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Effects of Some Haematological and Biochemical Parameters of Spermacoce Articularis

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Abstract: **- Medicinal plants and herbs have been used for ages for the purposes of enhancing and maintaining health and organic resistance against body infection. This is due to their ready availability and arguably efficacious state, therefore offering an alternative remedy in enhancing hematological and Biochemical parameters. Various chemical constituents of Spermacoce articularis are believed to possess therapeutic effects on hematological (WBC count and Hb Content) and Biochemical (SOD, CAT and GP X) parameters. These parameters were studied in mice that had different doses and duration of S. articularis pretreatment. 200mg dose of S.articularis per day for 20 days did not have any significant change(P>0.05) in the red blood cell count and packed cell volume. However at 400mg per day for the same duration, S. articularis increased red blood cell count and packed cell volume significantly (P<0.05). The same dose (200mg) of S.e articularis similarly and significantly increased total white blood cell count (WBC), neutrophil and lymphocyte counts (P&l;0.05). S. articularis thus promote leucopoiesis and increases neutrophil : lymphocyte ratio. Hematological profile reverted towards normal levels in extract treated mice. Treatment with S. articularis (200 and 400mg/kg) restored the serum biochemical parameters towards normal levels and decreased the levels of lipid peroxidation and increased the levels of reduced glutathione and other antioxidant enzymes (SOD, CAT and GPx).**

Keywords: **Spermacoce articularis, haematological parameters, Biochemical parameters.**

INTRODUCTION

The use of animals in scientific research has generated controversy. However, it has significantly contributed to the development of science, promoting several advances in understanding the metabolic machinery as well as the discovery of treatments and preventive measures applied to both human and veterinary medicine. Technological refinements in the generation of experimental models have led to a reduction in the number of animals per experimental group, principally due to reducing the variability observed in each experiment (RESTEL et al., 2014). Despite efforts to develop and use alternative methods, in some situations, the use of animals in accordance with ethical terms is still needed. Several animal species have been used, with mice the most heavily used because they are easy to treat, breed quickly, have a short life, a low cost of management and have a physiological similarity to the human cycle (FOSTER et al., 1982).
The similarities between the genomes of human and mouse model are the foundation of much of modern biology, with model organism experimentation permitting valuable insights into biological function and the etiology of human disease (EMES et al., 2003).

Therefore, it is important to know the normal ranges for many hematological and biochemical parameters of homeostasis assessment because pathological processes can influence at the metabolism and alter the results obtained in experimental procedures. The reference values for biochemical and hematological parameters are often sought to play up the metabolic status of the animals used in research. These values may vary according to the animal facility of origin, laboratories, age, sex, management conditions, diet and techniques used. So it is important to strive to generate a historical database benchmark based on the methods used.

Currently, there are few data that can be use in scientific research as reference values for mice from animal facilities. The aim of this study is to establish reference values for hematological parameters and Biochemical parameters.

MATERIALS AND METHOD

Collection and preparation of plant extract

The plant material (Leaves of Spermacoce articularis) was collected from Karur and was authenticated by authenticated by Botanical Survey of India (BSI/ SRC/ 5/23/2013-14/ tech, 1643) Coimbatore. The Leaves of plant were cleaned to remove impurities and shade dried. The coarselypowdered leaves were weighed and stored in air tight containers. The coarsely powdered shade dried leaves of the plant Spermacoce articularis (200g) was macerated with chloroform:water (5:95) by cold maceration process for 3 days. After completion of extraction the marc was filtered through muslin cloth followed

by filter paper and concentrated and dried on water bath to obtain aqueous extract of Spermacoce articularis and the extract was preserved in a refrigerator.
Cell lines EAC (Ehrlich Ascites Carcinoma) cells, obtained from Small Animals Breeding Station, Mannuthy, Kerala. The cell lines were maintained and propagated intra-peritonially by serial transplantation into adult Swiss albino mice.

Animals

The experiments were carried out on 8-10 weeks old Swiss albino mice of either six weighing 25 ± 5 gm and female Wistar albino rats weighing around 175±25gm. Animals used in the study were procured from a registered breeder. The animal care and handling was carried out in accordance to guidelines issued by the Institutional Animal Ethics Committee, Small Animals Breeding Station, Mannuthy, Kerala, India. Animals were acclimatized to the experimental room for one week prior to the experiment. Animals were maintained under controlled conditions of temperature (23 ± 30C) and humidity (50 ± 5%) and were caged in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of four animals in each cage. The mice were fed on standard food pellets and water ad libitum. The studies conducted were approved by the Institutional Ethical Committee, Small Animals Breeding Station, Mannuthy, Kerala.

Swiss albino mice were divided into 4 groups (n=6). All the groups were injected with EAC cells (0.2 ml of 2×106 cells/mouse) intraperitoneally except the normal group. This was taken as day zero. From the first day, Spermacoce articularis extract (100mg/kg) and 5-FU (25mg/kg) were administered intraperitoneally for 14 days to groups 3 and 4 respectively. After the administration of last dose followed by 18 h fasting, all the mice were sacrificed for the study of antitumor activity, serum biochemical, hematological and biochemical parameters. Antitumor effect of Spermacoce articularis was assessed by observation of changes with respect to body weight and abdominal circumference. All procedures described were reviewed and approved by the Institutional Animals Ethical Committee.

RESULTS AND DISCUSSION

The results are presented in the tables below separated by strains/stock. They are expressed in Range (minimum and maximum values) and Mean ± SD (mean and standard deviation).The results are shown in table 1, being considered as a reference for hematologic evaluation from Laboratory Animal Facility rodents used in experimental procedures. Differential leukocyte count consisted of evaluation of total and relative leukocytes number of different leukocyte populations (segmented neutrophils, lymphocytes, eosinophils, monocytes and basophils). The results are shown in table 2 and present small variations in Biochemical Parameters.

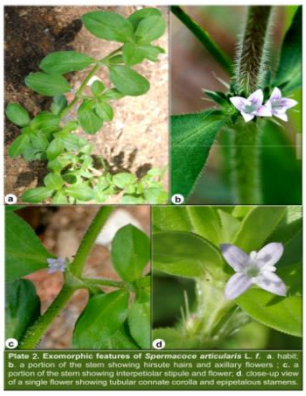

Plate 2. Exomorphic features of *Spermacoce articularis* L. f. a. habit; b. a portion of the stem showing hirsute hairs and axillary flowers ; c. a portion of the stem showing interpetiolar stipule and flower; d. close-up view of a single flower showing tubular connate corolla and epipetalous stamens.

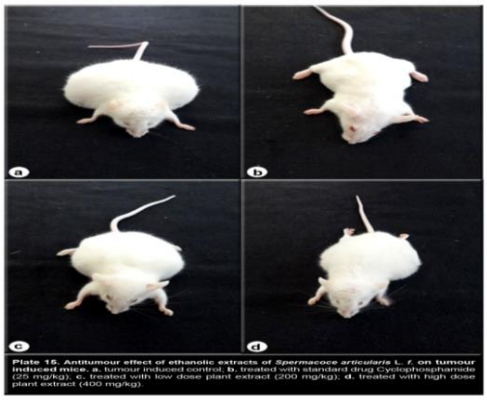

Plate 15. Antitumour effect of ethanolic extracts of *Spermacoce articularis* L. f. on tumour induced mice. a. tumour induced control; b. treated with standard drug Cyclophosphamide (25 mg/kg); c. treated with low dose plant extract (200 mg/kg); d. treated with high dose plant extract (400 mg/kg).

Haematological parameters

In order to assess the influence of treatment on the haematological status of EAC bearing
mice, blood was collected intra-cardinally from the animals into heparinised and EDTA treated micro centrifuge tubes on 10th day and following parameters were monitored.
1. White blood cell total count 2. Red blood cell total count 3. Haemoglobin contents.
Haemoglobin content and RBC count were significantly ($P<0.05$) decreased and total WBC count was significantly ($P<0.01$) increased in the EAC group as compared to the normal group. Treatment with Aloe vera significantly restored the RBC and haemoglobin levels towards the normal. In the differential count of WBC, the neutrophil count increased, while the lymphocyte count decreased in the EAC group as compared to the normal group. Treatment with Spermacoce articularis significantly restored the altered parameters towards the normal values.

Table 1: Effect of ethanolic extract on changes in hematological parameters of EAC tumor induced rats

Treatment group	Days	WBC (x103cells/mm3)	Hb (g/dl)	Differential count (DC) (%)				
				Lymphocytes	Neutrophils	Easnophils	Monocytes	Basophils
Control (i.p.)	0	7.43 ± 0.21	13.73 ± 1.10	81.17 ± 2.40	15.47 ± 0.67	2.33 ± 0.4	1.27 ± 0.25	1.07 ± 0.06
	20	13.17 ± 0.95	10.77 ± 0.45	54.13 ± 1.11	39.43 ± 0.93	2.60 ± 0.26	4.57 ± 0.31	1.20 ± 0.10
Cyclophosphamide 25 mg (i.p.)	0	7.30 ± 0.20	14.17 ± 0.31	81.60 ± 3.10	15.63 ± 0.23	1.33 ± 0.15	1.47 ± 0.25	2.43 ± 0.21
	20	8.10 ± 0.20	13.80 ± 0.40	78.33 ± 1.90	16.77 ± 0.35	0.27 ± 0.21	3.20 ± 0.20	3.37 ± 0.25
Ethanol extract 200 mg/kg (p.o.)	0	7.20 ± 0.30	13.77 ± 0.31	86.07 ± 3.57	10.60 ± 0.79	0.30 ± 0.20	2.57 ± 0.15	2.13 ± 0.11
	20	11.7 ± 0.26	12.10 ± 0.36	69.47 ± 0.86	24.63 ± 2.47	0.10 ± 0.10	4.67 ± 0.45	2.43 ± 0.21
Ethanol extract 400 mg/kg (p.o.)	0	7.37 ± 0.21	14.37 ± 0.86	82.87 ± 1.48	13.10 ± 1.47	1.57 ± 0.31	2.23 ± 0.31	2.23 ± 0.11
	20	9.47 ± 0.25	13.80 ± 0.36	78.63 ± 2.62	16.47 ± 1.96	1.67 ± 0.21	2.77 ± 0.31	2.47 ± 0.31

Table 2: Effect of ethanol extract on changes in biochemical parameters and antioxidant

Treatment group	SOD	CAT	GPx
Control (i.p)	0.76 ± 0.08	14.70 ± 2.08	61.79 ± 6.27
Cyclophosphamide 25 mg/kg (i.p)	1.71 ± 0.09	25.86 ± 0.82	89.29 ± 0.56
Ethanol extract 200 mg/kg	1.10 ± 0.03	19.40 ± 1.22	69.68 ± 2.66
Eethanol extract 400 mg/kg (p.o.)	1.36 ± 0.02	22.19 ± 5.52	83.87 ± 3.22

properties of EAC tumor induced rats

Values are MeMean ± SE_M of six independent analysis (n=6)

SOD – Superoxide dismutase (Units/min/mg protein)
CAT – Catalase (ì moles o H2O2 consumed/min/mg protein)
GPx – Glutathione peroxidase (ì moles of GSH oxidized/min/mg protein)

Biochemical Parameters (SOD, CAT and GPX):

There was a significant (P<0.05) decrease in SOD, CAT and GPx activities in EAC group. In the present study, the enzymic antioxidants were found to be significantly reduced in the inflammation induced animals, while the same elevated on treatment with ethanolic plant extracts of S. articularis (200 and 400mg/kg) and the cyclophosphamide. Spermacoce articularis treatment significantly restored the liver antioxidant enzymes levels towards normal.

Haematological Parameters

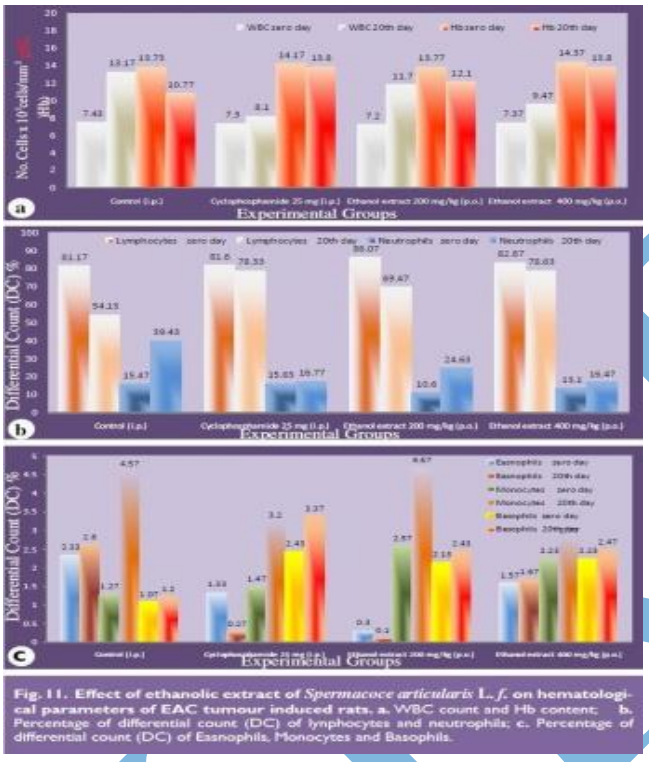

Fig. 11. Effect of ethanolic extract of *Spermacoce articularis* L. *f.* on hematological parameters of EAC tumour induced rats. a. WBC count and Hb content; **b.** Percentage of differential count (DC) of lymphocytes and neutrophils; **c.** Percentage of differential count (DC) of Eosnophils, Monocytes and Basophils.

Biochemical Parameters

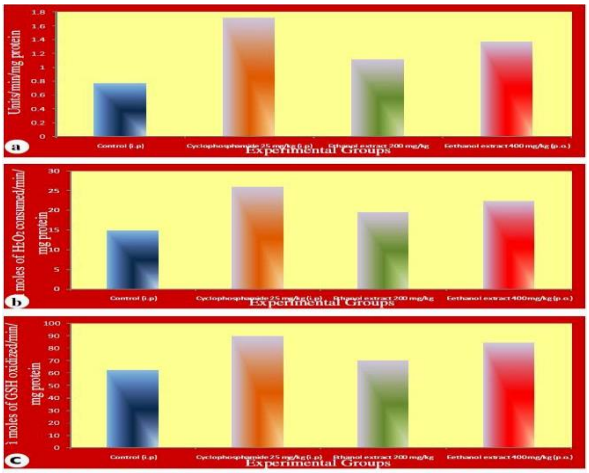

Fig. 12. Effect of ethanol extract of *Spermacoce articularis* L. *f.* on the antioxidant markers. a. Superoxide dismutase ; (Units/min/mg protein); **b.** Catalase (1 moles of H_2O_2 consumed/min/mg protein) ; **c.** Glutathione peroxidase (1 moles of GSH oxidized/min/mg protein)

The present study was carried out to evaluate the antitumor activity of 50% ethanol extract of Spermacoce articularis on EAC bearing mice. The extract treatment at the dose of 100 mg/kg inhibited the increase in body weight and abdominal circumference and also brought back the serum biochemical and hematological parameters towards normal levels. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as other antioxidant enzymes such as SOD, CAT and GPx in tumor bearing mice to near normal levels. In cancer chemotherapy the major problem are of myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. Treatment with Spermacoce articularis brought back the hemoglobin content, RBC and WBC cell count near to normal values. This indicates that Spermacoce articularis possess protective action on the haematopoietic system. The free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells and their function is to provide a defense against the potentially damaging reactions of superoxide and hydrogen peroxide. Sun et al reported a decrease in SOD activity in EAC bearing mice which might be due to loss of Mn-SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and CAT activities as a result of tumor growth was also reported. Similar findings were observed in the present investigation with EAC bearing mice. The administration of Spermacoce articularis increased the SOD and CAT levels, which may indicate the antioxidant and free radical scavenging property of Spermacoce articularis. Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells and antitumor activity in experimental animals. The increase in levels of SOD and catalase in Spermacoce articularis -treated group

indicates its potential as an inhibitor of EAC induced intracellular oxidative stress.

DISCUSSION

The task of establishing a range of reference values for rodents is very difficult, because many variables, such as gender, age, genetic variation, diet and environmental conditions in which these animals are submitted must be considered. The Institute for Clinical and Laboratory Standards suggests that values are defined by the result obtained with quantitative measurement of analytes based on the defined criteria (CLSI, 2000).

The present study was carried out to evaluate the toxicity, antitumor activity, lipid peroxidation and antioxidant status of Spermacoce articularis on EAC bearing mice.

The Spermacoce articularis treated animals at the different doses of 200; 400 mg/kg inhibited the body weight, tumor volume, packed cell volume, tumor cell count and also brought back the haematological parameters to more or less normal levels. The extracts also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as antioxidant enzymes such as SOD and CAT in tumor bearing mice to near normal levels.

On the basis of the above result it was suggested that, the in-vivo anticancer activity of aqueous extract of Spermacoce articularis leaves possess significant anticancer property with the dose dependent effect. This may probably due to the presence of phytochemicals such as alkaloids, phenols and flavonoids. Further isolation and purification of bioactive compound from Spermacoce articularis may reveal the presence of a potent novel anticancer agent and also to explore the exact mechanism of action of the anticancer activity.

CONCLUSION

The data presented in this study show a confidence range for hematological and biochemical parameters that are considered appropriate for mice used in scientific research.

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