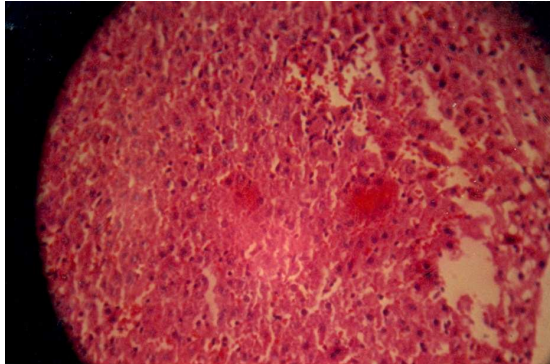


Fig A1: Section of liver treated With normal saline

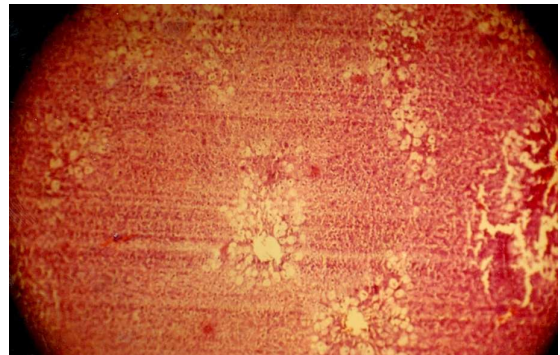


Fig A2: Section of liver treated with methanol extract of *Elytraria acaulis* (100 mg/kg)



Fig A3: Section of liver treated with methanol extract of *Elytraria acaulis* (750 mg/kg)

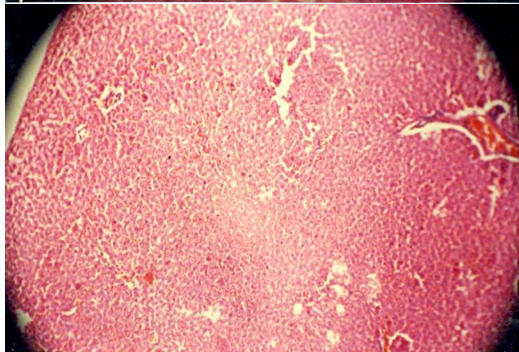


Fig A4: Section of liver treated with (400 mg/kg) methanol extract of *Elytraria*

Fig B: Effect of methanol extract of *Elytraria acaulis* on Histopathological Studies of Kidney in Sub acute Toxicity Studies

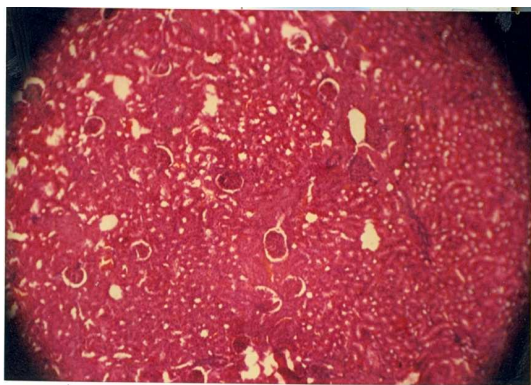


Fig B1: Section of liver treated with Normal saline

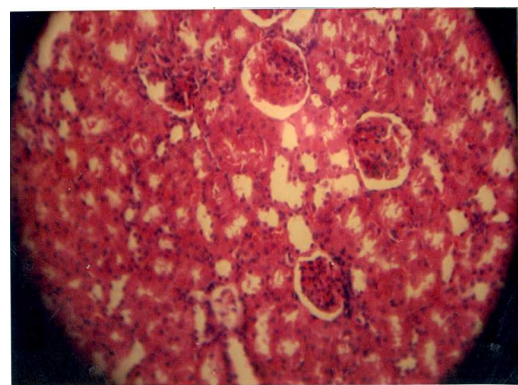


Fig B 2: Section of Kidney treated with methanol extract of *Elytraria acaulis* (100 mg/kg)

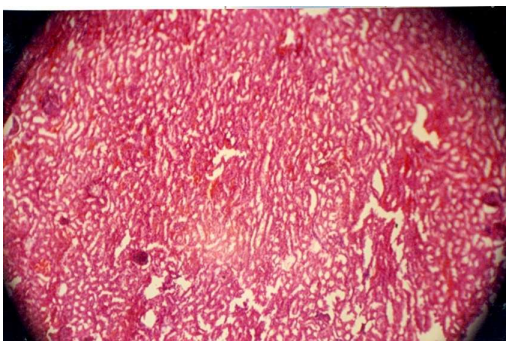


Fig B3: Section of Kidney treated with methanol extract of *Elytraria acaulis* (400 mg/kg)

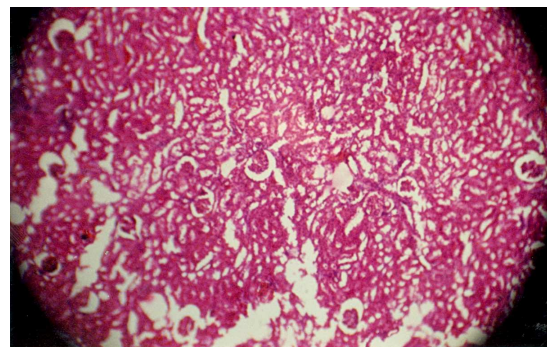


Fig B4: Section of Kidney treated with methanol extract of *Elytraria acaulis* mg/kg)

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