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RESEARCH ARTICLE

HEMATOLOGICAL RESPONSES OF AQUEOUS EXTRACT OF CANNABIS SATIVA INDICA LEAVES ON WISTAR RATS

Kiranmayi Varma Lanke¹, Sai Vardhan Myneni¹, Krishna Reethika Chokkakula¹, Kamala Vasanthi Karyamsetty¹, Mahalakshmi Meesala¹ and Suryanarayana Veeravilli²

1. Department of Biotechnology, KoneruLakshmaiah Education Foundation, Andhra Pradesh, India.
2. Department of Humanities and Basic Sciences, Aditya Engineering College, Surampalem, Andhra Pradesh, India.

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Abstract

Cannabis indica has widely reported traditional uses which include its use for stimulation and purification of platelets as well as body defense. Experiments were carried out to assess the impact of Cannabis indica leaf extract on hematological parameters of wistar rats. The results revealed that the extract (10–100 mg/kg p.o.) produced no significant change in the packed cell volume, haemoglobin, red blood cell count, total leucocyte count, monocyte, basophil, eosinophil, bleeding time and clotting time. Platelet count was significantly ($P < 0.05$) increased at the dose of 100 mg/kg p.o. The results did not support the traditional use of the plant leaf for stimulation of blood production. It however showed improvement of non-specific immune responses involving phagocytosis and inflammation.

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Introduction:-

Cannabis indica otherwise named as Marijuana of Cannabaceae family has been reported to have traditional uses in different parts of the world [1]. This noxious weed was first recorded in India [2], studies were carried out to elucidate the use of this plant owing to its antioxidant and anti-inflammatory properties [3]. Studies also confirmed about the aqueous extract of Cannabis indica, promotes benefits in migraine, headache, and pain [4] platelet count improvement [5], body defense system [6] as well as fertility [7]. Reports have shown that activation of the host's immunological system by any foreign stimulus leads to a spectrum of cellular and humoral events comprising of several effector mechanisms involving several cell types, cell products and soluble serum factors [8]. It is an indication that boosting the body's haematological level will possibly boost the immune state of a host [9]. Hence, it is worth to study of C. indica in the field of hematology concerning their traditional uses. Immunomodulatory therapy could provide an alternative to conventional chemotherapy against variety of diseased conditions to achieve desirable effects [10].

Material And Methods:-

Plant material:

Leaves of Cannabis indica were collected from Visakhapatnam district of Andhra Pradesh, India. They were then grinded in a mortar with one litre of milliQ water and homogenized for over 3h period on an orbital shaker (Remi, Mumbai) to ensure maximum mixing for preparation of smoothie [11]. The smoothie was spin at 14,000 rpm

Corresponding Author:- Kiranmayi Varma Lanke

Address:- Department of Biotechnology, KoneruLakshmaiah Education Foundation, Andhra Pradesh, India.

(Thermo, MicroCL 21 Microcentrifuge) in cold conditions for 12 min and supernatant was removed and stored at 4°C until further analysis. A yield of 25% w/w extract was obtained for subsequent studies.

Experimental Animals:

Wistar rats weighing between 83.6 – 128.5 g, from S R Biotechnologies, Bangalore and were used for the experimental studies. The animals were maintained under normal environmental temperature (26–28°C), approximately 12 h day and night illumination cycle. The animals were provided with commercial rat feed supplied by Hindustan Lever Ltd, Mumbai [12].

Acute Toxicity Studies:

The estimation of the median lethal dose (LD₅₀) for the extract was done in Wistar rats orally [13]. The extract was administered in biphasic manner using dosages ranging between 10 and 100 mg/kg. The animals were observed for 72 hours for behavioral effects such as nervousness, ataxia, excitement, alertness, dullness and death. The LD₅₀ was calculated as the geometric mean of the dose that caused 100% mortality and that which cause 0% mortality.

Haematological Studies:

Wistar rats were grouped into four of five rats each, first group received normal saline (10 ml/kg p.o.) and served as the control. The second, third and fourth groups received the extract (10, 50 and 100 mg/kg p.o.) once daily for 14 days. On the 15th day, all the animals were anaesthetized with chloroform, sacrificed and their blood collected in EDTA anti-coagulant bottles. Haematological methods which includes haemoglobin (Hb), packed cell volume (PCV), red blood cell count, total leucocyte count (WBC), differential leucocyte count, platelet count, bleeding time and clotting time were performed.

Statistical Analysis:

The results of the studies were expressed as mean ± SEM. The difference between the control and treated means were analysed using one-way analysis of variance (ANOVA). Student t-test was used where ANOVA showed significant difference. Statistical significance was established at P < 0.05. Results were presented as tables and diverse charts (histograms, line graphs).

Results And Discussion:-

Acute Toxicity Studies:

No overt toxicity signs or death were observed in rats and mice, 72 h post oral treatment with doses between 10 – 1000 mg/kg. Hence, the estimated oral LD₅₀ of *C. indica* leaf base extract in rats is ≥ 1000 mg/kg. The rats treated intraperitoneally (i.p.) with 10 – 1000 mg/kg doses showed no overt toxicity sign or death 24 h post treatment. However, all the rats treated with 1000 mg/kg i.p. dose became recumbent and died within 48 h of the intraperitoneal treatment while those treated with 10 – 400 mg/kg i.p. doses neither showed toxicity signs nor death 72 h post i.p. treatment. Hence, the intraperitoneal LD₅₀ based on 24 h and 48 h post treatment observation times were ≥ 1000 mg/kg i.p. and 714.2 mg/kg i.p. in rats. The mice treated intraperitoneally with extract doses ≤ 700 mg/kg showed neither toxicity signs nor death 24 h post treatment. At the dose of 800 mg/kg i.p., the mice were calm, dull, had increased respiratory rate with mortality of 66.7% and 100.0% occurring within 24 h and 48 h of i.p. treatment respectively. The mice treated intraperitoneally with 1000 mg/kg dose became calm, dull and recumbent with increased respiratory rate. A mortality of 100.0% occurred at this dose within 24 h. The estimated intraperitoneal median lethal dose in mice was 750.0 mg/kg i.p. and 850 mg/kg i.p. for 24 h and 48 h post treatment observations respectively.

Haematological Studies:

Both increases and decreases were observed at all the tested doses for such parameters as haemoglobin (Hb), packed cell volume (PCV), total red blood cells, total leucocyte count and bleeding time. However, none of these changes was significantly different from the control. Increases and decreases were also observed in neutrophil and lymphocyte indices. However, the increase observed for neutrophil was only significant (P < 0.05) at the dose of 100 mg/kg p.o. while the decrease observed in lymphocyte was only significantly (P < 0.05) different from control at dose of 100 mg/kg p.o. Also, there was a general but non-significant decrease in the monocyte count. The basophil and eosinophil counts replicated the control count. A general but non-significant decrease was observed in the clotting time while there was increased platelet count in all the doses. The platelet count elevation was significant at the dose of 100 mg/kg p.o. (Tables 1 and 2).

Table 1:- Effect of aqueous extract of *C. indica* leaf on some haematological indices of rats treated orally for two weeks.

Treatment (2 weeks)	Hb (g/dL)	RBC ($\times 10^{12}/L$)	WBC ($\times 10^9/L$)	Platelet ($\times 10^9/L$)	BT (sec)	CT (sec)
Control	12.72 \pm 0.38	4.84 \pm 0.16	7.78 \pm 0.59	468.0 \pm 14.5	31.4 \pm 4.1	43.2 \pm 2.7
Test (Ph)						
10 mg/kg	12.16 \pm 0.55	4.78 \pm 0.24	8.22 \pm 1.00	476.0 \pm 17.7	38.0 \pm 6.4	38.0 \pm 2.5
50 mg/kg	12.86 \pm 0.60	5.10 \pm 0.13	8.28 \pm 1.20	491.0 \pm 18.7	41.4 \pm 6.7	36.4 \pm 2.8
100 mg/kg	12.26 \pm 0.30	4.86 \pm 0.29	7.50 \pm 1.10	514.8 \pm 18.9*	23.2 \pm 3.1	38.4 \pm 3.7

Hb = haemoglobin; WBC = White Blood Cell; p.o. = Peros (per oral); RBC = Red Blood Cell; BT = Bleeding Time; CT = Clotting Time.

*= P<0.05, significantly different from control (One-way ANOVA; Student t-test).

Table 2:- Effect of 70% v/v aqueous extract of *C. indica* leaf on differential leucocyte count of rats treated orally for two weeks.

Treatment (2 weeks)	Differential Leukocyte Count (%)				
	Neu.	Lym.	Mon.	Eos.	Bas.
Control	11.8 \pm 1.2	87.8 \pm 0.9	0.4 \pm 0.4	0.0 \pm 0.0	0.0 \pm 0.0
Test (Ph)					
10 mg/kg	20.0 \pm 4.2*	79.8 \pm 2.2*	0.2 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0
50 mg/kg	19.0 \pm 4.3	80.8 \pm 2.0	0.2 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0
100 mg/kg	11.6 \pm 1.6	88.2 \pm 1.7	0.2 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0

Neu = Neutrophils; Lym= Lymphocytes; Mon= Monocytes; Eos= Eosinophils; Bas = Basophils. *= P<0.05, significantly different from control (One-way ANOVA; Student t-test).

Conclusion:-

The present study evaluated the extract for haematological and immunomodulatory properties of *C. indica* leaf part of which it is traditionally used. The immunological models adopted in the evaluation took into consideration both specific and non-specific types of immunity. The study revealed that the extract had no significant effect on the haemoglobin (Hb), packed cell volume (PCV), red blood cell count, total leucocyte count, monocytes, basophil, eosinophil, bleeding time and clotting time of rats treated for 14 days. The neutrophils and platelets of the 14-day treated rats however increased significantly (P<0.05) increased at 100 mg/kg p.o. and 400 mg/kg p.o. doses respectively, while their lymphocytes decreased significantly (P<0.05) at 100 mg/kg p.o.

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