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Influence of Manganese on Ochratoxin A Detoxification in Rats

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Abstract
The Influence of manganese on ochratoxin A detoxification was studied in 3- month old female white albino rats administered with 0.00, 500 and 500µg/kg body weight of ochratoxin A intraperitoneally daily for five days. In addition to the ochratoxin A, 0.5ml of 5% Manganese (II) chloride was administered to one of the test groups by intubation. The rats were monitored for mortality and clinical signs for five days before sacrifice. Serum samples were assayed for liver enzymes and kidney biomarkers. Histopathological examinations of the liver and kidney were conducted. The results showed that the administration of 500µg/kg body of ochratoxin A caused injury to the liver and kidney of the test groups though it was milder in the group given manganese and there was no mortality recorded. There was a significant increase (P< 0.05) in activities of the enzymes aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) in the test groups, while it was a non significant (P > 0.05) increase for creatinine level. Histopathological examinations showed degeneration of portal nuclei , walls of porteries and veins, pericholongitis, congestion of portal vessels by white blood cells, hepatocellular necrosis and perivascular cuffing in the liver and lymphocytic infiltration, haematin fragments, edema and tubular necrosis in the kidney of the test groups only. This study indicates clearly that manganese no significant influence on ochratoxin A detoxification.

Key Words: Ochratoxin A, Biochemical, Histological, Detoxification, Manganese, Rats.
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Introduction

Ochratoxin A is a toxic substance produced by fungi *Aspergillus ochraceus.* The spores of the fungi are ubiquitous and under favorable condition could germinate and secrete the toxin on the host substrate (Goldblatt, L.A., 1969). Though ochratoxin A is not the only toxin produced by the fungi, attention is mainly focused on it because of its toxicity as teratogenic, mutagenic, carcinogenic, nephrotoxic and hepatotoxic agent (Goldblatt, L.A., 1969)
Cereals, legumes, oil seeds, tubers, Hay, Silage etc on which livestock and Poultry farmers largely depend are found to be susceptible to ochratoxin A contamination, especially during storage (Van der merwe *et.al,* 1965). Furthermore, ochratoxin A is not substance that could be observed physically, but has to be chemically tested before its presence is noticed. It is clear from above that farmers could encounter problems of ochratoxin A toxicity caused by feeding animals with ochratoxin A contaminated feeds.

A lot of efforts have been made to detoxify Ochratoxin A in animals. Items used for the detoxification include, phenylalanine, activated charcoal and calcium aluminosilicate (Grukemeir,

A.,1990). It has been suggested that one of the ways through which ochratoxin A exerts its effect is through lipid peroxidation (Rahimtula *et.al.,*1988). Therefore, keeping antioxidant enzymes (such as Superoxide dismutase which destroys superoxide anions that initiate lipid peroxidation) active will reduce ochratoxin A toxicity in the body. Thus, the aim of this study is to keep the enzyme super oxide dismutase active by administering manganese which is its cofactor and monitor some biochemical and histological changes that take place during Ochratoxin A detoxification process.

Materials and methods

Twenty four apparently healthy 3-month old, female white albino rats with average weight of 204.70 ± 9.2g were obtained from Nigerian Institute of Trypanosomiasis research, Vom. The rats were assigned to four groups, A, B, C and D, each consisting of six rats. Group A rats were each injected intraperitoneally with 500µg/kg of Ochratoxin A per day. Group B rats were similarly treated but with additional 0.5ml of 5% Manganese (II) Chloride by intubation per day. The control group C rats were each treated with 0.5ml of 5% manganese (II) chloride by intubution per day, while the rats in control group D were each injected intraperitoneally with 1ml/kg of 1% $NaHC0_3$ (vehicle in which standard ochratoxin A was reconstituted) per day. The treatment period lasted for 5 days.

Ochratoxin A Standard: Five milligrams of standard ochratoxin A obtained from sigma was dissolved in 5ml of 1%$NaHCO_3$ to obtain stock standard of 1000 µg/ml.

Sample Collection

(i) Serum: Twenty four hours after the last treatment the rats were sacrificed and the blood samples were collected into clean and sterilized universal bottles. The bottles were kept in slanting position until serum separated and it was collected using Pasteur pipette.

(ii) Tissues: Liver and Kidney samples were collected after the rats were sacrificed.

Chemical Analysis/Statistical Analysis: The enzymes aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were assayed using method described by Reitman and Frankel (1957). The method reported by Kind and King (1954) was employed in Alkaline Phosphotase (ALP) assay. The method described by Verley (968) was employed in the analysis of creatinine. Atomic absorption spectrophotometer (Unicom 929) was used to analyse for Manganese. Duncan new multiple range test and student t-test were employed to analyse the data. All statements of significance were based on a significance level of 0.05.

Histological Examinations: The method described by Drury and Wallington (1967) was employed in sectioning and examining the tissues.

Results and Discussion

The results of serum enzyme changes are as shown in Table i. The enzymes AST, ALT and ALP showed significant increase ($P < 0.05$) in activities in groups A & B rats compared to the control groups C & D rats. The significant increase in activities observed in the test groups A & B rats might be due to the effects of ochratoxin A on the liver. The results are expected because the toxin is known to be hepatotoxic (Goldblatt, L.A., 1969). Similar results were reported by Cecil (Cecil, F. B. 1988) when he found that 500µg/kg of ochratoxin A he administered to rat elevated the activities of AST, ALT, & ALP significantly. In another study, (Kane *et al.,*1986) reported that administering 145µg/kg of Ochratoxin A per day to rats for 8 – 12 weeks caused the increase in activities of glutamyltransferase, Alkaline Phosphatase, lactate dehydrogenase and leucine aminopeptidase. Although in this study the increase in the activities of the enzymes in the test group A rats is higher than that of the test group B rats the difference is non significant ($P>0.05$). Since the dose of the toxin administered to group A & B rats is the same the difference in the increase in the enzyme activities may be attributed to the influence of the activity of

the enzyme super oxide dismutase on initiation of lipid peroxidation.

Table 1: Enzyme Changes (Mean ± Standard Deviation)

Group	AST (mmol/L) n = 6	ALT (mmo1/L) n = 6	ALP (iu/L) n = 6
A (OCHA)	146.74 ± 3.4 [a,b]	182.88±2.6 [a,b]	25.93±1.67 [a,b]
B (OCHA + Mn)	125.4 ± 2.90 [a,b]	157.93 ± 1.97 [a,b]	14,87 ± 1.13 [a,b]
C (Control Mn)	46.4 ± 2.9	41.23 ± 2.61	3.45 ± 0.53
D (Control $NaHCo_3$)	47.4 ± 3.2	41.06 ± 2.0	3.06 ± 0.31

AST = Aspartate aminotransferase
ALT = Alanine aminotrasferase
ALP = Alkaline Phosphatase
a- values are significantly different from C ($P<0.05$)
a- values are significantly different from D ($P<0.05$)

Similar conclusion can be drawn from the results of creatinine assay. The small increase in creatinine levels observed in the test group A and B rats (table ii) over that of the control groups C and D rats is an index of kidney injury. This conforms with the reports of Galtier *et.al.* (1974). They administered ochratoxin A to rats orally and observed that the toxin is nephrotoxic to the rats. However, the non significant increase ($p>0.05$) in creatinine level suggests that the injury done to the kidney by the toxin was mild.

Table 2: Serum levels of Creatinine (Mean ± Standard Deviation)

(Group)	mg/100ml	Significance
A (OCHA)	146 ± 0.02	NS
B (OCHA + Mn)	1.34 ± 0.03	NS
C (Control Mn)	1.15 ± 0.038	NS
D (Control $NaHCO_3$)	1.17 ± 0.034	NS

- $P > 0.05$

NS = non-significant

The results of histopathological examinations of liver (plate 1) showed degeneration of portal nuclei, perichlolongitis, perivascular cuffing and hepatocellular necrosis in the rats of groups A and B.

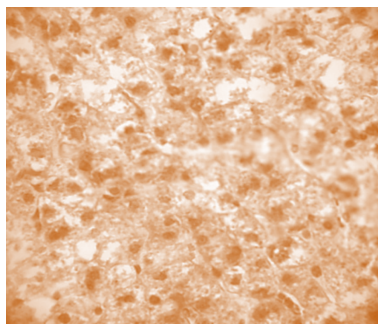
Plate 1: liver showing hepatocellular necrosis

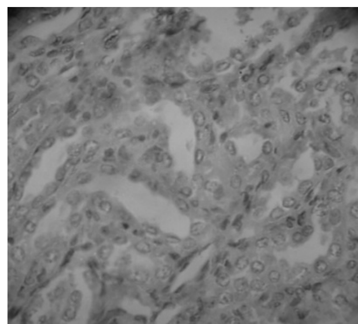
Plate 2: kidney showing tubular necrosis

Examinations of the kidney sections (plate 2) showed lymphocytic infiltration, congestion with haematin fragments, edema and tubular necrosis in the kidney of groups A and B rats. There were no such changes observed in either the kidney or liver of the control groups C and D rats.

The changes observed in liver and kidney of the test groups A and B rats are not surprising since Ochratoxin A is reported to be hepatotoxic and nephrotoxic (Goldblatt, L.A., 1969). Furthermore, the results support this discussion since the injury done to the kidney is mild thus concurring with the small increase in creatinine levels observed. Serum analysis results for manganese showed no detectable level of manganese in all groups of the rats. The inability to detect manganese residue in the rats serum may be attributed to its metabolism and excretion from the body or the low level of sensitivity of the instrument used, thus making it impossible to detect residual manganese

Conclusion

From this study it could be concluded that ochratoxin A inflicted injury on liver and kidney of the test group rats, but milder in test group B rats. Thus signifying that manganese has little influence on ochratoxin A detoxification in rats.

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