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Effect Of Alloxan Mixed Diet On Blood Parameters And Its Management By Silkworm Pupae Meal Diet In Common Carp

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45. EFFECT OF ALLOXAN MIXED DIET ON BLOOD PARAMETERS AND ITS MANAGEMENT BY SILKWORM PUPAE MEAL DIET IN COMMON CARP

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ABSTRACT

This paper presents a brief overview on the entry of alloxan into foods and its fatal link to blood parameter in carp. The management of silkworm pupae meal diet induced Alloxan blood parameter in carp. Five series of experiments were conducted to investigate the induced alloxan on blood parameter studied In the 8th series of experiments, utilization of Silkworm pupae meal diets on the reduction of alloxan toxicity on blood parameter, were estimated. The increase in the levels of alloxan decreases the Hb and RBC level where as WBC, Esr and Ht increases during the exposure period. Then treatment with silkworm pupae meal diet there an opposite trend was obtained. The administration of silkworm pupae meal might be beneficial for the restoration of hematological parameters, in the present study have revealed that incorporate silkworm pupae meal diet reduced the blood nonfunctional plasma enzymes and liver function parameters in Alloxan induced hyperglycemia in common carp. On the other hand Alloxan-induced diabetes could increase the liver enzyme levels. The increase in these enzymes may occur due to peroxidation reactions, arising from Alloxan biotransformation during diabetes and these reactions may inflict oxidative injury to cellular components. Our data shows that the silkworm is a good edible resource of natural Silkworm pupae meal diet with hypoglycemic activity which retards the ill effect of alloxan induced hyperglycemia.

KEYWORDS: Silkworm pupae meal, Alloxan, Hb, RBC, WBC, Ht, etc.

INTRODUCTION

Maida or refine wheat flour is the “heart” of the ingredients in making of the multivarious baked goods globally. It is also used to the fish pellet feed using as binding materials.. Alloxan is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution. Alloxan was discovered by von Liebig and Wohler in 1828 and has been regarded as one of the oldest named organic compounds that exist. In diabetes, this causes the level of glucose in the blood to be too high. This causes an insulin-dependent diabetes mellitus (called “Alloxan Diabetes”) in animals, with characteristics similar to type 1 diabetes in humans. Alloxan is one of the usual substances which is a toxic glucose analogue. It is stable in dry form, but is easily oxidized and selectively destroys insulin-producing cells in the solution in the presence of air. Oxidation is accelerated by pancreas when administered to rodents and many other heat, light, alkalis and traces of copper and iron. This causes an insulin-dependent acid is a molecule composed of six carbon atoms, six diabetes mellitus (called “Alloxan Diabetes”) in these oxygen atoms and eight hydrogen atoms, all linked animals, with characteristics similar to type I diabetes in together by chemical bonds (Ankur Rohilla,shahjad ali.,2012 and Federiuk et al,2004).

India has more than 40 million diabetic individuals which represents nearly 20% of total diabetes population worldwide. DM affects approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025. A number of currently existing anti-diabetic agents have number of unfavorable effects on the body. Therefore, regulation of diabetes without any side effects is still a difficult task for health care researchers. Consequently, the exploration for more successful and safer hypoglycemic agents with lesser side effects has unremitting to be a momentous area of study. Much diabetes related metabolic alterations are reported. Therefore, hematological parameters could be an important tool in the assessment of deleterious effect of drugs as well as medicinal plants Still though anti-diabetic action of crude extracts and purified bio- active components of many plants are identified, investigated related to the curative activity of medicinal plants with reference to the diabetes linked altered metabolic functions are very scanty. Diabetic models have provided considerable insight into physiological and biochemical derangement of the diabetic state (Dixit PK, Mittal S., 2013) Various hematological parameters and the immune system were also reported to be altered due to DM (Mansi k, Lahham J. 2008) Anemia is also caused in diabetic patients due to the hemolysis of red blood cells (RBCs).

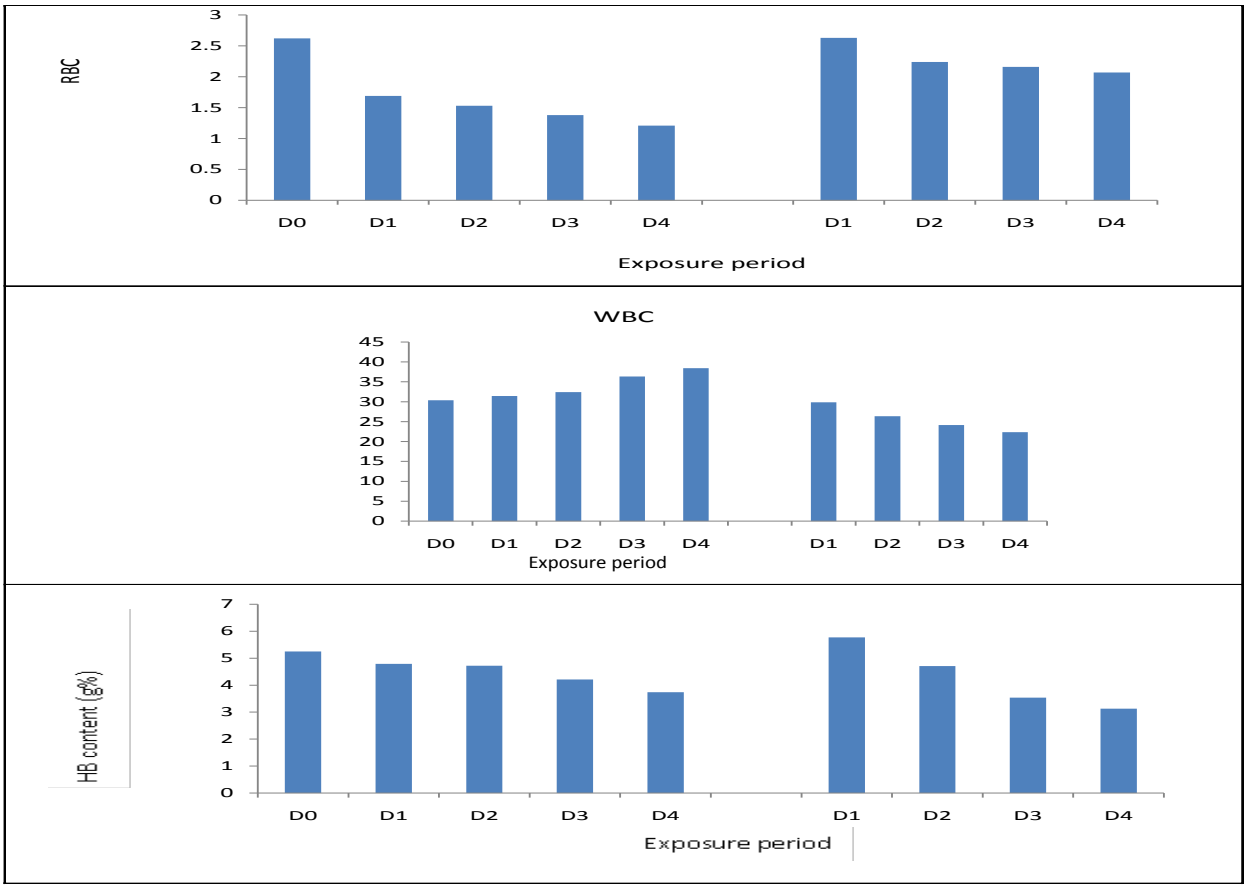
Moreover, we revealed common features between mammals and silkworm in the pharmacokinetics of antibiotics and toxic compounds (Hamamoto et al., 2009). These findings suggested us that evaluation of therapeutic activities of drugs based on pharmacokinetics using silkworms would be possible. In this paper, we introduce our recent findings on the application in the drug discovery by use of hyporglycemic silkworm In a long history of sericulture, mulberry leaves have been used for rearing silkworms. Nutrients contained in the mulberry leaves are absorbed from silkworm intestine to hemolymph and are transferred into the various organs like in mammalian animals. Silkworms have the organs such as intestine, fat body, and malpighian tubule, which function for exclusion of exogenously administrated chemicals. Moreover, silkworms can maintain glycogen as absorbed carbohydrates in the fat body and the muscle (Stake et al., 2000). Therefore, the systems for uptake of sugars and the storage mechanism show common features in silkworms and mammalian animals including humans.

MATERIALS AND METHODS

The blood parameter were studied by Routine clinical methods-Wintrobe.(1978) Five series oF experiments were conducted to investigate the induced alloxan on 0.01,0.1,1,10/1000g blood parameters in common carp. In next four series of experiments, utilization of Silkworm pupae meal diets on the reduction of alloxan toxicity on blood parameter.

RESULTS AND DISCUSSION

Fish exposed to alloxan elicited the time and concentrations dependent and significant (ANOVA : $P<0.05$) decrease in RBC count during the exposure period (Table 1). The RBC count of Common carp on days 0, 20, 40, and 60 exposed to the highest level of alloxan was 2.62, 1.69, 1.53, 1.38, and 1.18 × 106 mm-3 respectively (Table 1). The correlation coefficient was calculated for RBC count and exposure period to all the tested concentrations and it was found to be negative and significant ($P < 0.05$). However, the trend was reversed in WBC count 32.41, 34.43, 31.35, 38.43, 46.35 and ESR 3.85, 4.57, 5.27, 6.46, and 6.27 value of common carp exposed to alloxan levels (Fig. 1). Exposure of Common carp to alloxan also resulted a time and concentrations dependent ($P < 0.05$) decrease in Hb 5.25, 4.79, 4.72, 4.21, 3.74 content, calculated oxygen carrying of blood 5.57, 5.98, 5.90, 5.27, 4.68 and Ht 18.36, 15.27, 13.75, 13.24, 12.26 values. They also exerted a significant and negative between the



exposure period and concentrations of alloxan (Table 1; Fig.1). Two-way ANOVA test revealed that, alloxan levels hold significant($P < 0.05$) effect on RBC count, Hb content, ESR and Ht values while WBC count hold significant at alloxan levels and exposure period (Table1).

TABLE 1: Effects of silkworm pupae meal diets on blood parameters in alloxan exposed common carp, as a function of time. Each value is the mean ($\bar{X}$ ±SD) of three estimations.

RBC									
Rearing	Exposure Period					Treatment Period			
	D0	D1	D2	D3	D4	D1	D2	D3	D4
0	1.80 ± 0.09	1.80 ± 0.09	1.80 ± 0.09	1.80 ± 0.12	1.80 ± 0.22	1.69 ± 0.18	1.53 ± 0.34	1.38 ± 0.18	1.21 ± 0.09
20	2.15 ± 0.08	1.85 ± 0.04	1.72 ± 0.04	1.65 ± 0.16	1.58 ± 0.29	1.73 ± 0.16	1.86 ± 0.32	1.54 ± 0.17	1.35 ± 0.18
40	2.35 ± 0.14	1.76 ± 0.14	1.64 ± 0.08	1.54 ± 0.17	1.45 ± 0.14	1.95 ± 0.17	2.11 ± 0.18	1.77 ± 0.14	1.56 ± 0.16
60	2.62 ± 0.24	1.69 ± 0.18	1.53 ± 0.24	1.38 ± 0.18	1.18 ± 0.19	2.07 ± 0.14	2.16 ± 0.21	2.24 ± 0.12	2.63 ± 0.17
WBC									
0	23.01 ± 0.53	23.01 ± 0.53	23.01 ± 0.53	23.01 ± 0.53	23.01 ± 0.53	32.43 ± 0.32	31.35 ± 0.28	38.43 ± 0.19	36.35 ± 0.65
20	25.48 ± 0.23	24.46 ±0.29	26.38 ± 0.17	28.32 ± 0.27	29.33 ± 0.65	31.54 ± 0.41	28.84 ± 0.24	27.56 ± 0.21	32.69 ± 0.36
40	28.51 ± 0.81	29.27 ± 0.52	31.43 ± 0.20	29.56 ± 0.72	37.65 ± 0.50	29.16 ± 0.31	27.93 ± 0.31	26.85 ± 0.42	28.92 ± 0.51
60	32.41 ± 0.29	32.43 ± 0.32	31.35 ± 0.78	38.43 ± 0.39	36.35 ± 0.85	29.85 ± 0.21	25.07 ± 0.34	24.14 ± 0.31	22.35 ± 0.75
HB									
0	4.67 ± 0.15	4.67 ± 0.15	4.67 ± 0.15	4.67 ± 0.15	4.67 ± 0.15	4.79 ± 0.27	4.72 ± 0.22	4.21 ± 0.25	3.74 ± 0.13
20	4.77 ± 0.25	4.36 ± 0.21	4.59 ± 0.22	3.69 ± 0.64	3.78 ± 0.26	4.92 ± 0.24	4.87 ± 0.31	3.45 ± 0.15	3.95 ± 0.32
40	4.91 ± 0.23	4.58 ± 0.24	4.84 ± 0.19	4.34 ± 0.15	4.36 ± 0.15	4.51 ± 0.15	4.64 ± 0.19	3.50 ± 0.32	3.04 ± 0.13
60	5.25 ± 0.22	4.79 ± 0.17	4.72 ± 0.12	4.21 ± 0.05	3.74 ± 0.13	5.77 ± 0.03	4.71 ± 0.32	3.54 ± 0.18	3.13 ± 0.29
O2									
0	5.83 ± 0.21	5.83 ± 0.21	5.83 ± 0.21	5.83 ± 0.21	5.83 ± 0.21	7.31 ± 0.16	6.68 ± 0.29	5.51 ± 0.46	4.21 ± 0.52
20	5.95 ± 0.11	5.45 ± 0.13	5.73 ± 0.04	4.61 ± 0.12	4.73 ± 0.16	6.54 ± 0.31	6.94 ± 0.02	6.81 ± 0.74	6.27 ± 0.71
40	5.13 ± 0.13	5.73 ± 0.26	5.05 ± 0.11	5.43 ± 0.25	5.45 ± 0.24	7.72 ± 0.42	7.14 ± 0.11	7.54 ± 0.41	7.14 ± 0.68
60	5.57 ± 0.49	5.98 ± 0.18	5.90 ± 0.29	5.27 ± 0.46	4.68 ± 0.22	7.97 ± 0.88	7.14 ± 0.59	7.52 ± 0.81	7.26 ± 0.61
ESR									
0	2.53 ± 0.21	2.53 ± 0.21	2.53 ± 0.21	2.53 ± 0.21	2.53 ± 0.21	4.57 ± 0.12	5.27 ± 0.21	6.46 ± 0.24	6.27 ± 0.52
20	2.77 ± 0.39	2.97 ± 0.18	3.83 ± 0.33	3.96 ± 0.16	3.76 ± 0.16	3.65 ± 0.21	4.45 ± 0.32	5.58 ± 0.29	4.95 ± 0.41
40	3.29 ± 0.25	3.88 ± 0.24	4.56 ± 0.54	4.55 ± 0.54	4.36 ± 0.24	3.93 ± 0.25	3.68 ± 0.18	3.85 ± 0.38	5.95 ± 0.34
60	3.85 ± 0.54	4.57 ± 1.22	5.27 ± 1.25	6.46 ± 1.24	6.27 ± 1.32	2.15 ± 0.34	2.86 ± 0.31	3.36 ± 0.41	4.46 ± 0.52
HT									
0	15.80 ± 1.53	15.80 ± 1.53	15.80 ± 1.53	15.80 ± 1.53	15.80 ± 1.53	15.27 ± 0.11	13.75 ± 0.16	13.24 ± 0.29	12.26 ± 0.51
20	16.22 ± 0.25	15.82 ± 0.56	15.45 ± 0.17	14.83 ± 0.27	14.33 ± 0.32	16.27 ± 0.22	14.26 ± 0.31	13.26 ± 0.31	13.15 ± 0.39
40	17.24 ± 0.36	15.77 ± 0.52	14.27 ± 1.22	14.19 ± 0.82	14.08 ± 0.34	16.44 ± 0.52	14.73 ± 0.22	14.66 ± 0.41	14.24 ± 0.24
60	18.36 ± 0.14	15.27 ± 0.11	13.75 ± 2.16	13.24 ± 0.39	12.26 ± 0.51	16.83 ± 0.28	15.26 ± 0.27	14.73 ± 0.52	13.85 ± 0.54

Results of present study shows the levels of the heamatological changes in Common carp fed with different levels of alloxan incorporated diet. The results are as presented in Table1. The primary reasons for assessing the RBC is to check anemia and to evaluate normal erythropoiesis. Hemoglobin level indicates the amount of intracellular iron, while hematocrit, representing the volume of RBC in blood helps to determine the degree of anemia or polycythaemia. WBC was found to be increased in diabetic subject due to pathophysiological conditions including autolysis caused by some hydrolytic enzymes released by plasma under stress. SP strengthened hemopoetic system by supplying various constituent thus helps to control MCH, MCHC which was found to be decreased in diabetic subject.

The mean cell hemoglobin level is a significant index for folic acid and or Vit B12 need (Ganong.,1999) The resulting significant reduction in RBC levels and HCT levels in the treatment groups of ethyl acetate fraction and ethanol extract with significant increases in their MCV and MCH levels when compared with the diabetic control group may be due to hematotoxic effects associated with toxic substances on bone marrow depression caused by damage to multiple classes of hematopoietic cells and a variety of hematopoietic functions (Synder and Hadli, 1996). Reactive O2 species generated during alloxan metabolism is

implicated in red cell damage (Rao et al.,2003), diabetic rats forms glycosylated hemoglobin hence, decreased total hemoglobin (Sheela and Augusti, 1992). There was no change in the red cell indices of the diabetic rats treated with butanol, methanol, glibenclamide, n-hexane. This result was similar to that reported by some researchers (Mohammed et al., 2009; Edet et al., 2011). This was consistent with the report (Ajagbonna et al., 1999) on the ability of medicinal compounds or drugs in altering the normal range of hematological parameters. Alloxan monohydrate is known to induce diabetes by partial destruction of pancreatic beta cells of islet of langerhan.

This results in depletion of insulin levels and hyperglycemia leading to DM. The alloxan-treated mice, therefore, appear to represent a good laboratory model for DM. There is possibility for the survival of a few beta-cells and this has been proved by several workers who observed antihyperglycemic activity with oral hypoglycemic agents like glibenclamide, tolbutamide etc. in alloxan-induced diabetic mice (Sheeja et al,1993,Subramanian et al, 1996.) Similarly, significant decrease in the haemoglobin content and packed cell volume were also observed in mice due to pathophysiological condition when treated with heavy metals tartrazine, food colours chemical dye, sodium benzoateand fluorid]. These chemicals also caused elevation in blood glucose levels. (Chouhan S. Flora .,2008) investigation was to evaluate the efficiency of the aqueous leaves extract of S. cumini on alloxan-induced metabolic changes diabetic rats. Decreased Hb content was observed in diabetic rates might be due to increased formation of glycosylated Hb. Generally total hemoglobin levels is much below the normal levels in diabetic subject by (Chandaliam.,2002) and HbA1c levels has been reported to be increased in patients with diabetes mellitus. Alloxan-induced diabetic mice showed significantly reduced blood levels when compared to the normal control group. Alloxan is a well known chemical has been reported to suppress the immune system by destroying certain cells and organs in the body as was observed in this present study. The alteration of these parameters could be attributed to change in the number of leucocytes which may account for poor defensive mechanisms against infection, thus may have consequential effects on the immune system and phagocytic activity of the animals.

CONCLUSION

The administration of silkworm pupae meal might be beneficial for the restoration of biochemical and hematological parameters, in the present study have revealed that incorporate silkworm pupae meal diet reduced blood nonfunctional plasma enzymes and liver function parameters in Alloxan induced hyperglycemia in Common carp. On the other hand Alloxan-induced diabetes could increase the liver enzyme levels. The increase in these enzymes may occur due to peroxidation reactions, arising from Alloxan biotransformation during diabetes and these reactions may inflict oxidative injury to cellular components. Our data shows that the silkworm is a good edible resource of natural Silkworm pupae meal diet with hypoglycemic activity.

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