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Protective effects of aqueous extract of *Cryptolepis sanguinolenta* on endotoxin-induced oxidative stress in Wistar rats

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Abstract

The protective effect of oral co-administration of *Cryptolepis sanguinolenta* and *E. coli* endotoxin to rats was studied on liver and kidney function indices and antioxidant status in rats. Thirty-five (35) rats were randomized into 7 groups containing 5 rats each- Group A distilled water, Group Ascorbic acid and Endotoxin, Group C Endotoxin dose (50 EU/kg bw), Group D 50 mg/kg body weight (bw) of *Cryptolepis sanguinolenta* extract only and Group (E-G) 25, 50 and 100 mg/kg bw *Cryptolepis sanguinolenta* extract plus 50 EU/kg bw endotoxin respectively. The oral administration was repeated for 21 days at 11:30 am daily. Rectal temperatures of the rats were taken at an interval of 30 mins, 60 mins, 90 mins and 120 mins post administration. At the end of the 21 days, the animals were sacrificed and the serum, kidney and liver was obtained. Specific activities of alanine transaminase, aspartate transaminase, alkaline phosphatase as well as concentrations of creatinine and urea in the serum were determined. Activites of superoxide dismutase and catalase were also determined. Also assayed for was malondialdehyde concentration. Histopathological analysis was carried out on the liver and kidney from each test groups. Results show that exposure to 50 EU/ml endotoxin for 21 days plus extract at 50 and 100 mg/kg BW significantly ($P<0.05$) increased the specific activities of alanine aminotransferase and decreased the specfic activity of aspartate aminotransferase and alkaline phosphatase respectively. However, concentrations of creatinine in the serum significantly ($P<0.05$) decreased at a dose of extract 25 mg/kg. Urea concentration stayed stable at all doses and malondialdehyde significantly ($P<0.05$) decreased. Specific activities of superoxide dismutase and catalase significantly ($P<0.05$) decreased mostly at concentrations of 50 and 100 mg/kg BW of the extract. Histopathological study revealed inflamed hepatocytes and disrupted kidney functions in the untreated group and while organs of test groups administered 100 mg/kg bw of the extract were improved. This study hence supports the protective effects of the administration of *Cryptolepis sanguinolenta* prior to oral administration of *Escherichia coli* endotoxin as the extract at doses between 50 and 100 mg/kg protected the organs from the effect of reactive oxygen species induced by oral endotoxin on liver and kidney function indices.

Keywords: Endotoxin, escherichia coli, contaminated food, endotoxin-Induced oxidative stress

1. Introduction

Bacterial infections is involved in a large group of causative agents that elicit acute or chronic diseases. According to the world health organisation, they account for one of the major one of the major etiology of human morbidity and mortality. Some of these infections resulting in chronic diseases eventually leads to development of a range of pathologies including cancer, autoimmune diseases, diabetes mellitus, and the impairment of various organs. Over the past 20 years it has been clearly established that many of these infections actuate the synthesis of reactive oxygen (ROS) and nitrogen (RNS) species (Alexander *et al*., 2017) [4]. Plants have been in a symbiotic relationship with man since inception as they have established the basis of therapeutic drugs since they were less toxic when compared with synthetic drugs (Adaramoye *et al*., 2008) [2]. Folklore medicine is widespread and plants still rank as the top source of natural antioxidants that might serve as leads for the development of groundbreaking drugs. Several drugs like anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have been shown to posess antioxidant and/or anti-radical scavenging mechanism as part of their activity (Kimyai*et al*., 2010). In the course of numerous researches on the determination of sources of natural antioxidants and compounds with free radical scavenging activity during recent years, some have been found, examples were echinacoside gotten from Echinaceae root, anthocyanin, phenolic compound, water extracts of roasted Cassia, whey protein, and thioredoxin protein from sweet potato (Huang *et al*., 2005). Dietary mushrooms were also found to contain antioxidants which were suggested as possible protective agents to help the human body mop up free radicals without any interference. Now antioxidants were acknowledged in nutrition as essential functional foods and as a source of many physiological benefits. In developing countries (which would include most of the west african countries), food preservation for re-consumption is mostly done merely by re-heating the food. This action results in the food gathering endotoxin which upon digestion is activated, leading to a cascade of reactions to release of various cytokines, and also the production of nitric oxide synthethase which catalyzes the synthesis of nitric oxide leading to sepsis and subsequently multiple organ dysfunction (Dinarello, 2000) [12]. Lipopolysaccharide (LPS), is contained on the outer-membrane of Gram-negative bacteria and is a well characterized endotoxin that triggers activities of the immune system and in particular, induces inflammation (Rumpa, 2010) [25]. In some cases, endotoxemia progresses to severe sepsis, resulting in

multiple organ dysfunction, septic shock, and death (Lopez-Bojorquez *et al*., 2004). The rate of deaths associated with severe sepsis is high. There were one million deaths from sepsis worldwide, and approximately 25-30% of the cases were due to gram-negative bacterial infection (Rumpa, 2010) [25]. Reactive oxygen species (ROS) were known as highly reactive chemical molecules containing oxygenthat were synthesized during mitochondrial respiration (Bast and Goris, 1989; Bayir, 2005; McCord and Fridovich, 1978). Both intracellular and extracellular ROS were maintained at non-lethal concentrations by antioxidant enzymes like superoxide dismutase, catalase, and thiol-reducing buffer consisting of glutathione and thioredoxin (Gamaley and Klyubin, 1999; Nakamura *et al*., 1997). However, diseases and oxidative stress can fault a cell's ability to effectively regulate ROS. Elevated levels of free radicals can lead to the destabilization of structural proteins, DNA, RNA, and cell membranes by OH^- attack, inducing apoptosis and ultimately cell death (Bast and Goris, 1989; Machlin and Bendich, 1987; McCord and Fridovich, 1978). Exposure to LPS causes an upsurge in the synthesis of ROS in murine macrophages i.e rats and mice (Hsu and Wen, 2002; Kim *et al*., 2004), and the build-up of free radicals is a supporting factor in the development of sepsis in rats (Bayir, 2005). Thus, free radicals plays a major role in the induction of inflammatory response resulting from LPS ingestion and the subsequent incidence of sepsis. A previous study by Salawu *et al* in 2018 [26] involving the oral administration of *E. coli* endotoxin to groups of rabbits at different concentrations also resulted in the mortality of about $1/5^{th}$ of all the rabbits mostly of which were administered with higher dose of the endotoxin, there were increase in the activities of some enzyme markers like Alanine and Aspartate aminotransferase, alkaline phosphatase, acid phosphatase, activities of Superoxide Dismutase and Catalase also increased, confirming the presence of ROS in the system resulting from the orally ingested endotoxin. (Salawu *et al*., 2018) [26]. Therefore, this study is designed to determine if the antioxidant activity of extract from our plant of interest *Cryptolepis sanguinolenta* can neutralize the effects of the Endotoxin, Prevent the formation of ROS and to also determine the toxicity of the extract on some serum and tissue indices in Wistar Rats. *Cryptolepis sanguinolenta* the plant of interest is of the family Periploaceae and the order Apocyales. It is identified as a climbing shrub with blood-red colored juice in the cut stem. The leaves were glabrous, oblong-elliptic or ovalate, shortly acuminate apex, rounded, sometimes acutely cuneate base. The flowers were greenish-yellow, the fruit is a follicle, linear 17–31 cm long, the plant can be found growing in the rainforest and deciduous forest belt, known to the Yorubas as "Paran Pupa" and to the Hausas as "Gangnamau" (Iwu, 1993) and is found in secondary forest from Nigeria, Ghana to Senegal (Dokosi, 1998). A concoction of the stem and roots serve as herbal medicine in Ghana, West Africa to treat malaria, rheumatism and venereal disease (Boye-Yiadom, 1979; Boye and OkuAmpofo, 1983). The aqueous extract of *C.sanguinolenta*is a known antimalarial herbal medicine in many West African sub-regions (Boye and Ampofo, 1993), and there were several reports on the antimalarial activity of its major alkaloid Cryptolepine (CLP) (Kirby *et al*., 1995; Wright *et al*., 1996).

2. Materials and Method

2.1 Experimental Animals

Thirty-Five (35) Wistar male rats, with an average weight of 150g were obtained from the Animal Laboratory, Department of biochemistry, University of Ilorin,Ilorin, Kwara State. The rats were acclimatized in well ventilated steel cages and were maintained under standard laboratory conditions with free access to feed and water. Fresh *C. sanguinolenta* stems were gotten from Gegele Herb Market, Ilorin, Kwara state, identified, and authenticated at the Herbarium Unit of Department of Plant Biology, University of Ilorin, Kwara State, Nigeria with voucher number: UILH/101/1184.

2.2. Escherichia coli

Escherichia coli was cultured, isolated and Purified at the Microbiology Department laboratory, University of Ilorin, Kwara State.

2.3 Reagents and Assay Kits

All assay kits were obtained from Randox Laboratory Limited, Co-Antrim, United Kingdom. All other chemical reagents were prepared in the laboratory.

3. Methodology

3.1 Depyrogenation

All glass wares were depyrogenated by incubating inoven at 250ºC for 30 min.

3.2. Preparation of Endotoxin from purified e. coli

Sufficient quantity of purified *E. coli* was inoculated in250 ml of 5% Dextrose and mix well. 2ml of the Dextrosesolution was picked for initial microbial load sampling.Dextrose solution containing *E. coli* was then incubatedfor 370C for 48 hour. Two (2) ml sample was used for finalmicrobial load testing. After then, it was corked tightlyand sterilized in an autoclave for 21 min. Final samplecontain the pyrogenic solutions (endotoxin stock).

3.3. Determination of Endotoxin Content of theSolutions

To confirm the presence of endotoxin in the mixtureproduced, pyrogen test was carried out using the rabbittest method (Radhakrishna, 2010). The actualconcentration of endotoxin was estimated using astandard curve (Dalmora*et al*., 2004) correlatingtemperature rise and endotoxin content in injectedsolutions.

3.4 Animal Grouping and Treatments

Thirty-five Wistar rats will be randomly divided into 7 groups (A-G) of five rats each, and subjected to different treatments, the doses of plant extracts were determined from the LD50 results in the toxicity assessment of *C. sanguinolenta* (Ansah C. *et al*.,2008). Administration was done for 21 days, the First 7 days being exclusively Extract Administration to Group D-G, and the next 14 days' co-administration.

3.6. Qualitative Phytochemical Screening

Phytochemical analysis of the extract was carried out using the method described by Odebiyi and Sofowora (1978) for the

detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

3.7. Preparation of Serum
At the end of 21 days of the administration the animals were sacrificed and the serum, kidney and liver was obtained. The blood samples collected were allowed to clot and then centrifuged at 3,000 xg for 5 min for serum preparation using a table centrifuge. The clear supernatant was picked and then diluted with distilled water for biochemical analysis.

3.8. Histological Studies
The Liver and kidney were isolated in one rat from each group, fixed in 10% (v/v) formalin, dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene and embedded in paraffin wax (melting point 56ºC) (Krause, 2001). Tissue sections were prepared according to the method described by Drury and Wallington (1980) and stained with haematoxylin/eosin (H&E). The processed histology slides were read with a light microscope. Photomicrographs of the liver and kidney were captured at x400.

3.9. Biochemical Assays
Determination of alkaline phosphatase (ALP), Acid phosphatase (ACP) determined by the method of Wright *et al* (1972) [28], 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) [7].

Superoxide dismutase (SOD) was assayed as reported by (Mistra and Fridovich, 1975).Catalase was assayed according to the procedure described by Beers and Sizer (1952).The concentration of MDA was quantified according to the method of (Nelson, 2004).

3.10. Statistical Analysis
The results were expressed as mean ± SEM of four determinations. All results were statistically analyzed using one-way ANOVAand Duncan post hoc test. Difference between group means were considered to be significant at P<0.05 using Graphpadprism 6®.

4. Results
4.1. General Observation
Co-administration of C. sanguinolenta and endotoxin orally to rats within the 2nd week did not physically affectthe activity of the animals. Activity was however reduced in the last 7 days although they did not resist the co-administered sample. Food and water intake reduced in Groups C, E, F and G. there was no mortality case in the cause of the 21 days of administration and co-administration.

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 no significant (P<0.05) difference in serum specific ALT activity of the negative group and test groups administered C. *sanguinolenta* 50mg/kg bw in a dose dependent manner compared to the control, however there was a significant increase in serum specific ALT activity of Standard group and test groups administered *C. sanguinolenta* 25mg/kg bw compared with the control. Also there was a significant increase in serum specific ALT of test groups administered *C. sanguinolenta* 50 and 100 mg/kg bw in a dose dependent manner compared to the control.

4.2.2 Aspartate Transaminase (AST)
Oral administration of endotoxin led to a significant (P<0.05) decrease in serum specific AST activity of test groups administered *C. Sanguinolenta*50 and 100 mg/kg bw in a dose dependent manner compared to the standard and the control. However, there was a significant (P<0.05) increase in specific AST activity of groups administered *C.sanguinolenta* 25 mg/kg bw compared to the control. Also there was no significant (P<0.05) difference in serum specific AST activity of the positive group compared to the control.

4.2.3Alkaline Phosphatase (ALP)
Oral administration of endotoxin led to a significant (P<0.05) decrease in serum specific ALP activity ofstandard group and negative control group.However, there was asignificant (P<0.05) increase in the serum specific ALP activity of test group administered*C. Sanguinolenta*25mg/kg bw, compared to the control, but a significant decrease in the serum specific ALP activity of the test groups administered *C. Sanguinolenta*50 and 100mg/kg bw compared to the control.

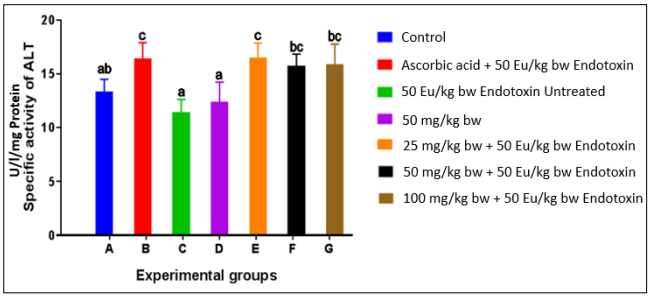

Fig 1: Specific activities of Alanine Aminotransferases (ALT) in the serum of rats orally co-administered Aqueous extract of C. sanguinolenta and E. coli endotoxin

Each values were a mean of Four ±SEM, values with different superscripts were significantly different (P<0.05)

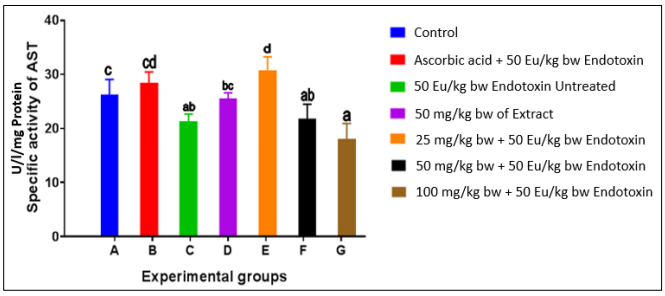

Fig 2: Specific activities of AspartateTransaminase (AST) in the serum of rats orally co-administered Aqueous extract of C. sanguinolenta and E. coli endotoxin

Each values were a mean of Four ±SEM, values with different superscripts were significantly different (P<0.05)

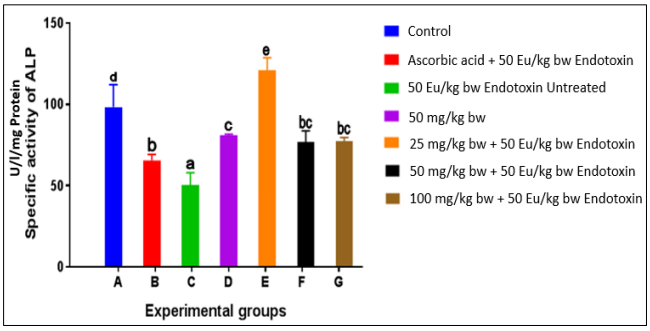

Fig 3: Specific activities of Alkaline Phosphatase (ALP) in the serum of rats orally co-administered Aqueous extract of C. sanguinolenta and *E. coli* endotoxin

Each values were a mean of Four ±SEM, values with different superscripts were significantly different (P<0.05)

4.2.4 Superoxide Dismutase (SOD)

Oral administration of endotoxin led to a significant (P<0.05) decrease in serum specific SOD activity of positive control group and test groups administered *C. sanguinolenta* 25, 50 and 100 mg/kg bw in a dose dependent manner compared to the standard and the control. However,there wasa significant (P<0.05) increase in serum specific SOD activity of Negative control group and a decrease in the standard group compared with the control group.

4.2.5 Catalase (CAT)

Oral administration of endotoxin led to no significant (P<0.05) difference in serum specific CAT activity of standard group, compared to the control. However, there was a significant (P<0.05) decrease in serum specific CAT activity ofnegative control, positive control and test groups administered C. sanguinolenta25 mg/kg bw in a dose dependent manner compared to the control. More so there was a significant increase in serum specific CAT activity of test group administered 50 and 100mg/kg bw compared with the control.

4.2.6 Reduced Glutathione (GSH)

Oral administration of endotoxin led to no significant (P<0.05) difference in serum GSH concentration of test group administered C. sanguinolenta 25mg/kg bw in a dose dependent manner compared to the control, However, there was a significant (P<0.05) decrease in serum GSH concentration of negative control, positive control and the standard group. Also there was a significant decrease in serum GSH concentration activity of test group administered 50 and 100mg/kg bw compared with the control.

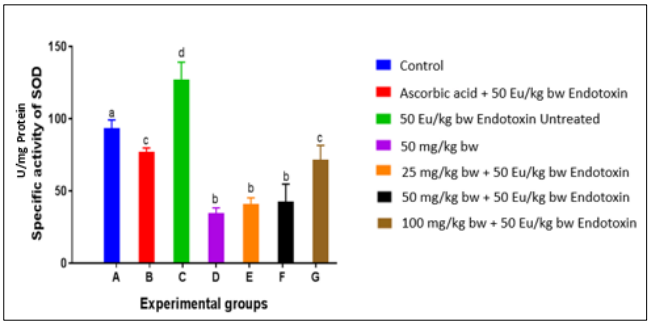

Fig 4: Specific activities of Superoxide Dismutase (SOD) in the serum of rats orally co-administered Aqueous extract of *C. sanguinolenta* and *E. coli* endotoxin

Each values were a mean of Four ±SEM, values with different superscripts were significantly different (P<0.05)

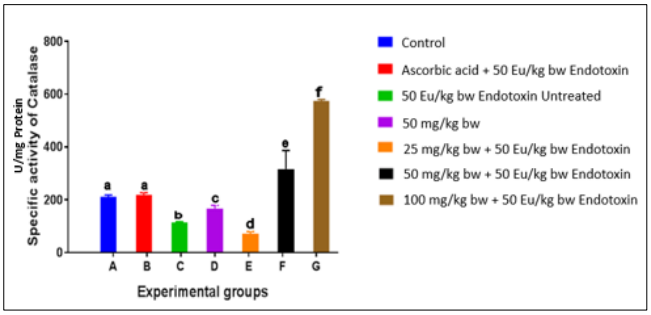

Fig 5: Specific activities of Catalase (CAT) in the serum of rats orally co-administered Aqueous extract of *C. sanguinolenta* and *E. coli* Endotoxin

Each values were a mean of Four ±SEM, values with different superscripts were significantly different (P<0.05)

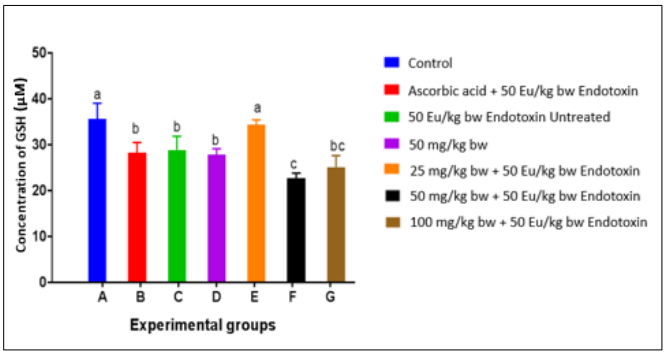

Fig 6: Concentration of Reduced Glutathione (GSH) in the serum of rats orally co-administered Aqueous extract of *C. sanguinolenta* and *E. coli* Endotoxin

Each values were a mean of Four ±SEM, Values with different superscripts were significantly different (P<0.05)

4.2.7. Malondialdehyde (MDA)

Oral administration of endotoxin led to no significant (P<0.05) difference in level of MDA of the standard group compared to the control,However, there was a significant (P<0.05) increase in level of MDA of thetest group administered 25mg/kg bw compared with the control and a significant decrease in the level of MDA of the test groups administered 50 and 100 mg/kg bwcompared with the control, the negative control also showed a significant increase in level of MDA when compared to the control, while the positive control shows a significant decrease in level of MDA when compared to the control.

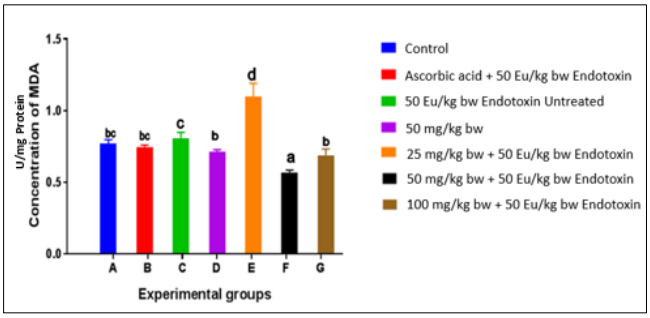

Fig 7: Concentration of Malondialdehyde in the serum of rats orally co-administered Aqueous extract of C. sanguinolenta and *E. coli* Endotoxin

Each values were a mean of Four ±SEM, Values with different superscripts were significantly different (P<0.05)

4.2.8. Creatinine

Oral administration of endotoxin led to no significant (P<0.05) difference in concentration of creatinine of the negative control compared to the control. However, there was a significant (P<0.05) increase in the concentration of creatinine in the standard group, positive control and test group administered 50 and 100mg/kg bw compared with the control and a significant decrease in the concentration of the test groups administered 25mg/kg bw compared with the control.

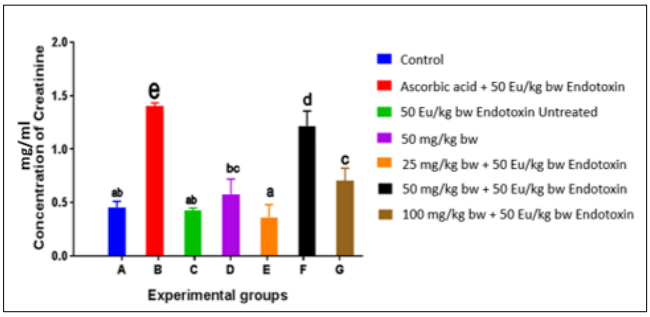

Fig 8: Concentration of Creatinine in the serum of rats orally co-administered Aqueous extract of *C. sanguinolenta* and *E. coli* Endotoxin

Each values were a mean of Four ±SEM, Values with different superscripts were significantly different (P<0.05)

4.2.9. Urea

Oral administration of endotoxin led to no significant (P<0.05) difference in concentration of Urea of the negative control, standard, and positive control compared to the control.Also, there was nosignificant (P<0.05) difference in the concentration of urea in the test group administered 25mg/kg, 50mg/kg and 100 mg/kg bw compared with the control. In summary there was no significant (P<0.05) difference in concentration of urea ofall the groups when compared with the control.

4.3 Histopathology

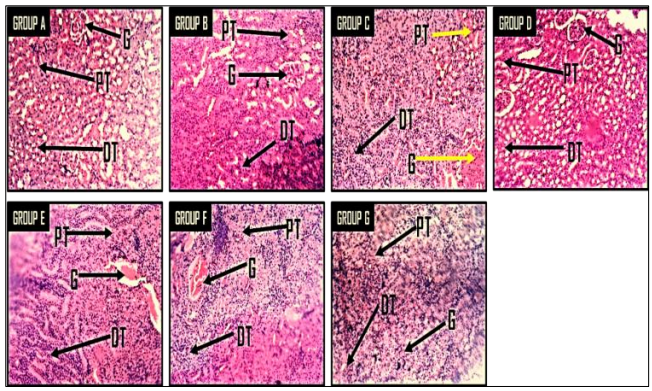

Fig 9: Photomicrograph showing the histo-morphology of the kidney across experimental animals stained with H&E at x100 magnification

Group A (control), Group B (Ascorbic Acid), Group C (Endotoxin), Group D (Positive control), Group E (25mg/kg bw of extract), Group F (50 mg/kg bw of extract), Group G (100 mg of extract). Normal histo-architecture can be seen in Group A, B and D as seen with the presence of normal glomerulus, distal and proximal tubules (Black arrows). Fragmented and inflamed glomeruli and proximal tubules (yellow arrow) were present in the Group C which depict cytotoxicity and disrupted normal kidney functioning. The group treated with 25 and 50mg/kg of extract shows slight significant improvement as there was normal cytoarchitecture with no disruption of both the distal and proximal tubules, however there was slight inflammation of the glomerulus. Group G shows histo-architecture that has no disruption and its similar to that of Group A, B, and D. (PT= Proximal tubules, DT= Distal tubules and G= Glomerulus).

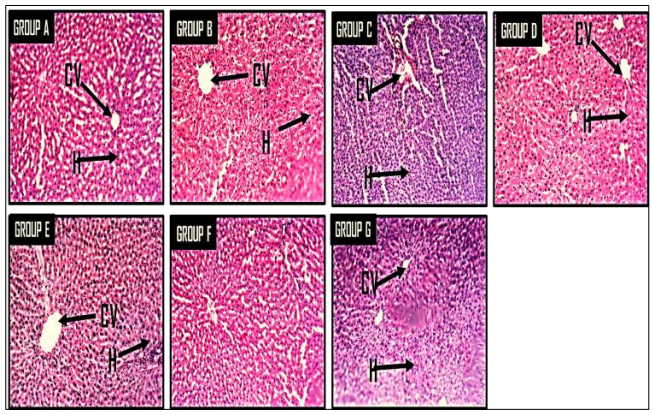

Fig 10: Photomicrograph showing the histo-morphology of the liver across experimental animals stained with H&E at x100 magnification

Photomicrograph showing the histo-morphology of the liver across experimental animals stained with H&E at x100 magnification. Group A (control), Group B (Ascorbic Acid), Group C (Endotoxin), Group D (Positive control), Group E (25mg of extract), Group F (50mg of extract), Group G (100mg of extract). Histology of the liver in Group A, B, and D shows normal central vein (CV) and hepatocytes (H). Experimental animals treated with endotoxin (Group C), reveals fragmented cytoarchitecture with disrupted and inflamed hepatocytes and central vein and accumulation of fatty substances. Groups treated with different doses of extract (Group E, F and G) after endotoxins have been administer shows histo-morphology that is similar to that of Group A, B and D.

5. Discussion

Consumption of Endotoxin occurs through various means. It can occur through ingested food and other substances such as oral drugs which at times might be contaminated with pyrogen however little effort is being made toward constant checking of endotoxin units in most food and pharmaceutical industry. In many homes in Nigeria for example, consumption of endotoxin can be a daily occurrence due to the constant re-heating of leftover foods to destroy the contaminating Gram negative bacteria and make the food more palatable to eat. However, lipopolysaccharides which is responsible for the pathological consequences of Gram-negative bacteria (Such as *E. coli*) which can be found on their cell wall become released therebyeliciting the deleterious effects of the endotoxin. Systematic exposure to endotoxin has been reported to cause 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 Failure Syndrome (MODS) (Dinarello, 2000) [12]. Co-administration of *Cryptolepis sanguinolenta* and endotoxin orally to rats within the 2nd week did not physically affect the activity of the animals; Activity was however reduced in the last 7 days although they did not resist the co-administered sample. Food and water intake reduced in Negative control group, and all 3 experimental groups receiving different doses of the aqueous extracts. There was no mortality case in the cause of the 21 days of administration and co-administration. In the course of this study, the various biomarkers used showed varying responses to the endotoxin administered via oral route. The liverbeing the mostimportant organ for endotoxin accumulation and also the largest organ containing monocytes/macrophage system. The degraded endotoxin by the macrophages is excreted from the liver, into the gut, bile and detected in faeces. Numerous metabolic activities in liver were altered as a result of endotoxin poisoning. Such changes include depletion of carbohydrate reserves, inhibition of particular enzyme inductions, and also inhibition of gluconeogenesis (Crespo *et al.,* 2009) [10]. Liver function tests (LFTs) were 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) [16]. Alanine transaminase (ALT) and Aspartate transaminase (AST) were enzymes found in the liver and have been widely used for diagnostic purposes as an indicator of liver damage (Whitehead *et al.,* 1999) [27]. 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, aqueous extract of *C.sanguinolenta* was extracted, freeze dried and diluted to different concentrations, then co-administered alongside endotoxin cultured from *E. coli*, and their temperature changes was monitored for the period of co-administration.This study evaluated the preventive effect of the oral administration of *C. Sanguinolenta*on oral endotoxin induced oxidative stress in wistar rats. Estimation of parameters in the serum and organs were done. The pyrogen level of the stock endotoxin was detected with the aid of rabbit pyrogen test. The significant temperature increase in the rabbits was 2.27^{o}C which ascertained that the endotoxin stock was Pyrogenic (Dalmora*et al.,* 2010), Temperature increase leads to fever/sepsis, which triggers induced ROS and subsequently leads to oxidative stress and damage. At the end of the pyrogen test the concentration of the stock solution was calculated to be 1962 Eu/kg (Table 1). The result of responses gotten from the mean of total responses per group for every 7 days of co-administration suggests that the aqueous extract of *C.sanguinolenta* is propyrexia. Superoxide dismutase helps protect the systemic tissues by converting superoxides into H_2O_2 and O_2 in a dismutase reaction, hence mopping up oxygen free radicals. Endotoxin induce macrophages which goes on to trigger induced Nitric Oxide Synthase, and production of NO which leads to the production of ROS i.e. H_2O_2 and O_2^-, this triggers the production of SOD and also Catalase with the aim of counteracting the ROS. It has been suggested that increase in antioxidant enzymes may represent a compensatory up regulation in response to increase oxidative stress (T. Atli*et al.,* 2004). There was a decrease in the serum SOD in all test groups with extract concentrations 25, 50, 100mg/kg body weight (bw), and positive control when compared to the control. This result suggests that the endotoxin was successfully mopped up and there was no need for over production of SOD. 25mg/kgbw of the extract test group also recorded a decreased activity of catalase, whereas at 50 and 100mg/kgbw of the extract, there was a significant increase compared to the control showing the effect of endotoxin triggered ROS generation which led to a high synthesis of catalase which may or may not have successfully counteracted. Reduced Glutathione is an extremely important cell protectant as it mops up hydroxyl free radicals, ROS and RNS. It's the essential cofactor for enzymes that require thiol reducing equivalent and helps keepredox sensitive active sites in the necessary reduced state.There was no significant difference in the GSH activity of the 25mg/kg bw of the extract group when compared to the control suggesting the extract was able to keep the system stable after endotoxin co-administration. There was however a decrease in the GSH activity of 50 and 100mg/kgbw of the extract group compared to the negative control and also the control group. This suggests the concentrations successfully mopped up the Free radicals hence the activity need not be increased as there was little or no free radical to counter. The concentration of Malondialdehyde (MDA) is a Lipid Peroxidation marker, hence its increase in blood serum can be associated with increased ROS generation as endotoxin have been known to induce oxidative damage as a result of Peroxidation of membrane lipids (Halliwell2006) [15]. There was a significant increase in the concentration of MDA in 25mg/kgbw of the extract group translating to an inability of the extract to mop up the generated ROS and prevent peroxidation, whereas

there was a significant decrease in concentration of MDA in groups administered 50 and 100mg/kgbw of the extract which will most likely mean that at these concentrations the extract was able to counteract against the endotoxin and prevent peroxidation of the membrane lipids. The ALT activity of the Liver resulted in a significant increase in serum ALT activity of the test group 25, 50 and 100mg/kg bw of the extract compared to the control. This increase in serum ALT activity could be as a result of over production of ALT in the liver r there have been leakages as a result of alterations in the integrity of the membrane, causing ALT from the Liver to leak into the serum. This study is quite similar to a study previously reported by Abd el Rahman *et al.*, (2005) [1] where he demonstrated that activities of endotoxin lead to an Increase in the ALT found in the serum. In the case of serum AST activity, there was a significant decrease in the AST activity of test groups 50 and 100mg/kg bw of the extract compared to the standard and control. This can be as a result of a positive effect of extract at the aforementioned doses in preventing the co-administered endotoxin from eliciting a damage, could also be that the extracts helped reinforce the immune response of the animals in the concerned groups hence limiting damages done to the liver and also enhancing cellular repair. At 25mg/kgbw of the extract the activity of AST increased compared to the control and this can be as a result of the negative effect of the endotoxin leading to oxidative tissue damage causing a leakage and the extract concentration not being enough to counteract the endotoxin. ALP is a marker enzyme that is associated with the plasma membrane and is assayed frequently to assess the structural integrity of the plasma membrane of the liver and can shed more light on whether the liver was damaged structurally or not (Akanji 1993) [3]. There was a significant increase in serum ALP in test group 25 mg/kg bw of the extract and this could be due to a prolonged systemic exposure of the organs to endotoxin causing a degradation and hence lysis of the plasma membrane releasing the enzyme into the blood (Reed 1999) [23]. A significant decrease in serum ALP activity of test groups 50 and 100mg/kg bw of the extract can mean that the effect of the endotoxin is been counteracted upon by the kuffer cells aided by the extract at those concentrations preventing the liver from losing any structural integrity. Creatinine a nitrogenous waste product is a cyclic derivative of creatine and is filtered in the kidney and any alteration in the ability of the kidney to filter will lead to accumulation of creatinine in the blood. There was a significant increase in the level of serum creatinine in test groups administered 50 and 100mg/kgbw of the extract and this can be associated with prolonged exposure of the kidney to endotoxin causing kidney dysfunction and leading to accumulation of nitrogenous waste in the blood serum. A decrease in the level of serum creatinine in group administered 25mg/kgbw of the extract can mean at lower dose the extract is able to keep the kidney functions at optimum condition as opposed to higher doses. Urea is the main end product of protein metabolism and from the liver the urea cycle ends with conversion of amino acids to urea which is then excreted in the urine, after glomerular filteration in the kidney. When there is a dysfunction in the kidney function or renal disease, this will cause a retention of urea in the blood serum (Rajan 2012) [26]. There was no significant difference in all test groups when compared to the control and this denotes that the administered endotoxin was not enough to have elicited any renal damage or impair the urea cycle, or that the extract at all concentration was sufficient enough to prevent any effect of the endotoxin on the kidney functions.

6. Conclusion

The results of the study support the preventive effects of the administration of *Cryptolepis sanguinolenta* prior to oral administration of *Escherichia coli* endotoxin as the extract at doses between 50 and 100 mg/kg bw of the extract prevented the organs from getting overridden by reactive oxygen species', as when compwere with the untreated group, the negative effect on the liver and kidney function indices were confirmed and also visualized in the results of the Histopathology. This suggest that the antioxidant properties of *Cryptolepis sanguinolenta*is sufficient and capable of protecting and counteracting against the deleterious oxidative effects of endotoxin..

7. Reference

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