Oral Administration of Escherichia coli Endotoxin Caused Liver - Kidney Dysfunction and Death in Rabbits

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Abstract— This study was carried out to determine the effects of ingested endotoxin on cellular and histological parameters in European albino rabbits - *Oryctolagus cuniculus*. Twenty-four (24) rabbits of either sexes weighing 1.5-1.8 kg each were randomly grouped into four of six (6) rabbits per group. The control group, A was orally administered with 5 % w/v dextrose containing 0 EU/ml and hence received 0 EU/kg body weight; groups B, C and D received 50, 500 and 1000 EU/kg body weight respectively, once daily by 10 am prior feeding for a total of 21 days. On a daily basis, blood samples were taken from each animal and serum levels of alanine transferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and catalase activities; bilirubin, malondialdehyde, total protein, albumin (ALB), urea, uric acid and creatinine levels were assayed for. At the end of the 21 days a mortality of 20.83 % was recorded in the groups administered with endotoxin (500 and 1000 EU/kg bw). Also, there was significant increase ($p < 0.05$) in the serum activities of ALT, AST and ALP; significantly reduced total protein and ALB levels with significant ($p<0.05$) increase in endotoxin doses and duration of the administration. There was significant increase ($p<0.05$) in bilirubin, urea and creatinine levels in the serum. Histopathology examinations show tissue degeneration in the liver, heart, kidney and lung related to increased dosages and durations of the orally administered endotoxin. We therefore conclude that oral ingestion of endotoxin causes damage to the liver, kidney, heart and the lungs in rabbits, especially when prolonged.

Keywords— Endotoxin, *Escherichia coli*, contaminated food, food poisoning

1. INTRODUCTION

Microbial contamination of food refers to the non-intended or accidental introduction of infectious agents like bacteria, yeast, mold, fungi, virus, protozoa or their toxins and by-products (Gabriel, 2008). This contamination can be natural via presence of microorganisms in food or drink by environmental contact. It could also be artificial such as through unhygienic handling of foods or drinks during processing. The organisms usually found as food contaminants include the Gram-negative bacteria such as *Escherichia coli* (*E. coli*).

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia*. They do not retain crystal violet stain used in Gram-staining method of bacterial differentiation (Baron et al., 1996). They are bacteria found in the environment, foods, water and intestine of people and animals. Most are harmless, some serotypes are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. They could cause serious food poisoning in their hosts and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005). They are characterized by cell envelopes which are composed of thin peptidoglycan cell wall sandwiched between an inner cytoplasmic cell membrane and a bacteria outer membrane containing lipopolysaccharides (LPS or endotoxin). Endotoxins are large molecules consisting of a lipid and polysaccharide composed of O- antigen, outer core and inner core joined by a covalent bond. It contributes greatly to the structural integrity of the bacteria, and protecting the membrane from certain types of chemical attack. Example of vehicle that has large endotoxin content includes animal feed, drinking water (Koppinen and Oijala, 1987) and organic dust (Donham et al., 1989).

Systemic exposures to endotoxin/ pyrogen have been reported to have adverse effects, such as hypotension, decreased cardiac output, increased pulmonary arterial pressure and vascular permeability in the lungs. Others include disseminated intravascular coagulation, activation and sequential damage to the heart, liver and lungs known as "Multiple Organ Dysfunction Syndrome". In response to endotoxin (pyrogen) released by bacteria or parenterally taken in, leads to release of various cytokines such as interleukins and gamma interferon; production of nitric oxide synthethase is induced within macrophages. This enzyme catalyzes the formation of nitric oxide which may produce hypotension of septic shock, which may lead to the multiple organ dysfunction syndromes (Dinarello, 2000).

However, presence of pyrogens or LPS in food items or orally administered drugs appears to be ignored as tests for pyrogens in food items or oral medications is not officially required. In the developing countries most homes could not rely on refrigerating food left-overs, due to shortage of electricity and have to re-heat or warm food remnants up each time they needed to eat the food which could have been loaded with pyrogens.

The aim of this study was therefore to investigate the effect of oral administration of endotoxin/pyrogen on liver and kidney functions indices of rabbits . The objective of this study was to assess the possible effect of oral administration of endotoxin which may be consumed unintentionally with contaminated food daily. Also, the study may provide information on whether orally-ingested endotoxin produce reactions similar to that produced by systemic administration of endotoxin (through injuries or injection). This study was approved by the Biochemistry Departmental Postgraduate Committee in 2015/2016 session on behalf of the University of Ilorin Research Ethics Committee.

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2. MATERIALS AND METHODS

2.1 Experimental animals

Twenty- four (24) adult rabbits (*Oryctolagus cuniculus*) of both sexes, with an average weight of 1.5 ± 0.05 kg were purchased from Biomedical Limited, Ilorin. The rabbits were housed in well ventilated wooden cages and maintained under standard laboratory conditions with free access to feed and water. They were maintained according to the University of Ilorin Ethical Committee Guidelines on the Use of Laboratory Animals.

2.2 *Escherichia coli*

Escherichia coli was cultured, isolated and purified by sub-culturing four times at Prince Consultancy Laboratory, Ilorin, Kwara State by a Microbiology Analyst, Mr. Anunu Emiko.

2.3 Reagents and Assay Kits

All assay kits were obtained from Randox Laboratory Limited, Co-Antrim, United Kingdom. Whatman syringe filter (0.2 micron), a product of GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK, was used for sterilization of the dextrose solution prepared. Dextrose monohydrate powder (Allenbury's Glucose D) was a product of Evans Medical PLC, Agbara Industrial Estate, Agbara, Ogun State, Nigeria.

3. METHODOLOGY

3.1 Depyrogenation

All glass wares were depyrogenated by incubating in oven at 250 0C for 30 min.

3.2 Preparation of Endotoxin from purified *E. coli*

Sufficient quantity of purified *E. coli* was inoculated in 250 ml of 5% Dextrose and mix well. 2ml of the Dextrose solution was picked for initial microbial load sampling. Dextrose solution containing *E. coli* was then incubated for 370C for 48 h. Two (2) ml sample was used for final microbial load testing. After then, it was corked tightly and sterilized in an autoclave for 21 min. Final sample contain the pyrogenic solutions (endotoxin source)

3.3 Determination of Endotoxin Content of the Solutions

To confirm the presence of endotoxin in the mixture produced, pyrogen test was carried out using the rabbit test method (Radhakrishna, 2010). The actual concentration of endotoxin was estimated using a standard curve (Dalmora et al., 2004) correlating temperature rise and endotoxin content in injected solutions.

3.4 Animal grouping

Twenty-four rabbits which had been acclimatized for 2 weeks were randomly grouped into four groups (A-D). Each group contained 6 rabbits. Group A (Control) was administered with pyrogen-free dextrose 5 % w/v solution (0 EU/kg bw). Group B contains animals that were administered 50 EU/kg bw. Group C contained animals administered with 500 EU/kg bw while Group D contained animals administered with 1000 EU/kg bw. The administrations were done orally with the aid of syringes daily at about 10 a.m., for 21 consecutive days while the rabbits were exposed to rabbit chow and water ad libitum. The animals were not fed daily until after the administration. The weights of the animals were monitored daily so as to ascertain the quantity of endotoxin solution to be administered. The administrations were done orally using oral canula.

3.5 Preparation of serum

The blood of the rabbits was sampled via ear marginal vein puncture at specific durations (0 hr, 1hr, 3 h, and 24 h, Day 3, Day 7, Day 14 and Day 21). The blood samples collected were allowed to clot and then centrifuged at 3, 000 g for 5 min for serum preparation using a table centrifuge. The clear supernatant was diluted with distilled water for biochemical analysis.

3.6 Collection of tissues for histopathology

After the 21 days of daily administration of endotoxin, one animal was sacrificed from each group by placing them in a jar containing wool soaked in diethyl-ether to make them unconscious. The jugular vein was sharply cut with clean sterile scalpel blade. The animal was dissected to remove liver, kidney, heart and lungs. It was cleaned free of blood and immersed in 10% formalin to maintain the integrity of the organs. It was then submitted for histopathology analysis.

3.7 Histopathological Examination

The tissues (liver, kidney, heart and lung) were fixed in 10% formalin for 48h, grossed, dehydrated through different grades of ethanol, xylene and embedded in paraffin. A section (3- 4 μm) of the tissues was stained with hematoxylin and eosin stains and mounted on a microscope (TP1020,USA) for photomicrography.

3.8 Liver and Kidney function indices

Determination of alkaline phosphatase (ALP), Acid phosphatase (ACP) determined by the method of Wright et al (1972), aspartate transaminase (AST), alanine transaminase (ALT) were determined according to the Reitman and Frankel (1957) method. Determination of urea was carried out according to method of Veniamin and Vakirtzi (1970). Creatinine were also assayed for according to the method described by Bartels and Bohmer (1972).

3.9 Statistics

The results were expressed as mean ± SEM of six determinations. All results were statistically analysed using two way Anova and Tukey post hoc test. Differences between group means were considered to be significant at $p<0.05$ using Graph Pad Prism 6.

4. RESULTS

4.1 General observation

Pyrogen level was determined using standard rabbit test method. The significant rise in temperature with 2.53ºC per rabbit administered stock endotoxin shows the Endotoxin stock was pyrogenic (Hurley, 1995). The endotoxin stock was found to contain 2,210 EU/ml

.Administration of endotoxin orally to rabbits within the first week led to reduced activity (sluggishness) in the animals. Also, food intake was reduced while there was increased water intake. During the second week, there was loss of fur on the body of the rabbits administered daily doses of 500 and 1000 EU/kg bw. Mortality during daily administration accounted for 20.83 % of the total animals and occurred in the groups administered 500 and 1000 EU/ Kg body weight.

Table 1. Concentration of creatinine in the serum of rabbits orally administered with *E. coli* endotoxin

Duration	Creatinine concentration (mg/dl) Control 0EU/kg bw	50EU/kg bw	500EU/kg bw	1000 EU/kg bw
0 hr	29.39±3.79^{a}	24.49±4.90^{a}	29.39±3.79^{a}	34.29±4.90^{a}
1 hr	22.04±3.29^{a}	36.74±3.29^{b}	24.49±4.90^{a}	35.46±2.91^{a}
3h	31.84±4.53^{a}	66.13±5.02^{b}	48.99±4.90^{c}	68.58±3.10^{b}
24 h	36.74±3.29^{a}	56.33±2.45^{b}	66.13±2.64^{b}	73.48±5.37^{bc}
Day 3	39.19±3.10^{a}	41.64±4.52^{a}	69.13±2.64^{b}	85.72±2.45^{c}
Day 7	26.94±4.52^{a}	85.72±4.52^{b}	93.07±3.10^{b}	115.12±2.45^{c}
Day 14	31.84±2.45^{a}	56.33±2.45^{b}	72.75±3.29^{b}	68.58±4.90^{b}
Day 21	39.19±3.10^{a}	83.28±3.10^{b}	105.32±2.45 C	129.81±2.45^{d}

Each value is a mean of six determinations ±SEM. Values on the same row with different superscripts are significantly different

Table 2. Concentration of Urea in the serum of rabbits orally administered with E. coli endotoxin

Duration	Urea concentration (mg/dl) Control 0EU/kg bw	50EU/kg bw	500EU/kg bw	1000 EU/kg bw
0 hr	6.22±0.19 ^{a}	7.06±0.20 ^{a}	6.55±0.35 ^{a}	6.97±0.23 ^{a}
1 hr	5.98±0.12 ^{a}	8.03±0.35 ^{b}	7.86±0.30 ^{b}	7.00±0.06 ab
3 h	5.95±0.23 ^{a}	7.78±0.28 ^{b}	6.59±0.37 ^{ab}	8.01±0.34 bc
24 h	6.76±0.24 ^{a}	7.38±0.58 ^{a}	8.77±0.62 ^{b}	9.47±0.70 ^{b}
Day 3	5.81±0.16 ^{a}	6.48±0.36 ^{a}	6.24±0.26 ^{a}	5.93±0.19 ^{a}
Day 7	5.82±0.23 ^{a}	6.66±0.16 ^{a}	6.27±0.35 ^{a}	7.44±0.44 ^{b}
Day 14	6.69±0.30 ^{a}	7.30±0.28 ^{a}	7.38±0.62 ^{a}	11.09±0.31 b
Day 21	6.80±0.41 ^{a}	9.40±0.68 ^{b}	11.43±0.58 c	15.24±0.66 d

Each value is a mean of six determinations ± SEM. Values on the same row with different superscripts are significantly different

4.2 Effect of oral administration of E. coli Endotoxin on enzymic hepatocellular markers

4.2.1 Alanine transaminase (ALT)

Oral administration of endotoxin led to significant ($p<0.05$) increase in specific ALT activity of the test groups administered 50, 500 and 1000 EU/kg bw in a dose-dependent manner compared to the control. At Day 21, a significant ($p<0.05$) reduction occured in specific ALT activity of test group administered 50 EU/kg bw compared to the other test groups. However, no significant ($p>0.05$) change occurred between the test groups administered 500 and 1000 EU/kg bw.

4.2.2 Aspartate transaminase (AST)

Oral administration of endotoxin led to significant ($p<0.05$) increase in specific AST activity of test groups administered 50, 500 and 1000 EU/kg bw in a dose-dependent manner compared to the control. At Day 21 there was a significant ($p<0.05$) increase in specific AST activity of test animals administered 50, 500 and 1000 EU/kg in bw compared to the control.

4.2.3 Acid phosphatase (ACP)

Oral administration of Endotoxin led to significant ($p<0.05$) increase in specific ACP activity of test groups administered 50, 500 and 1000 EU/kg bw in a dose dependent manner compared to the control. At day 7 no significant ($p>0.05$) difference occurred in specific ACP activity of test group administered 500 and 1000 EU/kg bw respectively compared control At Day 14 and 21 there is a significant ($p>0.05$) increase specific ACP activity of all test group administered 50, 500 and 1000 EU/kg bw respectively compared to control in a dose dependent manner.

4.2.4 Alkaline phosphatase (ALP)

At 24 hours, Day 3 and Day 7, there is a significant ($p<0.05$) increase in specific ALP activity of all test groups compared to control. At Day 14 no significant ($p>0.05$) difference in specific ALP activity between test groups administered 500 and 1000 EU/kg bw. At Day 21, there is a significant ($p<0.05$) increase in specific ALP activity in all test groups compared to control.

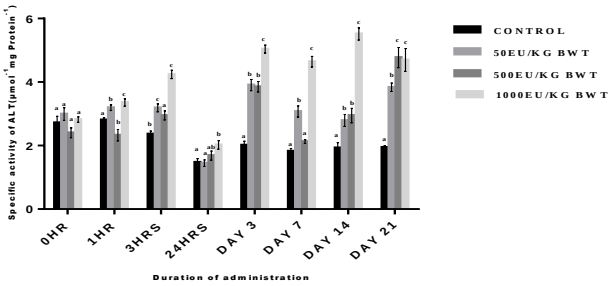


Fig. 1: Specific activities of alanine aminotransferase (ALT) in the serum of rabbits orally administered *E. coli* endotoxin
Each Value is a mean of six replicates ± SEM, Values with different superscripts are significantly different ($p<0.05$).

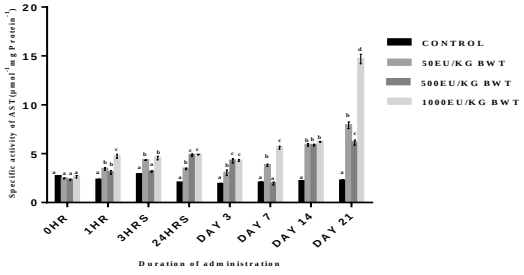


Fig. 2: Specific activities of Aspartate aminotransferase (AST) in the serum of rabbits orally administered *E. coli* endotoxin
Each Value is a mean of six replicates ± SEM, Values with different superscripts are significantly different ($p<0.05$).

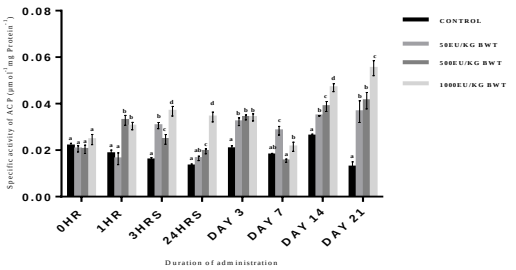


Fig. 3: Specific activities of Acid Phosphatase (ACP) in the serum of rabbits orally administered *E.coli* endotoxin
Each Value is a mean of six replicates ± SEM, Values with different superscripts are significantly different (p<0.05)

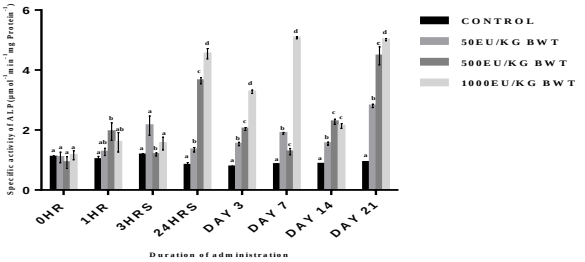


Fig. 4: Specific activities of Alkaline Phosphatase (ALP) in the serum of rabbits orally administered E.*coli* endotoxin
Each Value is a mean of six replicates ± SEM, Values with different superscripts are significantly different (p<0.05).

4.3.1 Creatinine

After one hour of oral administration of endotoxin, there is no significant (p>0.05) change in creatinine concentration in test groups administered 500 and 1000 EU/kg bw compared to control. A significant (p<0.05) change was observed in test groups administered 50 EU/kg bw compared to the control and the test groups administered 500 and 1000 EU/kg bw. At 3h, 24h, Day 3,7 and 14 there is a significant p>0.05increase in creatinine concentration of test animals administered 50, 500 and 1000EU/kg bw compared to control. At day 21, there was a drastic significant (p>0.05) increase in creatinine concentration in test animals administered 50, 500 and 1000 EU/kg bw compared to control. However, a significant (p<0.05) change was observed within the test groups as well.

4.3.2 Urea

After 1 hour of oral administration of endotoxin there was a significant (p< 0.05) change in urea concentration of test animals administered 50 and 500 EU/kg bw compared to control. However, there is no significant (p>0.05) change in test animals administered 1000 EU/kg bw compared to control. Similarly, there was no significant (p>0.05) change in urea concentration within the test groups administered 50, 500 and 1000 EU/kg bw. At 24 h, Day 7 and 14 there was a significant increase in test group administered 1000EU/kg bw compared to control and other test groups. At day 21, there was a significant (p>0.05) increase in urea concentration of test animals administered 50, 500 and 1000 EU/kg bw compared to the control.

4.3.4 Histopathology

The results of histopathological studies of selected tissues (Liver, Kidney, lungs and hearts) of rabbits orally administered doses (50, 500 and 1000EU/kg bw) *E. coli* Endotoxin daily for a period of 21 days are shown below.

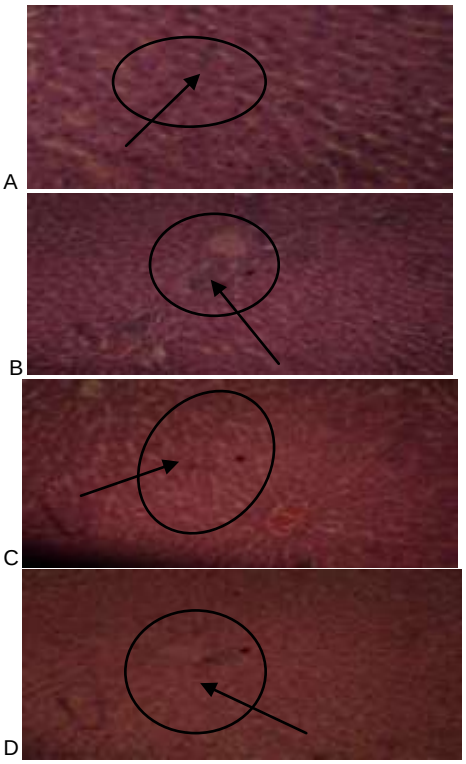
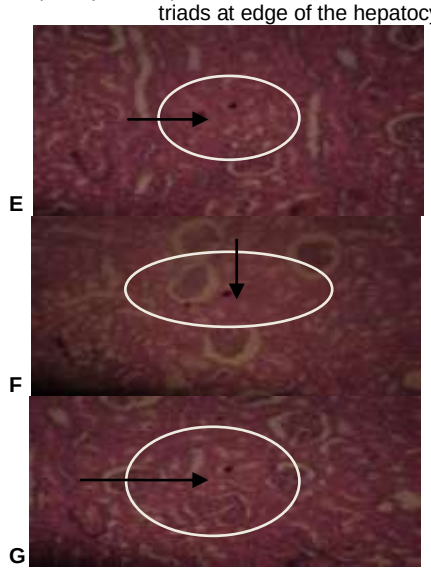


Fig. 5: Photomicrograph (X400) of the hepatocyte of A) Liver of control group liver shows a normal hepatic tissue B) Test group administered 50 EU/kg bw shows mild distortion of hepatic tissue with both interstitial/vascular congestion seen C) Test group 500 EU/kg bw shows an irregular hexagonal plate of hepatocytes, with central vein. The sinusoids at the lobule margin course between plates of hepatocytes to converge upon terminal venule. The hepatocytes are exposed to blood on both sides. D)Test group administered 1000 EU/kg bw shows an irregular hexagonal plate of hepatocytes with central vein. The sinusoids at the lobule margin. The plates of the hepatocytes are usually one cell thick and each hepatocyte is exposed to blood on both sides. There are portal triads at edge of the hepatocyte.



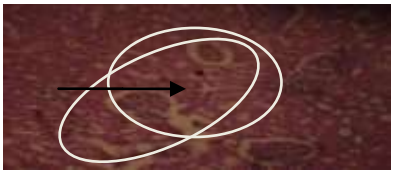


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Fig. 6: Photomicrograph (X400) of the Glomerulus of E) kidney of control shows normal Nephron and Glomeruli. F) Test group administered 50 EU/kg bw administered shows Normal nephron and glomeruli seen but with mild distortion. G) Test group kidney administered 500EU/kg bw shows a mild distortion in nephron and glomeruli with congestion. H) Test group administer red 1000EU/kg bw shows an abnormal nephron and glomeruli seen with narrow bowman's spaces. The tubules fill the bulk of the parenchyma between the corpuscles.

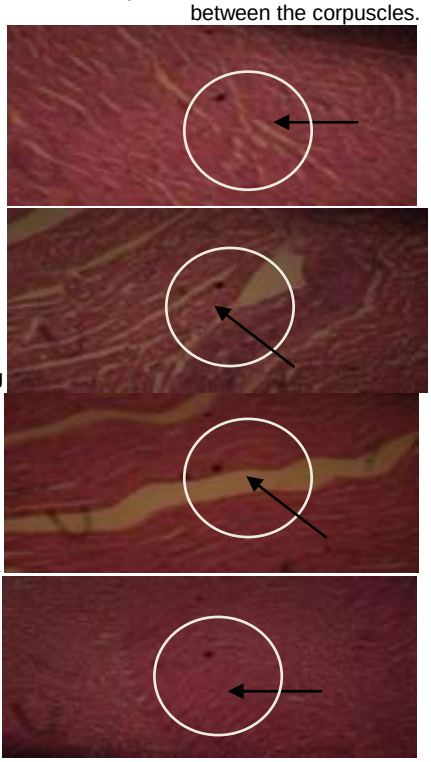

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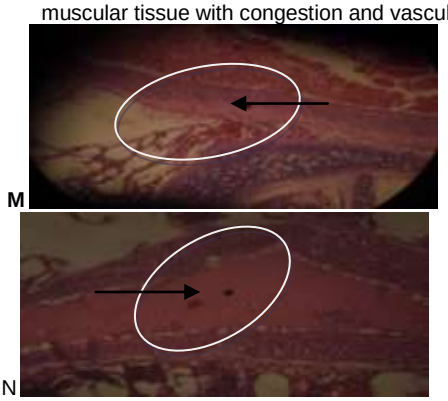
Fig. 7: Photomicrograph (X400) of the Heart of I) Heart of control group heart shows normal Cardiac Muscular tissue. J) Heart of Test groups administered 50 EU/kg bw shows a normal cardiac muscular tissue with mild vascular dilation K) Heart of test group heart administered 500 EU/kg bw shows mildly distorted branch of conducting fibers running in the interventricular septum just beneath the endocardium. The conducting fibers are separated from the myocardial fibers by layers' fibrous tissue. L) Heart of test group administered 1000EU/kg bw shows abnormal cardiac muscular tissue with congestion and vascular dilation seen.



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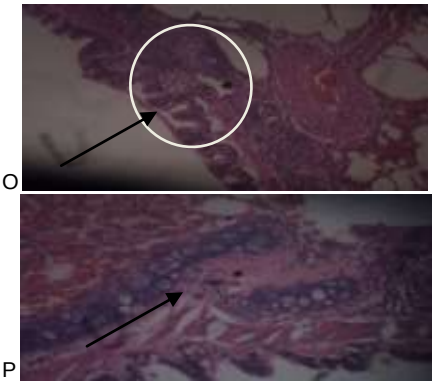

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Fig. 8: Photomicrograph (X400) of the Lungs of M) Control group shows normal external elastic lamina, intima with surface endothelial cells, normal smooth muscle cells, collagen and prominent elastic lamellae seen. N) Lungs of test groups administered 50EU/kg bw shows a normal external elastic laminae seen but the internal elastic laminae are mildly distorted. The intima has surface endothelial cells. Normal smooth muscle cells, collagen and prominent elastic lamellae seen. There are congestion/hemorrhage O) lungs of test group administered 500 EU/kg bw shows an abnormal external elastic laminar seen with mildly distorted internal elastic laminae with congestion P) Lungs of test group administered 1000 EU/kg bw shows an abnormal external lamina with distorted internal elastic laminae seen. There is congestion in areas.

5. DISCUSSION

Gram-negative bacteria, both pathogenic and non-pathogenic, contain lipopolysaccharide, being component of the outer membranes of the cells, which induce fever when introduced into the blood circulation of mammals. Consumption of endotoxin can occur through various means- food and other substances such as oral drugs which at times might be contaminated with pyrogen. This study evaluated the effect of oral administration of endotoxin on liver and kidney function indices of rabbits as well as the antioxidant status.

From this study, the manifested clinical signs and mortality could be as a result of the toxicity and prolong exposure to endotoxin, which might have caused multiple organ dysfunction and eventually death of the animal (Dinarello, 2000). It suggests that endotoxin orally ingested could have been absorbed into the blood stream and produced effects similar to injected endotoxin. Therefore, the death of the rabbits may be due to biochemical changes caused by endotoxin. It has been reported that after injection of LPS, level of the blood sugar begins to rise and reaches a maximum within 2 h, thereafter the level declines and hypoglycemia ensues and has been reported that LPS directly inhibits both glucose and lipid metabolism (Crespo et al., 2009). This is associated with the depletion of liver glycogen increased lactate and diminished pyruvate in blood and tissues, and an inhibition of succinate dehydrogenase activity in muscle and liver. It has also been reported LPS induces a transient hyperglycaemia followed by a marked hypoglycemia. Based on this present study the test animals might be hyperglycemic which causes the increased intake of water. The hypoglycemia develops as a consequence of both elevated uptake of glucose by peripheral tissues

and the failure of the liver to compensate for this by an augmented glucose output (Crespo et al., 2009).

In this study, the various biomarkers used showed varying responses to the endotoxin administered via oral route. The liver is the most important organ for endotoxin accumulation and largest organ that have monocyte/ macrophage system. The degraded endotoxin by the macrophages is excreted from the liver, into the gut, bile and detected in feces. Numerous metabolic activities in liver are altered after endotoxin poisoning. Such changes include depletion of carbohydrate reserves, inhibition of particular enzyme induction, and inhibition of gluconeogenesis (Crespo et al., 2009). Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of a known disease and determine the effect of potentially hepatotoxic agents (Harris, 2005). Alanine transaminase (ALT) and aspartate transaminase (AST) are enzymes found in the liver and have been widely used for diagnostic purposes as an indicator of liver damage (Whitehead et al., 1999). AST is present in both mitochondria and cytosol of liver cells while ALT is found in the cytosol only. (Al-Hashem et al., 2009).

In this study, after 1h and 3h of oral administration of E.coli Endotoxin to the rabbits, it resulted to a significant increase in ALT activity of all the test groups compared to the control. However, at 24h a reduction in ALT activity of test groups administered 50 and 500 EU/kg bw was observed although they were not significant to the control group (Figure 1). This could mean that there was a self-limiting immunological response initiated in the animals administered 50 and 500 EU/kg bw thus limiting damage caused by endotoxin and might be enhancing a repair. However, at high dosage of 1000 EU/kg bw could have caused some liver damage that leads to increase in ALT activity. This observation is in correlation to findings of Granado et al (2008), regarding injected endotoxin. After 24 h, increases in ALT activity persist till day 21 compared to control. This persistent increase may be as a result of over accumulation of the endotoxin in a dose dependent manner to a level that causes damage to the liver leading to leakage of the enzyme. This observation has a similar trend previously reported by Abd el Rahman et al., (2005) who demonstrated that endotoxins significantly increase serum ALT activity.

AST activity (Figure 2) shows no significant change in all test groups compared to the control group after 1 h till 24 h of oral administration of endotoxin. However, at Day 3, a slight significant ($P<0.05$) increase was observed in ALT activity of test groups administered 50, 500 and 1000 EU/kg compared to control. This increase could be due to the damage done to the membrane of the liver as a result of accumulated endotoxin due to daily administration, thereby causing alternation in hepatic function and possibly leakage of enzymes. At day 7, a significant reduction occurs in ALT activity of test groups administered 500 EU/kg bw compared to other test groups. This significant reduction is similar to ALT activity at Day 14 as well. This could mean that the immunological response of the animal in this group seem to adjusted to the assault caused by endotoxin therefore deactivating the denovo synthesis of ALT enzyme. However, at day 21, there was a drastic significant increase in ALT activity of all test groups compared to the control. This observation support previous studies by Abd el Rahman et al., (2005) who demonstrated that endotoxins significantly increase serum AST.

Acid phosphatase (ACP) catalyzes the removal of phosphoryl group from a phosphate ester in acidic medium. It is found throughout the body. (Nelson and Cox, 2000). However, damage to the liver or kidney causes moderate increase in serum level of acid phosphatase ACP (Moul, 1998). Increase in serum ACP activity as observed in this study may be an indicative of leakage of ACP from tissue to serum which can cause damage to cells of tissue. Significant ($P<0.05$) increase was observed in ACP activity of test groups administered 50, 500 and 1000 EU/kg body weight after 1hour of oral administration with a persistent increase in a dose dependent manner till Day 3. However, over time, at day 7 and 14, a significant reduction occur which could be as a result of self -limiting response elicited by the test groups as shown in the 500 and 50 EU/kg bw administered animal towards to the administered Endotoxin at day 7 and 14 respectively. Further administration of Endotoxin till Day 21 to test groups significantly increase ACP activity in a dose dependent manner compared to control. However, there was no significant change in ACP activity between test group administered 50 and 1000 EU/kg bw. This could be as a result of difference in biological response over time to endotoxin in different groups.

ALP is an enzyme in the cells lining the biliary ducts of the liver; it becomes elevated when there is a space occupying lesion in the liver. It is a marker enzyme for plasma membrane and endoplasmic reticulum (Kayode et al., 2011). It is assay frequently to assess the integrity of plasma membrane (Akanji et al., 1993). After 1h and 3h of oral administration of endotoxin to test animals, no significant change occurs in ALP activity of test animals compared to control group. This could mean that the endotoxin is being removed or detoxified by the Kupfer cells of the liver there by causing no significant harm to the liver. However, a significant increase occurs in ALP activity of all test groups in a dose dependent manner. This could be as a result of various immunological responses to endotoxin such as activation of macrophages and other mononuclear cells phagocytose the endotoxin, however, nitric oxide is induced within the macrophages. Nitric oxide is deleterious and could compromise the integrity of the liver membrane. This observation is related to that of reported by Reed (1999) that increase in ALP activity may be due to toxicity of endotoxin over prolonged systemic exposure causing lysis of animal cell membrane. However, at day 14, a reduction in ALP activity was observed in the test groups. Further exposure to endotoxin leads to

significant increase in ALP activity of tests groups in a dose dependent manner. The elevated ALP activity could be attributed to damaged structural integrity of the hepatic cells elicited by prolonged daily administration of endotoxin (Sallie et al., 1991). This observation is related to that of Suresh Kumar et al. (2006). Who observed that hepatic function is linked to ALP activity and increase in serum ALP activity is due to increase synthesis as a result of increase biliary pressure.

Oral administration of endotoxin to test animal shows a decrease in test group administered 1000 EU/kg bw compared to control after 1 hour. However, it was not significant to the test groups administered 50 and 500 EU/kg bw. This Reduction could be as a result of suppression in protein synthesis due to high dose of endotoxin administered to the group. After 3h and 24 h, a significant increase was observed in test group administered 500 and 50 EU/kg bw compared to control. This increase in total protein level could indicate that more protein was produced to help boost the immune system of the animals so as to fight back the endotoxin assault. However, increased production could not be controlled due to hepatic injury as notice in increase level ALT, AST and ACP therefore leading to the leakage of the enzymes. Significant reduction was observed in total protein from Day 3 till 21 in all test groups compared to control. This reduction could also be as a result of the suppressive effect in biosynthesis of protein due to endotoxin assault for a prolonged period as well as the hepatic dysfunction that occurred.

Kidney function indices (Urea and Creatinine) are indices used to assess the functionality ability of kidney (Naik, 2011). Urea is the main end product of protein metabolism. Amino acid deamination takes place in liver, which is also the site of urea cycle where ammonia is converted into urea and excreted through the urine (Alagammal et al., 2012). Production of Urea varies directly to protein intake and inversely with rate of excretion. Renal diseases diminish glomerular filtration leading to urea retention and decrease in urea excretion seen in severe liver disease with destruction of cells leading to impairment of Urea cycle (Rajan et al., 2012; Alagammal et al., 2012).

Oral administration of endotoxin to test animals significantly increased urea concentration after 1h till 24 h in a dose dependent fashion. However, at day 3, 7 and 14 no change was observed in test animals administered 50 and 500 EU/KG bw compared to control. However, increase in Urea concentration occurs in test groups administered 1000EU/kg bw in higher doses. Similarly, abnormal increase occurs in test groups administered 50, 500 and 1000EU/kg bw in a dose dependent manner compared to control at day 21. This increase could be as a result of severe impairment to the kidney leading to retention of urea as observed in increase urea concentration. This leads to accumulation of urea in the blood. This observation correlated to that of King Sung Kang et al., (2007) who observed that there was an increase in urea serum concentration of untreated rats induced with endotoxin. However, it was not orally administered but intraperitoneally.

Creatinine is a breakdown product in muscle tissues and may be defined as nitrogenous waste product. It is usually produced at a fairly constant rate by the body (Depending on body mass). In chemical terms, creatinine is a spontaneously formed cyclic derivative of creatine. If the filtering ability of the kidney is deficient, creatinine blood level rises. Oral administration of endotoxin shows no change in creatinine concentration of test group compared to control after 1 hour. However, drastic increase was observed after 3h till 14 days in 1000EU/ kg bw compared to control and other groups administered 50 and 1000EU/kg bw. However significant increase was observed in all test groups at Day 21. Increase in creatinine concentration in serum may be as a result of kidney dysfunction which was elicited by endotoxin stress over prolonging systemic exposure. Therefore, the nitrogenous waste is being accumulated and circulated in the blood lead to its increase level. This correlates to the increase in level of urea as well (Table 2). This observation correlated to that of King Sung Kang et al., (2007) who observed that there was an increase in creatinine serum concentration of untreated rats induced with endotoxin.

Histological changes in liver, kidney, lungs and heart are manifestation of chemical, physical and mechanical inflammatory assault in the tissue (Adesokan, 2007). Systemic exposure to endotoxin has been reported to cause "Multiple Organ Failure Syndrome (MODS) (Dinarello, 2000). Generally, it was observed from histological studies that severe distortion in respective tissues (Liver, Kidney, Heart and lungs) of test groups administered 500 and 1000 EU/kg body weight and less distortion in tissues of test groups administered 50 EU/kg body weight compared to the control group. This distortion is an evidence of tissue degeneration. This might be due to the toxicity of the orally administered endotoxin especially at higher doses. Crespo et al., (2009) reported that endotoxin directly inhibits glucose and lipid metabolism and causes hepatotoxicity, renal failure, and lipid peroxidation via the induction of free radicals. Our data agrees with these explanations of organ failure and metabolic alterations caused.

6. CONCLUSION

It is evident from this study that prolonged oral administration of endotoxin has negative effects on the liver and kidney functions of the rabbits which eventually has lead to death of some of the animals. Our findings also suggest that orally administered endotoxin gets absorbed into circulation from the gut, as the resultant effects are similar to those caused by systemic endotoxin.

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