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Alteration in hematology of European rabbit *Oryctolagus cuniculus* (Linn) due to exposure of *Ipomoea carnea* (Jacq) leaf extract

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ABSTRACT

The *Ipomoea carnea* is commonly known as Besharam, Behaya in India. The plant active ingredients are known to be reported medicinal importance. But hematological activities were lacking hence present task was assigned. In the present study ethanol leaf extract was evaluated on various fifteen haematological parameters at control, 2, 7, 14 and 21 days with weight loss. The blood parameters such as GRS, LY, MO, MCV, MCH and PWD were inclined in post treatment, whereas WBC, RBC, Hb, and blood indices such as HCT, MCHC, RDW, PLT and MPV were significantly declined in exposed rabbit ($P < 0.05$). Experimental rabbit showed 13.33 gm/day decline in body weight as compared to control. This indicates nutritional and medicinal values, but we cannot yet recommend its therapeutic use before detailed and depth of studies.

Keywords: Alteration, hematology, leaf extract, *Ipomoea carnea*.

INTRODUCTION

Ipomoea carnea Jacq is popularly known as Besharam, Behaya in India and morning glory in English. It belongs to family Convolvulaceae [1]. In India it is particularly distributed all over India [2]. It was used in ancient system of medicine in many countries but not to great extent due to toxicity. The plant has its immense potential in medicine and reported medicinal importance such as glycosidase inhibitor [3], inotropic cardiovascular [4], immunomodulation [5], wound healing [6], embryotoxic [7], antimicrobial [8], affect nervous system [9], antioxidant [10], anti-inflammation [11], antifungal [12], antidiabetic [13], antibacterial [14], hepatoprotective [15], anxiolytic [16], and anthelmintic activity [17].

Since literature on the effect of this plant on blood cells and loss in weight has been lacking behind, therefore the aim of the study was to investigate the effect of *Ipomoea carnea* leaf extract on haematological alterations and weight loss in European rabbits.

MATERIALS AND METHODS

Experimental animal: European rabbit *Oryctolagus cuniculus* (average weight 1890 gm) were used in this study. Weights of animals before and after experiments were measured using sensitive electronic balance.

Experimental plant: Plant leaf of *Ipomoea carnea* was collected from local area from Pravaranagar, Ahmednagar Maharashtra. The plant was identified based on its floral description

in the literature.

Preparation of extract: The plant leaves were washed, air dried under shade, left in ethanol (70%) for more than two days in Soxhlet apparatus. Then the 70% ethanol extract was dried in Rotatory Evaporator apparatus, weighted and dissolved in distilled water to give the final concentration and were administrated orally by 2 gm/body weight of experimental individuals for 28 days.

Collection of blood samples: Blood was collected from ear artery in capillary tubes coated with ethylene diamine tetra-acetic acid (EDTA) for further hematological studies. The blood was collected at control (Zero day), 2nd, 7th, 14th and 21th days of post-treatments.

Analysis of blood: The blood samples were analyzed to determine the haematological parameters on Automated Haematology Five part Cell Analyzer Coulter, Made by China. The machine is an automatic multipair blood cell counter for in vitro diagnostic use in clinical laboratory. It performs speedy and accurate analysis of blood parameters and detects the abnormal samples. The automated haematology analyzer reading correlated well with readings by the standard manual method. The following blood parameters such as white blood corpuscular count (WBC), granulocytes (GRS), monocytes (MO), lymphocytes (LY), red blood corpuscular count (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet blood test (PLT), procalcitonin (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) were recorded for further study.

Statistical analysis: Mean values of blood parameters and body weights were analyzed by student *t*-test using computer package program. The percent declined or inclined values were calculated over the control.

RESULTS AND DISCUSSION

European rabbit treated with ethanol extract showed significant decline in their body weight as compared to the controls ($P=0.014$). There was 13.33 gm/day loss of body weight in experimental as compared to control (Table 1). Post treatment rabbit showed low locomotion, weakness and erection of hairs. It is thus showed that reduction in weight in animal that created by plant. It may be any one of the causes that sheep, goat and other herbivorous animal's dose not feed on the *Ipomoea* leaves. Because the leaf of plant consist thirteen components in which any one or some or all may be toxic to animals. The significance reduction in body weight of rabbit against the captivity, where energy expenditure is minimal indicates anti-obesity activity. It indicates anti-obesity activity but the toxicity should be considered and detailed study should be necessary.

In leaf of *Ipomoea carnea* contains saponins [18]. The chemical constituents as saponins are toxic to animals. The ethanol extract of *Ipomoea carnea* leaves was proved for its toxicity against fish Guppy *Poecilia reticulata* [19]. Saponins also known to cause poisoning in animals. Sapogenins are metabolized in animals to glucuronide conjugates of epsimilagenin, which crystallize in bile, leading to biliary blockage, cholangitis, and secondary photosensitization [20]. In fact, herbivores are affected with signs of toxicity involving liver damage, with anorexia, mass loss, icterus, hepatoencephalopathy and photosensitization [21,22]. The mass loss could be a cause of body weight loss, which was observed in the present study.

It is revealed from table 2 that the blood parameters in treated and control group showed significant changes. The parameters such as granulocytes (GRS), lymphocytes (LY), monocytes (MO), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and platelet distribution width (PWD) were inclined in the post exposed rabbit. Some blood parameters such as white blood corpusculars (WBC), red blood corpusculars (RBC), haemoglobin contents (Hb), and blood indices such as haemacrits (HCT), mean coepuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet blood test (PLT), procalcitonon (PCT), and mean platelets volume (MPV) were significantly reduced in post exposed rabbit ($P<0.05$).

The present study showed that the leaf extract of *I. carnea* caused significant reduction in blood parameters i.e. RBC, WBC, Hb, and hematocrits. However, it showed that animals that browse the leaves of the plant might develop anaemia reflected due to reduction in RBC and Hb or both parameters. It is due to excessive red blood

corpuscular destruction or loss or reduction in production and is a manifestation of an underlying disease process, therefore the response to treatment of anaemia is transient unless the underlying disease process is addressed. In the study, anemia state of rabbit may be attributed to inhibition of erythropoietin coupled with enhanced rate of erythrocyte destruction, disturbed haemoglobin synthesis and haemodilution as also reported by other workers [23]. Some worker believed that reduction in total RBC count may be due to microlytic or normocytic anemia [24, 25].

WBC plays a major role in the defense mechanism in animals. An immediate reduction of the rabbit immune system was showed by increase in leucocytes throughout days of exposure. Decline in WBC is attributed to anti endomosis and consequent haemodilution on resulting increase in MCV through the exposure. The WBC indices such as lymphocytes and monocytes were found to be inclined in exposure rabbits. Platelet can retract and caused clot retraction due to their thrombostain in contains which help in coagulation it act as assist to homeostasis. In may be a one cause of reduction in platelets in present studies.

Hematocrit may be used for, diagnose, or monitor a number of conditions and diseases that affect the proportion of the blood made up of red blood cells. Some conditions affect RBC production in the bone marrow and may cause an increase or decrease in the number of mature RBCs released into the blood circulation. Other conditions may affect the lifespan of RBCs in the circulation. If there is increased hemolysis destruction of RBCs or loss of RBCs and/or the bone marrow is not able to produce new ones fast enough, then the overall number of RBCs and hematocrit will drop, resulting in anemia, which is observed in the present study.

The present task noticed increased in lymphocytic number in the post treated rabbit. A high lymphocyte count could indicate that animal are suffering from some sort of toxicant or should be cause of numbers to be higher.

The present study reported increased in MCV values as compared to control. The MCV in the present study showed 62.6 fl size in control which was increased on 2, 7, 14 and 21 days of post-exposed rabbits. MCV is the index used to measure the average volume of RBC. The MCV categorized RBC size and indicates normocytic, microcytic, and macrocytic anemia. Food deficiencies can cause anemia; the anemic, tests show a low MCH and low MCHC. MCH and MCHC are used to diagnose anemia, though a well-balanced diet helps prevent anemia and low MCH or MCHC. It indicates that during experimentation there was sufficient food but diet or plant extract has various thirteen chemical components in tested extracted plant which could be reduced weight in the experimental rabbits.

The average weight in RBC is measured by MCH. The MCH values usually rise or fall as the MCV increased or decreased. The MCHC measured the average concentration of hemoglobin in a RBC. The MCHC categorized RBC to their concentration of hemoglobin in a RBC. The MCHC categorized RBC to their concentration of hemoglobin. It provides information about normochromic, hypochromic but not hyperchromic category.

The PWD was increased in the study. It revealed 2.20 μm in control and increased up to 20.0 μm on 2 and 14 days exposure. PWD gives information about the range of platelet size in blood sample. MPV refers the average volume of individual platelets. PWD is dispersion of the size of the platelets in blood volume. The PLT were declined in the study. They declined on 2, 7, 14 and 21 days of exposure. These two indicators can be done automatically in automatic blood cell counter. The platelet parameters such as MPV and PWD have been available and usefulness for clinical was not understandable [26], which may be influenced by the delay between blood collection and analysis.

In the study PLT was declined in exposure rabbit. In human/animals platelets are used for investigation of diseases [27]. Generally MPV has an inverse, nonlinear relation with platelets count, while platelet volume heterogeneity has direct, nonlinear relation with MPV. Compared with the patients with chronic lymphocytic leucomia, atherosclerotic heart disease, diabetic mellitus and chronic schizophrenia had normal platelet volume mean and heterogeneity [28]. The large platelet has been reported in animals with MPD after examination of stained films of peripheral blood [29]. The quantification of such platelets, however, remains subjective. Several authors [30, 31] tried to discriminate thrombocytosis in MPD from RT by using platelet parameters on condition that by blood analyzers.

The present data revealed a relation between both PLT and MPV and PDW. Therefore, it is suggested that these three parameters be subjected to a combination interpretation, through the calculation of a PDW. The increased PDW probably reflects a deregulation in thrombopoiesis, which is also translated by the multiple abnormalities of platelet reactivity and the change in platelets membrane and adenine nucleotide content [30, 32, 33]. The reason mentioned above, I did not compared with PDW with these tests. The physio-pathological considerations were well

beyond the scope of this study. Several workers have used a series of platelets indices measured by haematological analyzer given the fact that platelet activation causes morphological changes of platelets. The MPV most extensively studied platelet activation marker [34, 35]. Recently, platelet indices such as mean platelet component (MPC) and platelets component distribution width (PCDW) have been investigated as prospective platelet activation markers [36, 37]. However, not all hematological analysis examines these indices.

In the study RDW was noticed as increased in trends. RDW is elevated in accordance with variation in red cell size (anisocytosis), i.e. when elevated RDW is reported on complete blood count; marked anisocytosis (increased variation in red cell size) is expected on peripheral blood smear review [38]. The parameter PCT was revealed declined trend in post exposure rabbit.

Table 1. Showing body weightlossin European rabbit

Parameters	Experimental specie
Initial weight (g)	1890 ± 5.33
Final weight (g)	1610 ± 5.12
Differences in weight (g)	280 ± 2.82
Differences in weight (%)	14.81 ± 2.11
Weight loss (per/day)	13.33 ± 1.12

Table 2. Haematological changes in rabbit exposed to leaf extract of *Ipomoeacarnea*

Parameters	Post exposure (days)				
	Control	2	7	14	21
WBC (cumm ⁻³)	2.3 (100)	2.2 (-4.35)	2.1 (-8.69)	2.2 (-4.35)	5.4 (134.7)
GRS (%)	27.8 (100)	24.2 (12.95)	29.3 (5.40)	39.2 (41.01)	31.7 (14.03)
LY (%)	60.4 (100)	62.9 (4.14)	64.1 (6.13)	47.7 (-21.03)	53.9 (-10.76)
MO (%)	11.8 (100)	12.9 (9.32)	13.1 (11.02)	13.5 (14.41)	14.4 (22.03)
RBC (cumm ⁻⁶)	6.30 (100)	5.57(-9.52)	5.61 (-10.95)	5.64 (-10.47)	6.11(-3.02)
Hb (gm/dl)	12.5 (100)	12.2 (-2.40)	11.1 (-11.2)	11.2 (-10.4)	11.3 (-9.60)
HCT (%)	39.5 (100)	37.4 (-5.32)	36.9 (-6.58)	39.1 (-1.01)	41.2 (4.30)
MCV (fl)	62.6 (100)	67.1 (7.19)	71.2 (13.74)	69.3 (10.79)	67.4 (7.67)
MCH (pg)	19.8 (100)	21.9 (10.60)	20.5 (3.53)	19.8 (0.00)	18.4 (-7.07)
MCHC (g/dl)	31.6 (100)	32.8 (3.79)	29.5 (-6.65)	28.6 (-9.49)	27.4 (-13.29)
RDW μ m	26.0 (100)	17.1 (-34.23)	17.3 (-33.46)	17.6 (-32.31)	17.9 (-31.15)
PLT (cumm ⁻³)	541 (100)	562 (3.88)	488 (-10.80)	405 (-25.14)	315 (-41.77)
PCT (%)	0.82 (100)	0.81(-1.22)	0.73 (-10.98)	0.53 (-35.37)	0.23 (-71.05)
MPV (fl)	15.1 (100)	11.2 (-25.83)	12.7 (-15.89)	13.2 (-12.58)	7.3 (-51.66)
PDW μ m	20.20 (100)	20.6 (10200)	10.2 (5000)	20.6 (10200)	8.9 (4350)

Keys: WBC, white blood cell count; GRS, granulocytes; MO, monocytes, LY, lymphocytes, RBC, red blood cell count; HCT, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; PLT, platelet blood test; PCT, prolactin; MPV, mean platelet volume; PDW, platelet distribution width.

Figures in parenthesis are percent incline or decline over control.

CONCLUSION

The results of this study supported that the plant *I. cornea* in having haematological effects in relation to some health problem associated with nutritional problems. It was indicted that its negative effect on some blood parameters and body weight loss in the experimental animals. This showed that plant has toxic potential and their continued presence in nature could lead to plant toxicity, especially if these plants are consumed. The effect of this toxicity in livestock production could be direct (death) and indirect losses have high economic consequences on livestock production for farmers. On the basis of results, we would like to recommend investigation on the histopathology and blood parameters in prenatal rabbits and other animal's models. This work gives access to new hematological indices which can be used as a diagnostic indicator of pathologies. I would like to focus on future plan as: To evaluate biochemical estimation in tissue level. To evaluate histopathology on various organs level. To evaluate and separate the biochemical constituents present in leafand then remove the toxicity and then confirmed as anti-obesity activity.

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