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Hormonal profile and haematological parameters of male wistar albino rats treated with methanloic extract of *Parthenium hysterophorus* L.

Priyanka Saha, Neha Yadav, Suruchi Kumari, Bharti Singh Raipat[1] and M. P. Sinha*

Department of Zoology, Ranchi University, Ranchi – 834008, Jharkhand, India.
[1]Department of Zoology, St. Xavier's College, Ranchi – 834001, Jharkhand, India

Abstract

Parthenium hysterophorus world's seven most notorious weed is a nuisance weed and causes harm to the system it invades. Haematological and hormonal profiles are used to determine normal anatomy, physiology, mood and sexual behavior of an organism. To show its impact on wistar albino rat's experiments were done. Changes in hormonal and haematological level were assessed in male wistar albino rats treated with methanolic extract of *Parthenium hysterophorus* L. The result showed that methanolic extract treatment caused a significant ($p < 0.01$) reduction of 20 % and 40% in total RBC count (6.25 ± 0.025 to 5 ± 0.5 x 106/µL) and haemoglobin (17.1 ± 0.1892 to 10.2 ± 0.79 g / dL) respectively in treated rats over control. Unlike haematological parameters, hormonal profile showed a significant increase of 40% ($p < 0.05$), 200% ($p < 0.01$), 100% ($p < 0.01$) and 45.08% ($p < 0.001$) in follicle stimulating hormone, leutinizing hormone, prolactin and testosterone respectively. The reduction of blood parameters is due to less haemopoiesis or induction of anemia. The increase in hormone level may be a cause of prostate cancer in wistar albino rats.

Keywords: *Parthenium hysterophorus*, prostate gland, Haematology, oncogenes, dihydrotestosterone.

INTRODUCTION

Haematological values are widely used to determine systemic relationships and physiological adaptations including the assessment of general health condition of an organism. [1, 2] . Hormonal profile regulates sexual behavior, growth of the cellular components of tissues and organs. Alteration of blood parameters disrupts normal physiological functions likewise changes in the concentration of hormones can have profound effects on mood, behavior, anatomy and physiology in humans. Inhalation exposure to kerosene, petrol fumes and gasoline has been reported to alter the level of hormone and different components of blood [3,4].

Parthenium hysterophorus L., commonly known as gajar ghass, congress weed and feverfew, an obnoxious weed is considered to be one of the world's seven most notorious weed and it is estimated that about 35 million hectares area in India has been invaded by it [5]. In Australia and India *Parthenium* has achieved the status of "worst weed" which causes harm to agriculture, environment and human health in the world. *Parthenium* hysterophorus, an r-selection species, is an extremely prolific seed producer (up to 25000 seeds per plant) [6]. The chemical analysis has indicated that all plants parts including trichomes and pollen contains toxins from the chemical group of sesquiterpene lactones [7]. The major components of toxic being parthenin and other phenolic acids such as caffeic acid, vanillic acid, ansic acid, p-anisic acid, chlorogenic acid, and parahydroxy benzoic acid are lethal to human beings and animals [8,9].

The toxicity caused by the toxic chemicals of plants affects the beneficial microorganisms of soil [10, 11]. Tudor [12] and Towers and Subba Rao [13] have studied the impact of weed *Parthenium* in tainting of meat of sheep grazing on them and on human affairs. Aqueous extracts of 10, 30 and 50% obtained from aerial parts of *Parthenium hysterophorus* has been reported to inhibit germination and seedling growth of Helianthus annuus L. [14]. The impact of methanolic extract of *Parthenium* on haematological parameters of wistar albino rat has also been reported [15]. Uboh et al. [4] have studied the impact of gasoline vapours on serum total and prostatic acid phophatase, alkaline phosphatase and testosterone level in wistar albino rats. The review of literature reveals the paucity of information on impact of the weed on mammalian endocrine system and particularly hormone level. This study assessed the effect of methanolic extract of *Parthenium hysterophorus* on total RBC count, Haemoglobin amount, Follicle stimulating hormone (FSH), Leutinizing hormone (LH), Prolactin (PRL) and testosterone level in the blood of male wistar albino rats.

MATERIALS AND METHODS

Experimental animals

As experimental material, 12 male wistar albino rats (Rattus norvegicus) weighing 120 – 200g were taken. They were kept in metallic cages (40 x 15 x 16 cm) under laboratory conditions for one week of acclimatization and were divided into two groups control and treated. The rats were fed with normal rat chow (guinea feeds product) and tap water ad libitum. They were kept in

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*Corresponding Author

M. P. Sinha
Department of Zoology, Ranchi University, Ranchi – 834008,
Jharkhand, India.
Tel: 91- 9431360645
Email: m_psinha@yahoo.com

well ventilated room at ambient temp. of 30 ± 5ºC under 12 hr light / dark cycle. The animals in both control and treated groups were maintained in normal diet while animals in the treated group were administered orally 20 mg / 100 g body weight of methanolic extract of *Parthenium hysterophorus* (MEPH) by using a curved needle and tuberculin syringe [16].

Preparation of extract

The shed dried plant of *Parthenium* was powdered and was subjected to soxhlet extraction with methanol for 24 hr. The blackish green extract thus obtained was evaporated to dryness in a flask evaporator at room temperature and the residue designated as methanolic extract of *Parthenium hysterophorus* (MEPH) was used as toxicant for further studies.

Haematological analysis

After 14 days of oral treatment, blood sample was collected by cardiac puncture in sterilized vials separately for control and treated group of rats. Sample was analysed for total RBC count and haemoglobin amount by fully automated bi – directional 5 part differential analysers technology [17].

Hormonal analysis

The peptide hormones namely Follicle Stimulating Hormone (FSH) and Leutinizing Hormone (LH) were measured by fully automated bidirectionally interfaced chemi luminescent immune assay and concentration was expressed as mIU / mL for FSH and LH while for PRL as ng / mL. The steroid hormone testosterone was measured by radioimmunoassay as described by Banu et al.[18] in ng / mL.

The results were statistically analyzed by Microsoft Office-Excel (2007 version).

RESULTS AND DISCUSSION

The effects of oral administration of Methanolic extract of *Parthenium hysterophorus* (MEPH) on haematological parameter and hormonal profile are shown in Table 1 and 2. It was observed that oral administration of MEPH caused a significant ($p < 0.01$) reduction of 20 % in total RBC count between control and treated group of rats. Its value was 6.25 ± 0.025 in control and 5 ± 0.5 x 106/µL in treated group. Likewise haemoglobin amount of control was 17.1 ± 0.1892 g / dL which also decreased significantly ($p< 0.01$) to 10.2 ± 0.79 g / dL in treated group with 40% reduction. The result revealed that the rats become anemic after oral treatment of MEPH. KamalShah [1] also found similar deteriorating trend in haematological parameters in female rabbits following the treatment with cypermethrin. MEPH might have inhibited RBC formation [1] which resulted in reduction of RBC contents and leads to decreased haemoglobin content [19]. The depletion in RBC count and haemoglobin content may be attributed to defective haemopoiesis [1, 20]. Other possible factors affecting adversely may be reduced food intake by animals or internal haemorrhages [21], but no such obvious sign was noticed during the study. Significant decrease in Haemoglobin (Hb) concentration may be due to impaired oxygen supply to various tissues, resulting in slow metabolic rate and low energy production [22], or may be due to increased in metabolic rate, which may have led to decrease Hb concentration level [23].(Reddy and Bashamohideen 1989).

Table 1: Effect of doses of MEPH on total RBC count and haemoglobin

S. No.	Parameters	Control	Treated	Change in %
1	Total RBC (106 / µL)	6.25 ± 0.25	*5 ± 0.5	20↓
2	Haemoglobin (g / dL)	17.1 ± 0.1892	*10.2 ± 0.79	40↓

* = significant ($p < 0.01$)

Fall in haemoglobin content and RBC count can be correlated with induction of anemia in experimental animals after exposure to toxic compounds [24, 25]. Ammonium metavanadate in the dose range 0 – 10 mg per kg caused both dose and duration dependent effects on the haemoglobin and packed cell volume of female wistar rats [26]. It was reported that 7 to 28 days of persistant treatment with Vanadate, Ammonium metavanadate caused a dose dependent significant decrease from 11.29 ± 1.2 to 5.67 ± 0.9 and 11.350 ± 1.4 to 4.245 ± 1.02 g / dL respectively [26]. Ashour et al. [27] also observed oral administration of 1000 or 2000 ppm lead acetate significantly decreased red blood cell count, hemoglobin level and hematocrit value at 20, 40 and 60 days compared with control groups of male wistar albino rats. A shortening of erythrocyte survival time was observed in the rats exposed to lead [28]. According to Terayama et al. [29] lead could affect the rat erythrocyte membrane and decrease their mobility, it may also induce oxidative stress in RBCs [30]. Similarly parthenin content of MEPH could be the reason behind significant decrease of RBC count and haemoglobin content. The results are also indicative of maturation arrests of haematological cells or decreased heme biosynthesis by inhibiting aminolevulinic acid dehydratase and ferrochelatase activity [31, 32]. Egbung, [33] reported decreases in RBC counts and adverse effect on hematopoietic status of all treated wistar albino rats which were fed with trans fatty acids. The likely explanation for the effect is destruction of the membrane structure of RBC. Failure of erythropoietin may have caused the decrease RBC count in the groups fed with test diets. The inhibitory response (anemia) were consistent with the earlier works of Knecht et al. [34], showing that the results obtained with haematological parameters are dependent on the time of harvesting of the blood cells. From the result it could be said that parthenin content of weed may induce anemia both by interfering with haem biosynthesis and by diminishing RBC survival.

Result of hormonal profile of control and treated group (Table 2) showed that level of FSH was 1.15 ± 0.1 mIU / mL in control and 1.61 ± 0.09 mIU / mL in treated group showing 40 % increase ($p < 0.05$) from control condition. Similarly LH and PRL showed significant ($p < 0.01$) increase of 200 % from 0.06 ± 0.01 mIU / mL (control) to 0.18 ± 0.03 mIU / mL (treated) and 100 % increase from 0.15 ± 0.025 ng / mL (control) to 0.30 ± 0.05 ng / mL (treated). The steroid hormone, testosterone also revealed significant increase ($p < 0.001$) of 45.089 % and its value was 24.64 ± 4.1 ng / mL in control and 35.75 ± 3.300 ng / mL in treated.

Table 2: Effect of doses of MEPH on peptide and steroid hormonal profile

S. No.	Parameters	Control	Treated	Change in %
	Peptide hormone			
1	Follicle stimulating hormone (mIU / mL)	1.15 ± 0.1	**1.61 ± 0.09	40 ↑
2	Leutinizing hormone (mIU / mL)	0.06 ± 0.01	*0.18 ± 0.03	200 ↑
3	Prolactin (ng / mL)	< 0.15 ± 0.025	*< 0.30 ± 0.05	100 ↑
	Steroid hormone			
4	Testosterone (ng / mL)	24.64 ± 4.1	***35.75 ± 3.300	45.08 ↑

* = significant ($p < 0.01$), ** = significant ($p < 0.05$),*** = significant ($p < 0.001$)

Uboh et al. [35] reported that exposure to gasoline vapours increased serum FSH, LH and testosterone in male rats and decreased serum FSH, LH and estradiol and progesterone in female rats. Testicular androgens (testosterone) are responsible for the growth and development of male urogenital system and the accessory sex organs. The prostate gland tissue is also known to be a testosterone dependent organ and when the testosterone level decreases as a result from an increasingly pronounced metabolic syndrome [36, 37], the growth-stimulating effect on the prostate gland by other aberrations might possibly be reduced. Niu et al. [38] also stated that the hormonal anatomical and functional growth of the prostate is mainly controlled by androgens. The hormonal hypothesis seems to be one of the most important hypotheses in prostatic cancer etiology, and efforts are continuing to improve the understanding of androgen action in prostatic cancer [39]. Although evidence from epidemiological studies of an association between circulating levels of androgens and prostatic cancer risk has been inconsistent, the traditional view that higher testosterone represents a risk factor for prostatic cancer appears to have little evidentiary support [40].

The results of Uboh et al. [4] experiment showed that exposure to gasoline vapours caused increase in serum total and prostatic acid phosphatase, alkaline phosphatase, gamma glutamyl transferase, and testosterone in male rats. These results gave an indication that exposure to gasoline vapours may adversely affect the functionality of the prostate glands. The elevation of serum level of these bio-indices have been reported to be associated with prostatic cancer [41 - 44]. This indicates that these enzymes and the androgen can be used as biomarkers for the assessment of prostatic cancer. The observations made from the result of this present study therefore suggest that treatment with MEPH may be considered to be one of the predisposing environmental risk factors for prostatic disorders leading to cancer. Although the specific mechanism(s) through which administration of MEPH causes increase in follicle stimulating hormone, prolactin, leutinizing hormone and blood testosterone in male rats is not understood. However, Hadley [45] emphasized that PRL augmented the effects of testosterone on the prostate gland greatly. On the contrary, a lack of LH production and secretion or a LH receptor defect would also result in failure of testosterone production. Several intriguing reports, especially the report by Ross et al. [42], suggest that the endogenous level of androgenic hormones, either testosterone, or dihydrotestosterone, may play a pivotal role in the cause of prostate cancer. Bruchovsky [46] reported that androgens can be metabolized to DHT (active androgen) in rat ventral prostate. An enhanced uptake of DHT in the gland may be due in part to enhanced intracellular binding of the androgen. Several studies have identified relationships between pretreatment serum levels of testosterone and both clinical stage of prostate cancer and patient survival, suggesting that pretreatment serum testosterone level has potential as a prognostic factor for prostatic cancer [47, 48].

The increasing trend of blood hormones FSH, LH, PRL and testosterone on oral administration of MEPH were novel since no works has earlier reported these. Raji et al. [49] found the mean serum testosterone level of rats treated with 400 mg/(kg·d) of the Morinda lucida leaf extract for 4 and 13 weeks significantly increased ($p < 0.01$) compared with the controls. He also stated that the extract caused an increase in the weight of the testes, which was accompanied by an increase in the serum levels of testosterone. Similar changes have been reported with the extract of Zingiber officinale and Pentadiplendra brazzeana in rats [50, 51]. Others have reported testicular weight reduction accompanied by decreased serum testosterone levels in male rats treated with the extracts of Quassia amara [52], Azadirachta indica [53] and gossypol, a phenolic compound extracted from the cotton seed [54]. The MEPH treatment may lead to carcinogenesis.

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