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Effect of Therapeutic and Toxic Doses of Ivermectin (Mectizan) on Total Serum Proteins and Hepatic Enzymes of Wistar Albino Rats

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Abstract: The toxicities of the therapeutic and harmful doses of an anthelmintic drug Ivermectin (5-0-demethyl-22, 23-dihydrobenzohydrofuran) are studied in rats. Doses of 0.4 and 4.0 mg kg⁻¹ b.wt. of Ivermectin (Mectizan) given at the rate of four repeated doses at daily interval within 21 days to normal rats on a stock mash protein diet produced total serum protein concentration (g L⁻¹) of 72.61±4.54 and 75.51±3.82, respectively, in comparison to 70.94±8.13 for the control thereby showing no significant effect of Ivermectin dosage on serum proteins. The corresponding test values for the activities of the serum enzymes (μ L⁻¹): alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltranspeptidase (GGT) were 90.22±2.96, 94.20±1.81; 32.08±3.87, 43.98±1.64 and 47.16±2.43, 56.73±4.05; 15.29±1.48, 25.88±0.77 against control values of 84.15±4.34, 28.83±1.66, 2.19±1.64 and 4.58±0.93, clearly indicating Ivermectin induced toxicity in rats which is dose dependent. The significance of the role of Ivermectin in toxicity studies and its relation to serum enzymes is discussed. Short term administration of Ivermectin (Mectizan) at therapeutic and toxic doses to albino rats did not affect total serum proteins but has marked effects on some liver function enzymes such as AST, ALT and GGP.

Key words: Ivermectin, toxic dose, glutamyl transpeptidase, aminotransferases, onchocerciasis

INTRODUCTION

Ivermectin (Ivm), a semi synthetic macrocyclic lactone marketed under the name mectizan is a widely used drug in veterinary medicine. It was introduced in 1987 for the treatment of human Onchocerciasis. It is a derivative of avermectin (C₁₄H₇₂O₄), a fermentable product of streptomyces avermitilis. As an anti parasitic agent, it is claimed to have considerable promise in the treatment of Onchocerciasis (river blindness) (Schroder *et al.*, 1986; Chui *et al.*, 1987; Lankas *et al.*, 1989).

Onchocerciasis is a disease of public health importance, about 18 million people world wide are infected with filarial nematode *O. volvulus*-transmitted from person to person by the black fly of the genus Simulium. About 1 million of these are blind or have severe visual impairment from Onchocerciasis and more than 80 million living in endemic areas of sub-Saharan Africa, central and South America are at risk of the disease (Abiose *et al.*, 1993).

Onchocerciasis is a disease found in all states of Nigeria including Abuja (Budden, 1956; Crosskey, 1979). In Cross River State of Nigeria, the prevalence of the disease has been established in 42 of the 46 villages (Braide *et al.*, 1990; Brain *et al.*, 1992; Campel, 1985).

Ivermectin is the new drug receiving wide usage in the treatment of endemic Onchocerciasis distributed free of charge.

This study was targeted on 36 rats fed on normal diet (Growers Mash Stock) which attained 150-250 g adult body weight. Group 1 and 2 were administered intra-peritoneal (i.p.) Ivermectin (Mectizan) therapeutic and toxic doses of 0.4 mg and 4.0 mg kg⁻¹ b.wt., respectively at daily intervals within 7 days (4×7). Group 3 was the control. Each group had consisted of 12 animal rats. Total serum protein and hepatic enzymes were monitored at the end of 21 days. The experimental model and feeding protocol was established on the basis of drug nutrient interaction.

The objective of this study was to investigate possible toxicity of Ivermectin using therapeutic and toxic doses in normal rats fed on growers marsh diet.

MATERIALS AND METHODS

Drug and Animal Treatment

Ivermectin (Mectizan) was obtained from Meek Sharp and Dohme (MSD) Company New Jersey USA. Dimethyl sulphoxide (DMSO), the solvent of the drug was bought from BDH chemicals Ltd., Poole England in 2002. A stock solution of 0.4 mg mL⁻¹ equivalent was prepared using 25 mL DMSO and made up to 25 mL with physiological saline.

Ivermectin is a micro filaricide and administered in repeated doses (Green *et al.*, 1991). From the weight of the animal rat, a calculated equivalent in millimeters of Ivermectin-therapeutic (400 µg or 0.4 mg kg⁻¹ b.wt.) and toxic (4.0 mg kg⁻¹ b.wt.) was administered 4×7 days within a 21 days interval. At the end of 21 days period, the animals were killed by diethyl ether in suffocation, 24 h after the last injection.

Collection of Samples

Blood was collected by cardiac puncture and stored in sterile disposable 10 mL plastic containers from which serum was emptied into labeled bottles after centrifugation at 25000x g using MSE top centrifuge. The serum was kept in deep freezers for a short period (within one week) and used for the determination of serum enzyme activities.

Standardization

To obtain reproducible results, assays were carried out prior to actual collection of data. Mectizan is extremely insoluble in water. To overcome this, normal saline and DMSO was used to dissolve the drug. The rationale for choosing the drug doses was based on epidemiological studies. At the preliminary stage EDTA plasma was used for the assay in the estimation of the liver enzymes. But this gave inconsistent results, reason being that EDTA may have chelate Ca⁺⁺ ions in the plasma, hence blood serum was obtained for the assay. Total serum proteins were determined by the Biuret method using diagnostic Kit obtained from BIOLABO SA BP14F (France).

Measurement of Enzyme Activities (AST, ALT, ALP and GGT)

The aminotransferase (AST, ALT); alkaline phosphates and glutmyl transpeptidase were determined using appropriate kits from Randox Laboratories Ltd., England with standard procedure. Total proteins were determined by Biuret reagent with Standard Bourine Albumin (7g mL⁻¹); while AST, ALT, ALP and GGT were estimated using kits (Randox Laboratories Ltd., Crumlin UK). Sample volumes (0.1 mL) used, were those of serum mixed, incubated for 30 min at 37°C. Enzyme activities (UL⁻¹) of AST, ALP and GGT were read for the absorbance against reagent blank and read from the table.

The data obtained were analyzed statistically using student t-test at 95% (p≤0.05) level where test groups were compared with the control for significant differences.

Data Analysis

The result are presented as the mean standard values of three replicates (n = 12). Student t-test was used to compare results of each of the test groups with control at 95% probability level.

RESULTS

The results for rats fed growers mash and the effect on serum protein and blood serum activities of ALP, ASP, ALT and GGT are shown in Table 1-5, respectively.

The mean±SD values of the therapeutic (0.4 mg kg⁻¹ b.wt.) and toxic (4.0 mg kg⁻¹ b.wt.) doses of Ivermectin on TSP (g L⁻¹) were 72.61±4.54 and 75.51±3.82, respectively while the control value is 70.94±8.13 g L⁻¹.

The mean±SD values of ALP, AST, ALT and GGT enzymes activities (μ L⁻¹) were 90.22±2.96, 94.20±1.81; 32.08±3.87, 43.98±1.64 for the therapeutic dose and 47.16±2.43, 56.73±4.05; 15.29±1.48, 25.88±0.77 for the toxic regimen, respectively. The corresponding

Table 1: Effects of Ivermectin (Mectizan) administration (i.p.) at 0.4 and 4.0 mg kg⁻¹ b.wt. on total serum protein (g L⁻¹) in rats fed growers mash diet

Group	Values
1	72.61±4.54
2	75.51±3.82
3	70.94±8.13

n = 12; Values are Mean±SD. The values are not statistically significant (p = 0.05) in comparison with the control

Table 2: Effect of Ivermectin (Mectizan) administration (i.p.) at 0.4 4.0 mg kg⁻¹ b.wt. on blood serum activity (μ L⁻¹) of alkaline phosphatase (ALP) in rats fed on growers mash stock diet

Group	Values
1	90.22±2.96
2	94.20±1.81
3	84.15±4.39

n = 12; Values are Mean±SD. The values are SS (p = 0.05) in comparison with the control

Table 3: Effect of Ivermectin (Mectizan) administration (i.p.) at 0.4 and 4.0 4.0 mg kg⁻¹ b.wt. on blood serum activity of AST in rats fed on growers mash stock diet

Group	Values
1	32.08±3.87
2	43.98±1.64
3	28.83±1.66

n = 12; Mean±SD values are highly SS (p = 0.001) in comparison with each other and with the control

Table 4: Effect of Ivermectin (Mectizan) administration at 0.4 and 4.0 4.0 mg kg⁻¹ b.wt. on blood serum activity (μ L⁻¹) of ALT in rats fed growers mash stock diet

Group	Values
1	47.16±2.43
2	56.73±4.05
3	2.19±1.64

n = 12; Mean±SD values are highly SS (p = 0.001) in comparison with each other and the control

Table 5: Effect of Ivermectin (Mectizan) administration at 0.4 and 4.0 4.0 mg kg⁻¹ b.wt. on blood serum activity of gama glutamyl transpeptidase: GGT (μ L⁻¹) in rats fed growers mash stock diet

Group	Values
1	15.29±1.48
2	25.88±0.77
3	4.58±0.93

Values are Mean±SD. The values are SS (p = 0.001) in comparison with each other and with the control

enzymes control values were 84.15 ± 4.34 , 28.83 ± 1.66 , 2.19 ± 1.64 and 4.58 ± 0.93 . The results showed that there was no statistically significant dose dependent effect of Ivermectin on total serum protein concentrations in rats fed on a normal mash diet ($p = 0.05$) (Table 1).

The result in respect of activities in liver function enzymes of ALP, AST, ALT and GGT (μL^{-1}) showed on the other hand that there were dose dependent of Ivermectin (Table 2). In comparison with the control, ALP activities (μL^{-1}) was significantly higher ($p = 0.05$) while AST, ALT and GGT were highly significant ($p = 0.001$; Table 3). The response of ALP, AST, ALT and GGT to the therapeutic dose was significantly lower ($p = 0.001$) than for the toxic dose (Table 4). While each value of ALP in serum was statistically significant in respect with each other in comparison with the control ($p = 0.05$), the AST, ALT and GGT values were highly statistically significant ($p = 0.01$; Table 5). Therefore, the result indicated that administration of Ivermectin at therapeutic and toxic doses did not exhibit any effect on the total serum proteins, but the reverse effect was shown for rat serum activities as exemplified by ALP, AST, ALT and GGT.

DISCUSSION

Instinctively, animals and humans exhibit and evolve characteristics which tend towards food/nutrition and medication. These characteristics, either in normal or disease conditions are of great importance to health and are mutually interrelated. An example in a study (Utu-Baku *et al.*, 1999) that short term ascorbic acid influences RNA/protein ratio and hepatic lipids in Ivermectin (Mectizan) treated rats and guinea pigs. Ivermectin is a semi synthetic macrocyclic lactone currently used in the treatment of endo- and ecto-parasites in animals, as well as of human Onchocerciasis. This disease coexists in malnourished conditions in large areas of sub-Saharan Africa, Southern Mexico and Brazil (Thylefors and Roland, 1995). It is speculated that the prevalence as well as the epidemiological reports on River Blindness are as equally threatening as those of HIV/AIDS today. Therefore, Onchocerciasis continues to threaten the efforts of medical and paramedical scientists particularly from the stand point of chemotherapy nutrition.

This study on blood total serum proteins and the activities of hepatic enzymes in Wistar albino rats fed growers mash and treated with therapeutic and toxic doses of Mectizan is relevant. Severe Protein Energy Malnutrition (PEM) is characterized by decreased concentration of serum proteins (Shetty *et al.*, 1979). However, the concentration of some proteins may not reflect protein nutritional status accurately because malnutrition is invariably accompanied by concurrent deficiencies in trace elements and vitamins and the presence of infections or pathological conditions that may affect the concentrations of plasma proteins. The condition may be more complex with drug administration. The results (Table 1) indicated that there were no effects of dose dependents Ivermectin on Total Serum Protein in rats fed on growers mash. The concentrations of total serum proteins in the therapeutic and toxic groups of rats were not significantly different ($p = 0.05$) from the control. This shows that total serum protein concentrations in albino rats do not appear to be affected when fed on normal growers mash diet and treated with Ivermectin. It could mean that the rate of synthesis of plasma proteins was unaffected and can not be predicted from measurements of total serum protein concentrations alone. This leaves the possible reasoning that some specific plasma proteins (the rapid turn over protein i.e., retinal binding proteins and transthyretin) may have to be considered as among sensitive index in assessing this effect of dietary status on drugs: using Mectizan and determining several proteins (Shetty *et al.*, 1979).

Proteins and intracellular enzymes appear in serum or plasma as a consequence of normal cell turnover. However, an increased level may be due to cell damage as a result of drugs or cell proliferation. The present findings from the results and illustrations in the activities of ALP, AST, ALT and GGT in Wistar rats fed on growers mash and treated with therapeutic and toxic doses of Ivermectin indicate that all the four liver enzymes were increased and statistically significant ($p = 0.01$) both with the therapeutic and toxic doses, but more with the toxic dose when compared with the control. The GGT activities in rats fed on growers mash, administered with therapeutic and toxic doses show high statistical difference ($p = 0.001$) in comparison with each other and with the control (Table 5). Similarly, ALT, a hepatic enzyme is relatively higher while AST (Table 3), is moderate. Elevation of ALT appears to reflect toxic hepatitis and is more specific that is true of AST (Zimmerman, 1974). Determination of AST enzyme is useful in early recognition of a toxic hepatocellular origin of some drugs (e.g., halothane and carbon tetrachloride (Israel and John, 1974). Because the values of ALT are higher than those of AST (Table 3) in intra-hepatic cholestasis, this study therefore agreed with these findings and indicated that Ivermectin has a dose dependent influence on activities of these enzymes. It could therefore be said that Ivermectin at a toxic dose (4.0 mg kg^{-1}) can trigger increase in rat serum activities.

CONCLUSION

The effect of a therapeutic and toxic dose of Ivermectin (Mectizan) on the total serum proteins and marked liver enzymes was elucidated in rats fed mashed stock diet. Ivermectin, being an anthelmintic drug and a microfilaricide must be administered repeatedly. In this study, short term administration of Mectizan at therapeutic and toxic doses did not appear to have any effect on total serum proteins in rats maintained on normal protein diet but had a dose dependent influence on serum activities of alkaline phosphatase, aspartate aminotransferase, alkaline aminotransferase and γ -glutamyltranspeptidase.

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