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EFFICACY OF INSULIN PLANT (*COSTUS IGNEUS* NAK) ON BLOOD SUGAR AND PARAMETERS OF NEW ZEALAND WHITE RABBIT (*ORYCTOLAGUS CUNICULUS* L.)

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Abstract*: The intent of the study was assigned to evaluate blood sugar level in New Zealand white rabbit (Oryctolagus cuniculus L.) exposed to the insulin plant leaves (Costus igneus). Diabetes mellitus is a metabolic disorder that has highly affected human health and quality of life. Conventional agents are being applied to control this disorder along with lifestyle management. The leaves of insulin were feed to rat regularly and blood contents were evaluated. The blood sugar level was declined as 0.27 mg/day in the exposed rabbit. The consumption of leaves of Costus igneus helps to prevent diabetes mellitus by decreasing the blood glucose level. Considering this ability of Costus igneus, the plant contents have a high therapeutic potential, should use as anti-diabetic. These bio-compounds deserve special attention pharmaceutically. Attempts are being made to adapt the model for special studies concerning the early state of diabetes.*

Key Words: *Blood glucose, Blood paramètres, Zetland white Rabbit, insulin plant, efficacy.*

1. INTRODUCTION:

Diabetes mellitus is a chronic metabolic disorder in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. It is of two types, i.e. Type 1 and Type 2. Type 1 diabetes is also called as insulin-dependent diabetes or juvenile-onset diabetes due to its appearance in childhood. Type 2 is mostly concerned due to its deleterious effects. Statistical reports are available on death rates of diabetic patients like in 2012 diabetes caused 1.5 million deaths around worldwide. Many of these deaths (43%) occur under age of 70 [1]. This number may be increased up to 592 million people by 2030, i.e., 9 % of the country's population is likely to be affected from diabetes [2]. Hence, it is important to control diabetes. The management of diabetes is still major demand for research on natural products with anti-diabetic properties [3]. In India, the herb *Costus igneus* is popularly known as insulin plant. Due to consumption of one or two leaves of *Costus igneus* a day keeps diabetes away [4].

2. LITERATURE REVIEW:

The plant *Costus* belongs to the family Costaceae, which is found in tropical Africa, Asia, Australia, and North, Central and South America. In India, it is cultivated in coastal area, Uttar Kannada district of Karnataka. In this area, people take traditionally 2-3 leaves of this plant twice a day for the management of diabetes [5]. The rhizome of insulin plant is considered as a bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant and useful in burning sensation, constipation, leprosy, worm infection, skin diseases, fever, asthma, bronchitis, inflammations, and anemia [6]. Recently, a number of researches have been carried out phytoconstituents, pharmacological activities [7], antimicrobial [8], antioxidant [9] anticancer activities [10], etc. However, the hematological information's was not notice. Considering the medicinal importance of this widely available plant species, the aim of the present study was to identify the blood sugar and hematological changes in rabbit due to exposed to *Costus igneus.*

3. MATERIALS: Collection of plant:

The plant material fresh *Costus igneus* leaves used in this experiment were collected from the garden of P. V. P. College, Pravaranagar (Loni), Ahmednagar, MS.

Plant used: Two leaves were feed regularly to exposed group regularly up to 28 days.

Test Animals: An adult male, New Zealand white rabbit weighing 1.05 to 1.20 kg were used in the study. The animals were housed in polypropylene cages (55 cm L x 45 cm W x 30 cm H) under well-regulated light/dark (12 h: 12 h) cycle at standard temperature (22±1 ^{0}C). The animals were maintained as per guidelines of the committee for the purpose of control and supervision of experiments on animals' regulations. A control group provided only water and food, while treatment groups provided insulin plant leaves at 2.2 mg/kg body weight according. The treatment followed up to 28 days.

Experimental design: Adult New Zealand white rabbits were grouped in to two groups. The rabbit group-I were treated with vehicle (distilled water) and served as control. The rabbits in group-II were exposed to insulin plant leaves up to 28 days respectively. A dose prepared was used 2.2 mg/kg body weight to animal. Regularly treatment was applied up to 28 days. The acute oral study was performed according to the office of prevention, pesticide and toxic substance (OPPTS) guidelines following the limit test procedure. The animals were accustomed an overnight prior to the study.

Collection of blood: Blood samples were collected by nurturing the ear artery using heparinized microsyring (2 mm D). The animal was nurtured on control, 2nd, 5th,7th, 14th, 21st and 28th day post treatment. The blood was collected with the help of micro syringe in the sterilized vial containing anticoagulant EDTA as anticoagulant and used for determination blood parameters

Haematological analysis: Blood parameters such as blood glucose, red blood corpuscles (RBC), Haemoglobin (Hb), white blood cell (WBC), platelets count (PLT), lymphocytes (LYM), Monocytes (M), Eosinophils (E), Basophils (B), Neutrophils (L) and Lymphocytes (L) values by automatic hematology analyser.

4. DISCUSSION:

Stress can alter many different haematological parameters (e.g., blood glucose). Prolonged stress, such as transportation, unfamiliar noises, smell, chronic pain, and poor environment, can induce heterophilia, lymphopenia, and leucocytosis. Simple handling does not induce this response, but several muscle enzymes including lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and creatine kinase elevate after physical restraint of the rabbits, especially if they are fractious or unfamiliar with handling. Sedation or general anaesthesia can be helpful. Isoflurane anaesthesia does not appear to affect blood parameters in rabbits.

Medicinal plants have been used for greater extent for control of diabetes mellitus in various traditional system of medicine worldwide as they are of natural origin and many of them are known to be effective against diabetes. *Costus igneus* is known as an elegant plant. In India, it is popularly known as insulin plant due to the consumption of leaves of *Costus igneus* helps to prevent diabetes mellitus by decreasing the blood glucose level. Considering the ability of the valuable things present in *Costus igneus*, this review is aimed to summarize the information of the biochemical constituents, their chemical structures, and mode of action. Due to the high therapeutic potential, these bio-compounds deserve special attention. *Costus igneus* contains various phytochemicals like flavonoids, alkaloids, terpenoids and it was traditionally used in India to control diabetes and in experimental diabetic rats [15-17]. Chemical structures of various anti-diabetic components are mentioned. The actual anti-diabetic prospective associated with active bio-compounds like triterpenoids, steroids, alkaloids, flavonoids, proteins, fatty acids, carbohydrates, etc. are usually large as a result of their modulatory effects on blood sugar level by increasing insulin secretion and promoting proliferation of pancreatic cells and by reducing insulin resistance. The molecular mechanism underlying the glucose metabolism in diabetes would provide require insights in the field of drug development, future inventions may one day yield therapeutic benefit [11].

5. RESULT:

The result of partial changes in blood sugar and blood parameters were reported in table1. The rabbit exposed to *Costus igneus* resulted as significant decrease in sugar level as compared to control, while red blood corpuscles (RBC), Haemoglobin (Hb), white blood cell (WBC), platelets count (PLT), lymphocytes (LYM), Monocytes (M), Eosinophils (E), Basophils (B), Neutrophils (L) and Lymphocytes (L) values were within the standard limits as compared to standard levels to Table 1.

Blood glucose: In the present study, there was increase in the treated rabbit blood sugar level as compared as control. There was average 0.27 mg/day loss of blood glucose level during the study period when compared to controls [$P < 0.001$]. The blood glucose level was within reference range in the treated rabbits. Plant products have been used in folk medicine and traditional healing systems and are being evaluated for their hypoglycaemics effects. The study was

planned to evaluate the insulin-sensitizing effects of the insulin plant (*Costus igneus*) in dexamethasone-induced hyperglycaemia in male albino rats. The study was simple and create a database to further anti-diabetic properties in details [11].

Hb content: After exposure to *Costus igneus* rabbit showed haemoglobin values with the reference range. For the interpretation of the results it was important to ascertain whether the rate of blood formation and/or the blood volume was constant in normal rabbits. Rabbit haemoglobin is a native protein with full length. This protein is purified from rabbit erythrocytes.

RBC count: The RBC values reported to be in between 6.42 to 7.42 cumm^{-3} range when exposed to insulin leaves. It is in between the standard referenced values reported in rabbits. The erythrocyte life span of rabbits showed a 50 per cent survival between 45 and 50 days. The longest cells lived about 80 days.

WBC count: the WBC values reported to be 9.12 to 11.5 cumm^{-3} range when exposed to insulin plant leaves. It is in between the standard referenced values reported in rabbits. Rabbits under 12 weeks of age have lower red blood cell (RBC) and white blood cell (WBC) counts. The total WBC and lymphocyte counts are lowest in the late afternoon and evening, when the heterophils and eosinophil counts rise.

Platelet: In the present study none of the depletion or increase was noticed in platelets rabbit after exposed to insulin. It was in between the prescribed range. Platelet can retract and caused clot retraction due to their thrombostain contains which help in coagulation it acts as assist to homeostasis. In may be a one cause of reduction in platelets.

Neutrophil: It reported from the study that neutrophils were in between 44.6 to 52.5 %. This range is in between the standard referenced range. The insulin plant not reported any effect on neutrophils. Neutrophils are important effector cells in the innate arm of the immune system. They constantly patrol the organism for signs of microbial infections, and when found, these cells quickly respond to trap and kill the invading pathogens [12].

Basophils: It reported from the study that neutrophils were in between 4.1 to 6.8 %. The study reported that basophils were within standard reference range. The insulin plant leaves not reported any effects on neutrophils. The diameter of rabbit basophil measures 8 to 12 μm. Its nucleus is less segmented than the eosinophil or heterophil and difficult to see because of the many deep purple granules that obscure the light gray cytoplasm. As in other species, the function of the rabbit basophil is not fully understood, but these cells are often present in large numbers of rabbit blood smears. Basophilia with concurrent eosinophilia has been described in rabbits with chronic skin problems [13].

Eosinophilia: Similarly, to other WBC indices eosinophils were within the referenced range**.** In rabbits can occur when tissues rich in mast cells, such as the skin, lungs, GI tract, or uterus, are involved in disease. Eosinophilia can indicate the presence of an abscess and may be found during wound healing. In other species, eosinophilia is linked to parasitic diseases, especially when larvae are moving through tissues, but this is rare in domestic rabbits. Encephalitozoon cuniculi does not stimulate an eosinophilic response. In clinically healthy rabbits, a very low eosinophilic count, even 0, is a common finding. High levels of cortisol (chronic stress) can induce eosinopenia.

Lymphocytes: The lymphocytes were within standard reference range which lies in between 37.3 to 51.6 %. The primary role of the lymphocytes is to respond to those activities that stimulate the immune system. In rabbits, lymphocytes are primarily found in the blood, spleen, bone marrow, lymph nodes and the lymphatic tissues in the GI tract. The number of circulating lymphocytes is a balance between the cells entering and leaving the bloodstream and does not necessarily reflect a change in lymphopoiesis. In rabbits, it has been shown that an increase in adrenaline levels (acute stress) induces lymphocytosis, whereas raised cortisol levels (chronic stress) leads to lymphopenia. Viral diseases may result in a normal or higher lymphocyte count. Other causes of lymphocytosis are lymphoma [14].

Monocytes: The lymphocytes were within standard reference range which lies in between 8.4 to 12.31 %. Monocytosis is linked with chronic inflammation (e.g., abscesses, mastitis, tympanic bulla empyema). However, the absence of a monocytosis does not rule out inflammation. Monocyte counts within the normal range are a common finding in rabbits with osteomyelitis due to dental disease.

6. RECOMMENDATIONS:

The authors should recommend as insulin plant is better source of diabetic control and there should be more detail study on haematology.

Table 1. Showing referenced ranges of normal haematological parameters in the New Zealand white rabbit (*Oryctolagus cuniculus*).

Sr. No.	Blood parameters	Range	
		Male	Female
1.	Glucose mg/l	76.0	150.0

2.	RBC (cumm^{-3})	5.46-7.94	5.11-6.51
3.	Hb (g/dL)	10.4-17.4	9.8-15.8
4.	Platelets (cumm^{-3})	304-656	270-630
5.	WBC (cumm^{-3})	5.5–12.5	5.2–10.6
6.	N (%)	38–54	36.4–50.4
7.	L (%)	28–50	31.5–52.1
8.	E (%)	0.5–3.5	0.8–3.2
9.	B (%)	2.5–7.5	2.4–6.
10.	M (%)	4–12	6.6–13.4

(**Source:** Moore *et al*, 2010)

Table. 2. Showing changes in blood parameters in rabbit exposed to colistin.

Sr. No.	Blood Parameters (Units)	Control	Treated (Exposed days)					
		0	2	5	7	14	21	28
1	Glucose mg/dL	103.0(5.07)	101.9(5.04)	99.8(4.99)	98.5(4.96)	97.1(4.93)	95.4(4.88)	94.1(4.85)
2	RBC (cumm^{-3})	6.42(1.27)	6.61(1.29)	6.89(1.31)	6.85(1.31)	7.32(1.35)	7.22(1.34)	7.42(1.36)
3	Hb (g/dL)	12.4(1.76)	11.4(1.69)	12.8()1.79)	13.4(1.83)	14.4(1.89)	14.9(1.93)	16.8(2.05)
4	Platelets (cumm^{-3})	451(10.6)	422(10.3)	467(10.8)	556(11.8)	605(12.3)	586(12.1)	610(12.3)
5	WBC (cumm^{-3})	9.12(1.51)	10.2(1.59)	11.9(1.72)	10.8(1.64)	11.2(1.67)	12.8(1.79)	11.5(1.69)
6	N (%)	44.6(3.34)	42.3(3.25)	48.6(3.49)	47.5(3.45)	51.5(3.59)	54.3(3.68)	52.5(3.61)
7	L (%)	37.3(3.05)	40.6(3.19)	38.6(3.10)	44.3(3.33)	49.6(3.52)	52.3(3.62)	51.6(3.59)
8	E (%)	2.2(0.74)	2.6(0.81)	2.9(0.85)	3.0(0.87)	2.5(0.79)	2.7(0.82)	3.4(0.92)
9	B (%)	4.1(1.01)	4.0(1.0)	5.8(1.2)	6.4(1.3)	6.9(1.3)	5.9(1.2)	6.8(1.3)
10	M (%)	8.5(1.46)	7.9(1.40)	9.5(1.54)	10.3(1.60)	10.9(1.70)	11.7(1.70)	12.31.(75)

Keys: RBC- red blood cell count, Hb-haemoglobin; PLT-platelets, WBC-white blood cell count, M-monocytes, L-lymphocytes, E- eosinophils, B-basophils, N- neutrophils. Figures in parenthesis are poison values.

7. CONCLUSION:

The medicinal value of this plant lies in some chemical substances that produce a definite physiological action on the human body. The qualitative analysis of leaf extract of *Costus igneus* reveals the presence of medicinally valued as a reducing sugar. *Costus igneus* are used for discovering sugar reducing constituents which are very helpful for the manufacturing of new drugs for treatment of diabetic diseases. The research is in progress to discover its biological activity and enhance the pharmacological profile of it in the area of traditional medicine.

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