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HEMATO-BIOCHEMICAL PARAMETERS ON THE DEFENSIVE ROLE OF GRAVIOLA IN RABBITS TREATED WITH DIMETHOATE

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ABSTRACT

Organphosphorus insecticide, dimethoate (DM), is a systemic insecticide widely used in agriculture and domestic pest control. Graviola may be a natural product tree with numerous employments in conventional and elective pharmaceutical. Therefore, the present experiment was carried out to determine the effectiveness of graviola in alleviating the toxicity of dimethoate (DM) on certain hemato-biochemical parameters and lipid peroxidation of male New Zealand white rabbits. Five rabbits per group were assigned to 1 of 4 treatment groups: 0mg graviola and 0mg dimethoate/kg body weight (BW) (control); 100mg AA/kgBW; 43.2mg dimethoate/kgBW (1/50 LD50); 43.2mg dimethoate plus 100mg graviola/kg BW. Rabbits were orally administered their respective doses every other day for 6 weeks. Evaluations were made for lipid peroxidation and hemato-biochemical parameters. Results indicated that treatment with graviola did not affect red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV), while, increase platelet count (PLT), and hemoglobin (Hb). On the other hand DM decrease RBC, Hb, PCV and PLT, while increase WBC. The presence of graviola with DM returned the values of the previous parameters to near to the control values. Treatment with DM resulted in significant ($P<0.05$) increase in the activities of blood plasma AST and ALT, (ɣ-GT), urea, creatinine and TBARS while ALP were significantly ($P<0.05$) decreased compared with control group. Graviola alone caused significant ($P<0.05$) decrease in the activities of AST and ALT, (ɣ-GT) urea, creatinine and TBARS while AlP caused insignificant ($P<0.05$) increase in these parameters compared with control. Also, the present study showed that graviola can be effective in the protection of dimethoate-induced toxicity.

KEYWORDS

Graviola, Dimethoate, Biochemistry and Rabbits.

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INTRODUCTION

Wide spread utilize and transfer of organophosphorus compounds for bug control have come about within the discharge of their buildup into common water, hence actuating an natural issue and have been widely recognized as a health

hazard[1]. Other than fatalities, caused by tall dosage, presentation of creatures to moo measurements organophosphorus bug sprays has been found to cause far reaching impact on body counting organ particular injuries in central nervous system[2], liver[3], kidneys and generalized effects like immunosuppression, teratogenesis, carcinogenesis and metabolic disorders[4]. Organophosphorus insecticide, DM, is a systemic insecticide widely used in agriculture and domestic pest control[5]. It acts by interfering with the activities of cholinesterase activities and is toxic to insects, rodents, fish and humans[6]. Its chronic exposure has been associated with the critical increase in hepatopathy, nephropathy as well as diabetic mellitus in humans[7] and has been recognized as a possible human carcinogen[8]. Several studies addressed the toxic effect of DM on the functions of several mammalian organs including liver and kidney.

Dimethoate was reported to alter the level of the marker parameters related to the liver and kidneys in rats and mice[3]. Significant increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyltransferase (ɣ-GT) as well as the decrease in the levels of cholinesterase, bilirubin, total protein and albumin in the plasma were the major diagnostic symptoms of liver diseases in animals and human[9]. The increase in the uric acid and creatinine in the plasma are the major symptoms of glomerular filtration damage[10]. Some studies were shown that acute and sub chronic exposure etestes of rats[11] and human erythrocytes[12].

Antioxidants are widely needed to prevent deterioration of other oxidisable goods, such as cosmetics, pharmaceuticals and plastics Polyphenols are the major plant compounds with antioxidant action, in spite of the fact that they are not the as it were ones.. In addition, other biological properties such as anti-carcinogenicity, anti-mutagenicity, anti allergenicity and antiaging activity have been reported for natural and synthetic antioxidants[13]. Annona. muricata Linn, which belongs to the Annonaceae family, is commonly known as soursop, graviola or guanabana. It is native to sub Saharan countries though it is now widely cultivated in many tropical countries in the world such as India, Malaysia and Nigeria[14]. Regularly, this plant is looked for for its restorative impacts.. Each part of the tree i.e. the root, stem-bark, leaves, fruit and even the seed is used in traditional medicines around the world[15]. The supposed therapeutic benefits of the soursop has attracted intensive research on the chemical composition of the leaves and seeds that has led to the finding of acetogenin compounds[16]. These isolated compounds, which are secondary metabolites/antioxidants, answer the potential of the soursop for possessing anti-cancer, insecticidal, sedating as well as pain and immunosuppressing properties[17].

In the past, several studies focused on antioxidant activity of extracts from pulps, leaves and peel of graviola[18]. Referred to a comparative study on antioxidant properties of the peel and pulp of ripe Graviola that detailed that the antioxidant potential in soursop peel was found to be altogether higher than within the mash, as decided by ferric lessening antioxidant control (FRAP), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Fe2+ chelation and hydroxyl scavenging tests. Additionally, graviola leaf aqueous extract was found to alleviate the pancreatic B-cells of Streptozotocin treated diabetic rats by directly quenching lipid peroxides and indirectly enhancing production of endogenous antioxidants, thus addressing its antioxidant potential[19,20]. Reported that the hepatoprotective and antioxidant activity of graviola stem bark extract against oxidative stress in rats induced by DM as determined from plasma enzyme markers. According to[16], ethanol-induced gastric injury in rabbits could be treated by ethyl acetate extract of graviola leaves, which provide a suppressive effect against oxidative damage and a preservative effect on gastric wall mucus. Despite extensive research into the antioxidant level and activity possessed by graviola and its effectiveness in treating disease, a comparative study of the antioxidant level and activity of graviola obtained from different

locations has not been reported. Nonetheless, previous studies have shown that there are different levels of antioxidant/phenolic content among plants of similar species[21].

MATERIAL AND METHODS

In this study dimethoate (DM) and graviola were used. DM (purity 400g/L) was purchased from B and W agrochemichals (China) and graviola was purchased from maximum international company, Brasil. Each capsule contains 3g powder and the content of each capsule was dissolved in corn oil just before use. Mature male New Zealand White rabbits age of 6 months and initial weight of (1.641 ± 27.2Kg) were used. Creatures were independently housed in cages and weighed week by week all through 6-weeks test period. Feed and water were provided ad libtum. Rabbits fed pellets which consisted of 30% berseem (Trifolium alexandrinum) hay, 25% yellow corn, 26.2% wheat bran, 14% soybean meal, 3% molasses, 1% $CaCl_2$, 0.4% NaCl, 0.3% mixture of minerals and vitamins, and 0.1% methionine. The vitamin and mineral premix per kg contained the following IU/gm for vitamins or minerals: vit A-4000,000, vit D3-5000, 000, vit E-16,7 g, K-0.67 g, vit B1-0.67g, vit B2-2g, B6-0.67g, B12-0.004g, B5-16.7g, Pantothinc acid-6.67g, Biotein-0.07g, Folic acid-1.67g, Choline chloride-400g, Zn-23.3g, Mn-10g, Fe-25g, Cu-1.67g, I-0.25g, Se-0.033g, and Mg-133.4g (Rabbit premix created by Holland Nourish Associate. Co). The chemical analysis of the pellets[22] showed that they contained 15.8% crude protein, 11.3% crude fiber, 3.7% ether extract, 7.2% ash, 92.9% organic matter and 62.4% nitrogen free extract % as DM basis.

The first group was used as control. While, groups 2, 3 and 4 were treated with graviola100mg/kg BW[23-25] and dimethoate by gavage at a dose of 43.2 mg/kg B.W/day (1/50 of DM) lethal dose[26] and the combination of dimethoate and graviola, respectively. Rabbits were given with DM daily at a dose of 43.2mg/kg B.W./day by gavage like group III and given the graviola concurrently daily at a dose of 100mg/kg B.W./day by gavage like bunch II for 6 progressive weeks.

Blood samples were collected from the ear vein of all animals every week throughout the 6-weeks experimental period. Heparin was used as anticoagulant. Plasma was obtained by centrifugation of samples at 860*g for 20 min, and was stored at -20C until used for analysis. Hematological parameters including White blood cells (WBC), red blood corpuscles (RBC), hematocrit (HCT), Hemoglobin (HG), and platelets were evaluated using an automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American, Inc).

Urea concentration was determined by utilising enzymatic determination according to reaction that ammonia ions react with the salicylate and hypochlorite in an alkaline medium to form a green coloured indophenols[27]. Plasma creatinine concentration was measured according to the method of[28]. The principle of this method is based on the reaction of creatinine with picrate in alkaline solution to form a coloured complex. The activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of[29]. Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma according to the method of[30]. Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the method of[31]. **Statistical analysis**

Where applicable, statistical analysis was carried out in Minitab software (version17) statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the information and suitable $P < 0.05$ consider significant.

RESULTS AND DISCUSSION

General health of rabbits Observation of animals DM-fed rabbits showed varying degrees of clinical signs few minutes after dose. The signs included disorientation, drowsiness, uncoordinated movements, mild tremor, Redness around eyes, blindness and diarrhea. Concerning morphological changes, DM treated rabbits showed hair loss

especially in the fifth and sixth weeks of the experiment whereas control and graviola animals did not display such change. The livers of dissected rabbits also showed scars of depression in response to DM administration whereas those of the control animals showed normal appearance (Figure No.1).

Table No.1 Represent the hematological parameters of male rabbits treated with graviola, DM and their combination. Results indicated that treatment with graviola did not affect red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV), while, increase platelet count (PLT), and hemoglobin (Hb). On the other hand DM decrease RBC, Hb, PCV and PLT, while increase WBC. The presence of graviola with DM returned the values of the previous parameters to near to the control values.

Table No.2 Illustrated the activities of aspartate trans aminase (AST), alanine trans aminase (ALT), alkaline phosphatase (AlP), gamma glutamyl transferase activity (ɣ-GT), urea and creatinine in blood plasma as affected by treatment with graviola, DM and/or their combination throughout the 6week experimental period. Treatment with DM resulted in significant ($P<0.05$) increase in the activities of blood plasma AST, ALT, (ɣ-GT), urea and creatinine while ALP were significantly ($P<0.05$) decreased compared with control group. Graviola alone caused significant ($P<0.05$) decrease in the activities of AST and ALT, (ɣ-GT), urea and creatinine while AlP caused insignificant ($P<0.05$) increase in these enzymes compared with control. The presence of graviola with DM caused significant ($P<0.05$) decrease in the induction in the levels of AST ALT, (ɣ-GT), urea, creatinine and significant ($P<0.05$) increase in the reduction AlP due to treatment with DM, and this means that graviola had protective effect against the toxicity of DM.

Discussion

General health of rabbits (Figure No.1) the livers of DM-treated rabbits showed scars of depressions also in the last two weeks of the experiment which may be due to distortion in the liver cells. DM is known to induce morphological changes in the liver[32]. The anti-oxidative properties of graviola and its components have been explored in various in vitro and *in vivo* tests. Strengthening the body's defenses by improving the antioxidant status will undoubtedly protect human against many chronic diseases[33].

The results indicated that treatment with graviola did not affect red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelet count (PLT), hemoglobin (Hb), mean cell volume mean cell hemoglobin and mean cell hemoglobin concentration. On the other hand DM decrease RBC, WBC, PLT, and Hb. Similar to the results of this study[34], also did not observed any significant difference in blood parameters analyzed. Although platelet showed a significant increase with increasing dosage, levels for all parameters were within the normal range as reported by[35]. Graviola extract contains high total antioxidant which is good in promoting health. Blood hematology results in the present study did not show any abnormalities[36,37]. The findings from present studies suggested that the administration of Annona muricata extract did not cause any toxicological effect since the values were in the normal range as reported by[38]. Pesticides exposure poses a serious risk to all domestic animals, to the environment and the public health[39]. The results of this study showed that, the hematological parameters RBC and Hb were significantly decreased in DM treated rabbits when the erythrocytes sedimentation rate was highly significant increased as compared to control. The effect of organophosphorus pesticides on the Hb of other workers has been studied by[40,41]. The decrease in the Hb along with the decrease in the RBC might be due to the effect of pesticides on blood forming organ (bone marrow and liver), and inhibition of many steps of heme biosynthesis in rabbits, as the result of pesticides exposure[41]. The poisoning by pesticide residues leads to the development of anemia due to interference of Hb biosynthesis and shortening of the life span of circulating erythrocytes[42,43]. The increase of ESR indicates to inflammation caused by organophosphorus pesticides[44]. In this study

significant decrease alterations were observed in Hb concentration in rabbits which were intoxicated. However, one study has observed decrease in Hb concentration due to a direct effect on bone marrow as reported in rabbits[7]. The discrepancy might be attributed to short period of study with intact compensatory mechanisms. Significantly decreased PCV might be due to malabsorption of nutrients or the hyperactivity of the animal[45].

The present study showed that (DM) caused changes in the activities of marker enzymes like ALT, AST, AlP and ɣ-GT in plasma. This study showed that the mean levels of serum ALT, AST, ɣ-GT and ALP in the DM-treated rabbits were significantly higher than those in the controls. Such elevation of liver enzymes as a result of DM administration was documented by other authors[46-48].

Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as DM poisoning[32]. Serum ALT, AST and, ɣ-GT are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity[11].

In contrast to elevation of transaminases, ɣ-GT and ALP was markedly decreased in DM-treated rabbits compared to controls. Such inhibition in ChE in response to organophosphorus DM administrated was obtained by[26]. The decrease in plasma AST, ALT, AlP and activities in the present study is in agreement with the results of[35]. It has been shown that graviola extract contains high total antioxidant which is good in promoting health. The results from this study have shown that the plant graviola has moderate anti-hepatotoxic ability. Generation of free radicals in the body beyond antioxidants capacity leads to oxidative stress which has been implicated in diseases such as cancer, cardiovascular disease, aging and several other chronic diseases because of their ability to induce oxidative damage to biomolecules such as lipid, DNA and protein[35]. With regards to serum biochemistry, a significant decrease in liver function test parameters such as serum ALT, AST, GGT and ALP were noted[35]. Generation of free radicals in the body beyond antioxidants capacity leads to oxidative stress which has been implicated in diseases such as cancer, cardiovascular disease, aging and several other chronic diseases because of their ability to induce oxidative damage to biomolecules such as lipid, DNA and protein[49].

The influence of DM on kidney function in DM-treated rabbits was assessed throughout the measurement of urea and creatinine concentration. Such findings are in agreement with that reported in other studies[7,11,48]. A creatinine level raised out of proportion to the urea may indicate a pre-renal problem[50] (Delanghe *et al*, 1989). Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function. The decrease in protein profile observed in the present study may support this explanation. Creatinine is break-down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys[49]. Creatinine has been found to be a reasonably dependable pointer of kidney work. As the kidneys become impaired for any reason, for example in case of DM poisoning the creatinine level in the blood will rise due to poor clearance by the kidneys. A rise in blood creatinine level was observed with damage to functioning nephrons and impaired renal function[51,52].

Table No.1: Changes in red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelets count (PLT) and hemoglobin (Hb) of male rabbits treated with graviola, DM (DM) and/or their combination (means ±SE)

S.No	Parameter	Experimental groups			
		Control	Graviola	DM	Graviola+DM
1	RBC $\times 10^6$ (µl)	6.20 ± 0.06^a	6.38 ± 0.09^a	4.92 ± 0.09^b	5.29 ± 0.96^b
2	WBC $\times 10^3$ (µl)	8.46 ± 0.27^b	8.55 ± 0.33^b	11.56 ± 0.18^a	8.53 ± 0.09^b
3	PCV$\times 10^3$ (mm^3)	40.16 ± 0.46^a	41.15 ± 0.64^a	32.35 ± 0.35^b	40.43 ± 0.61^c
4	PLT $\times 10^3$ (µl)	293.6 ± 15.9^b	468.4 ± 51.5^a	217.8 ± 20.62^{bc}	297.3 ± 37.48^c
5	Hb (g/dl)	12.7 ± 0.18^a	13.5 ± 0.50^a	10.51 ± 0.58^b	12.6 ± 0.21^b

Values are expressed as means ± SE; n=10 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$

Table No.2: The overall means (±S.E.) of plasma biochemistry during treatment of male rabbits with graviola, DM (DM) and/or their combination

S.No	Parameters	Experimental groups			
		Control	Graviola	DM	Graviola+DM
1	AST (U/L)	43.08 ± 1.12^{ab}	29.37 ± 3.0^b	50.91 ± 3.85^a	42.93 ± 4.31^a
2	ALT(U/L)	46.19 ± 2.07^a	34.61 ± 3.27^b	48.92 ± 2.86^a	44.85 ± 3.42^b
3	AlP(U/L)	142.55 ± 4.7^a	152.43 ± 6.9^a	137.1 ± 3.8^a	140.7 ± 10.3^b
4	ɣ-GT(U/L)	7.17 ± 0.10^a	6.45 ± 0.36^a	7.55 ± 0.18^a	7.35 ± 0.75^a
5	Urea (mg/dl)	37.89 ± 0.66^a	28.89 ± 2.33^b	39.85 ± 0.92^a	39.35 ± 1.80^a
6	Creatinin(g/dl)	0.65 ± 0.01^b	0.56 ± 0.04^b	0.74 ± 0.03^b	0.64 ± 0.12^a

Values are expressed as means ± SE; n=10 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.

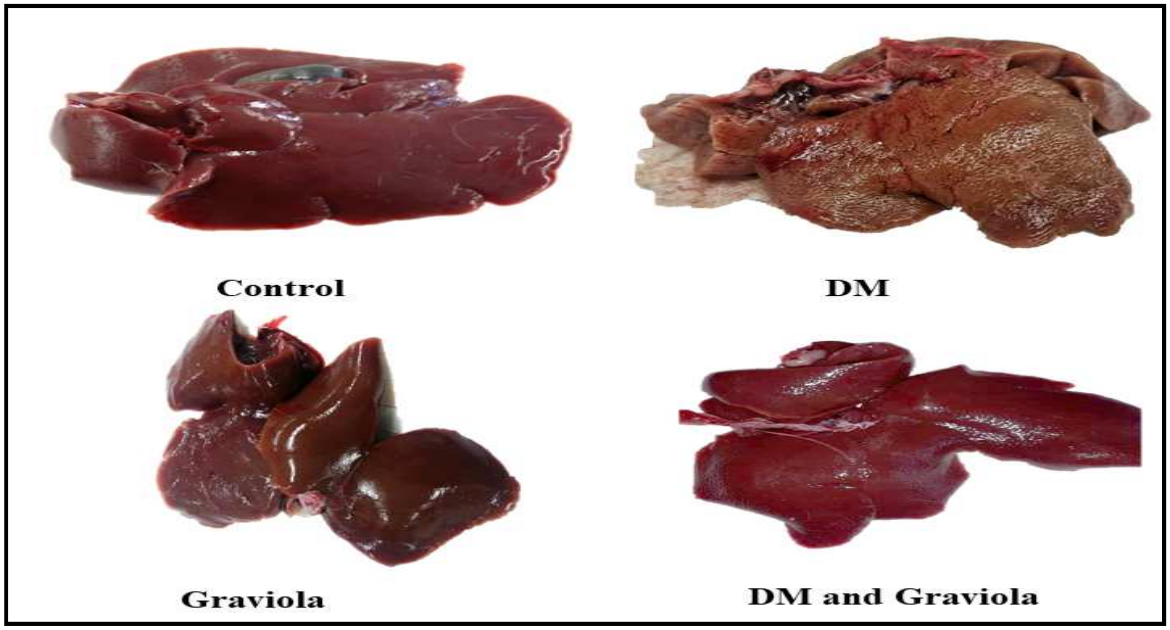

Figure No.1: Morphological effect of DM graviola, and/or their combination after 6 weeks on liver of male rabbit

CONCLUSION

The results of the present study convincingly demonstrated that dimethoate exposure resulted in varying degree of changes in hemato- biochemical parameters in plasma of rabbits. Annona muricata is broadly utilized in conventional medication to treat sickness. Using Annona muricata capability to alleviate the harmful effect of dimethoate.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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