

Hepatoprotective effects of ethanolic leaf extract of *Emilia coccinea* in doxorubicin-induced liver injury in Wistar rats

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Abstract

Background

Doxorubicin (DOX) can cause liver injury, and *Emilia coccinea* has a history of traditional medicinal use. This study evaluated the phytochemical profile and potential hepatoprotective effects of its ethanolic leaf extract in rats. Methods: Qualitative phytochemical screening and acute toxicity observations were conducted. Twenty-one Wistar rats were assigned to normal-control, DOX-control, and DOX-plus-extract groups ($n = 7$); serum liver enzymes and biochemical markers and liver histology were assessed. Results: The extract tested positive for alkaloids, flavonoids, saponins, tannins, and steroids. Compared with the DOX-control group, the extract group had lower AST and ALT activities ($p < 0.05$); ALP, bilirubin, total protein, and albumin differences were not significant in the reported comparisons. Histological findings described less tissue damage in the treated group. Conclusions: The findings suggest a possible protective effect of the extract in this rat model, but should be interpreted cautiously pending confirmation of study details and underlying data.

1. Introduction

There is a growing interest in the pharmacological evaluation of various plants used in the system of medicine. Historically, plants have been used in folk medicine to treat various diseases and most of the plants used are rich sources of antioxidants. Many researchers have examined the effects of plants used traditionally by indigenous people to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience by discovering the modes of actions of these plants and the liver is a major organ saddled with almost all biochemical processes including xenobiotic metabolism. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. But the liver is constantly abused and exposed to poor drug usage, excessive alcohol and prescribed over the counter drugs which can eventually lead to various liver ailments like hepatitis, jaundice, cirrhosis etc.. Thus, liver diseases are some of the most fatal diseases in the world today. Modern medicine has little to offer for the alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders

1.1. Description Of *Emilia Coccinea*

Emilia coccinea commonly known as tassel flower is an annual herb commonly found throughout the plain of the central Africa and in dry up areas up to 2000m altitude in the eastern of Africa (Olorede, 1984). This species belong to the genus of *Emilia* represented by about 100 species, with 50 of them found in Africa. A captivating little plant for the front of the border, tassel flower produces small, scarlet-orange yellow pompos which, when viewed from a distance, seem like they are floating in the air. The flowers cluster at the top of wiry stems that rise from a basal rosette of blue-green leaves. Plants occasionally self-sow when sited in a good location. Its delicate and airy nature looks best with bowed-leaves or showy plants in the background, creating a see through effect (Chillendon, 1956). An ideal

plant for hot dry areas and costal soils. *E.coccinea* known as yellow tassel flower or shaving bush is locally known as Ntiele (Igbo), Aninge (tiv), Ichokolo (Idoma), Odundunodo (Yoruba) in Nigeria (Agishi, 2010). In traditional medicine, the plant is used for the treatment of ulcers, gonorrhea, measles, cough and convulsion in children (Edeoga et al., 2005). The leaf sap is used to treat vertigo (Oliver, 1960). The crushed green leaves are used for the treatment of wounds, sores and sinusitis while dried powdered leaves is used to manage sores. The leaf is used for treatment of epilepsy in Ivory Coast and in Congo for managing gonococcal infection, hernia and for syphilis. The dry leaves are also used to treat jaundice, abdominal pains and gastritis.

1.2. Oxidative Stress

Oxidative stress is defined as excessive generation of reactive oxygen species such as superoxide and hydrogen peroxide, beyond the body's innate ability to counter their noxious effects.

1.3. The Role Of Antioxidants And Oxidative Stress In Liver Diseases

In biological system, oxygen and nitrogen based radicals are two types of free radicals. Oxygen free radicals such as superoxide, hydroxyl radicals and peroxy radicals with the addition of non-radicals such as hydrogen peroxide, hypochlorous acid and ozone, are known as reactive oxygen species (ROS), which are generated during the metabolism process of oxygen. Reactive nitrogen species (RNS), including nitrogen based radicals and peroxynitrite, are derived from nitric oxide and superoxide via double nitric oxide synthase and nicotinamide adenine dinucleotide phosphate oxidase respectively. Due to their special chemical characteristics, ROS/RNS can initiate lipid peroxidation, cause DNA strand break and indiscriminately oxidize all molecules biological membranes and tissues resulting in injury. However, since the body is able to remove ROS/RNS to a certain degree, these reactive species are not necessarily a threat to the body under physiological conditions. As a matter of fact ROS are required at a certain level in the body to perform its important physiological conditions. The generation of ROS is a natural part of aerobic life, which is responsible for the manifestation of cellular functions including signal transduction pathways, defense against invading microorganisms and gene expression to the promotion of growth or death. Oxidative/nitrosative stress represents the bodies' imbalance in the production and the elimination of reactive oxygen and nitrogen species as well as decreased production of antioxidants. In terms of oxidative stress, in specific physiological conditions, it is actually useful. For example, it could strengthen biological defense mechanisms during appropriate physical exercise and ischemia, and induce apoptosis to prepare the birth canal for delivery. However, this is confined to particular situations, and in most other cases, large levels of ROS and oxidative stress will induce cell death through necrotic and/or apoptotic mechanisms, leading to cellular and tissue injury.

Liver is a major organ attacked by ROS (Sanchez-Valle et al., 2012). Parenchymal cells are primary cells subjected to oxidative stress induced injury in the liver. The mitochondrion, microsomes and peroxisomes in parenchymal cells can produce ROS, regulating on PPAR α (peroxisome proliferator activated receptor α), which is mainly related to the liver fatty acid oxidation gene expression. Moreover, Kupffer cells, hepatic stellate cells and endothelial cells are potentially more exposed or sensitive to

oxidative stress-related molecules. A variety of cytokines like TNF- α can be produced in Kupffer cells induced by oxidative stress, which might increase inflammation and apoptosis. With regard to hepatic stellate cells, the proliferation and collagen synthesis of hepatic stellate cells is triggered by lipid peroxidation caused by oxidative stress (Wu and Cederbaum, 2009). In mammals, a sophisticated antioxidant system has been developed to maintain the redox homeostasis in the liver. When the ROS is excessive, the homeostasis will be disturbed, resulting in oxidative stress, which plays a critical role in liver diseases and other chronic and degenerative disorders. The oxidative stress not only triggers hepatic damage by inducing irretrievable alteration of lipids, proteins and DNA contents and more importantly, modulating pathways that control normal biological functions. Since these pathways regulate genes transcription, protein expression, cell apoptosis, and hepatic stellate cell activation; oxidative stress is regarded as one of the pathological mechanisms that results in initiation and progression of various liver diseases, such as chronic viral hepatitis, alcoholic liver diseases and non-alcoholic steatohepatitis (Singal et al., 2011). It has also been suggested that there are complicated cross-talks among pathological factors, inflammation, free radicals and immune responses (Medina and Moreno-Otero, 2005). Moreover, systemic oxidative stress arising during liver disease can also cause damage to extra- hepatic organs, such as brain impairment and kidney failure (Palma et al., 2014). It was suggested systemic oxidative stress might be a significant “first hit”, acting synergistically with ammonia to induce brain edema in chronic liver failure (Bosoi et al., 2012). With regard to kidney failure, systemic oxidative stress is considered to play a critical role in the pathophysiology of several kidney diseases (Valente et al., 2012).

2.2.2 OXIDATIVE STRESS CAUSED BY DRUGS

The liver is a frequent target of drug toxicity. Reactive oxygen and nitrogen species have been proposed as early events in drug-induced hepatotoxicity and as indicators of hepatotoxic potential (Videla, 2009). A study of paracetamol-induced hepatic injury in mice reported increased lipid peroxidation markers and reduced antioxidant enzyme activity (Mladenovic et al., 2009). Oxidative stress generated by anticancer drugs, including doxorubicin, paclitaxel and docetaxel, has been reported in rat liver; combined administration produced greater changes in oxidative stress-related measures than individual agents (Pieniazek et al., 2013). Nimesulide treatment in mice was associated with increased serum ALT, AST, ALP and bilirubin and decreased liver antioxidant enzyme activity (Nguyen et al., 2015).

Although hepatotoxicity induced by various drugs in humans has been demonstrated in a great number of clinical trials, report concerning the role of oxidative stress in patients with drug induced liver disease is limited by far. For example, mitochondrial dysfunction and DNA are found to be critical events in the underling mechanism of paracetamol induced hepatotoxicity in patients, which is supposed to partly attribute to oxidative stress, but, accurate and direct evidence to show the status and role oxidative stress in patients is lacking. As a matter of fact, currently, in addition to animal model study, the investigation of hepatotoxicity induced by drugs is mainly based on results of retrospective study, whereas there are few clinical studies with large numbers of patients. Moreover, models using human cells have been attempted to mimic pathogenesis of drug induced hepatotoxicity in humans. Overall,

clinical data and appropriate experimental model, which could closely resemble human pathophysiology, is critical for future study of antioxidant treatment for hepatotoxicity caused by drugs (Stine and Chalasani, 2015).

1.4. The Liver

The liver is the largest organ of the human body weighing approximately 1500g and is located in the upper right corner of the abdomen on top of the stomach, right kidney and beneath the diaphragm. The liver performs more than 500 vital metabolic functions. It is involved in the synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the bloodstream. It is also involved in the production of bile excreted in the intestinal tract. Bile serves as a filter that separates out harmful substances from the bloodstream and excretes them. It regulates blood levels of amino acids. Liver parenchyma serves as a storage organ for several products like glycogen, fats and fat soluble vitamins. Smooth endoplasmic reticulum of the liver is the principal metabolic clearing house for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins and exogenous substances like drugs and alcohol. Thus, the central role played by the liver in the clearance and transformation of chemicals exposes it to toxic injury.

1.5. Clinical Significance Of Liver Enzymes

The following are common biomarkers for clinical diagnosis of liver diseases; ALT, AST and ALP.

1.6. Alanine Amino Transferase (Alt)

Alanine amino transferase or serum glutamic pyruvic transaminase (SGPT) activity is the most frequently relied biomarker of hepatotoxicity. It is a liver enzyme that plays an important role in amino acid metabolism and gluconeogenesis. It catalyzes the reductive transfer of amino group from alanine to alpha ketoglutarate to yield glutamate and pyruvate. Normal levels in humans are in the range of 5-50 U/L. Elevated level of this enzyme is released during liver damage. The estimation of this enzyme is specific for detecting liver abnormalities since it is primarily found in the liver (Dufour et al., 2000).

1.7. Aspartate Amino Transferase (Ast)

Aspartate amino transferases or Serum Glutamic Oxaloacetate Transaminase (SGOT) is another liver enzyme that aids in producing proteins. It catalyzes the reductive transfer of amino group from aspartate to alpha ketoglutarate to yield oxaloacetate and glutamate. Besides liver, it is also found in other organs like heart, muscles, brain and kidney. Injury to any of these tissues can cause an elevated blood level (Nathwani et al., 2005). Normal levels in humans are in range of 7-40 U/L. It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker for hepatocellular injury as it can also signify abnormalities in heart, muscle, brain or kidney (Dufour et al., 2000). The ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage (Nathwani et al., 2005).

1.8. Alkaline Phosphatase (Alp)

Alkaline phosphatase is a hydrolase enzyme that is eliminated in the bile. It hydrolyses monophosphates at an alkaline PH. It is particularly present in the cells which line the biliary ducts of the liver. It is also found in other organs like the bone, placenta, kidney and intestine. It may be elevated if bile excretion is inhibited by liver damage. Hepatotoxicity leads to elevation of the normal values due to the body's inability to excrete it through the bile due to congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the gallbladder through the pancreas that empty into the duodenum. Increase in ALP with little or no increase in ALT is primarily a sign of hepatobiliary effects of cholestasis. In humans, increased ALP levels have been associated with drug induced cholestalsis (Wright and Vandenberg, 2007).

Other important biochemical parameters for assessing liver integrity are; Total bilirubin, albumin and total protein.

1.9. Total Bilirubin

Bilirubin is an endogenous anion derived from the regular degradation of hemoglobin from the red blood cells and excreted from the liver into the bile. It is a chemical normally present in the blood in small amounts and used by the liver to produce bile. Normal bilirubin levels in the blood range between 0.2 to 1.2 mg/dl. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, causing build-up of bilirubin in the blood and extracellular fluid. Serum bilirubin could be elevated if the serum albumin increases and the bilirubin shifts from tissue sites to circulation. Increased levels of bilirubin may also be due to hepatic clearance and this may lead to jaundice and other hepatotoxicity symptoms. In acute human injury, total bilirubin can be a better indicator of the disease severity compared to ALT (Dufour et al., 2001). Bilirubin is measured as total bilirubin and direct bilirubin. Total bilirubin is a measurement of all the bilirubin in the blood while direct bilirubin is a measurement of water soluble conjugated form of bilirubin made in the liver and its normal range is 0-0.3 mg/dl. Indirect bilirubin is calculated by the difference in the total and direct bilirubin as a measure of unconjugated fraction of bilirubin.

1.10. Albumin

It is the main protein in the blood and is made by the liver. Hepatotoxicity leads to decrease in albumin production (Thapa and Walia, 2007). It can be used as a supplementary test for hepatic biosynthetic dysfunction.

1.11. Total Protein

The estimation of total protein in the body is helpful in differentiating between a normal and a damaged liver function as majority of plasma proteins like albumins and globulins are produced in the liver (Thapa and Walia, 2007). Normal range of total protein is 6.0 to 8.3g/dl. Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury (Thapa and Walia, 2007).

1.12. Drug (Doxorubicin Hydrochloride)

Doxorubicin is an anthracycline glycoside antibiotic that possesses a potent and broad spectrum antitumor activity against a variety of human solid tumors and hematological malignancies. The DOX antitumor effects include mechanisms related to alterations of DNA and the production of free radicals. However its use in chemotherapy has been limited largely due to its diverse toxicities, including cardiac, hepatic, hematological and testicular toxicity.

Several studies have shown that the combination of the inflammatory process, free radical oxidative stress and lipid peroxidation are frequently associated with liver damage, induced by toxic agents such as DOX. Persistent and irreversible liver damage has been a side effect of DOX therapy. It has been observed that there is an increase in apoptotic processes in liver tissue after a single dose of DOX. DOX mediated hepatotoxicity includes focal damage in hepatocytes, vascular damage and steatosis. Oxygen free radicals produced during the metabolic activation of DOX may have effects on the heart muscles, which is provided with poor mechanisms of detoxification of such species. DOX is likely to have toxic effects on the liver by increasing the levels of superoxide dismutase (SOD), catalase(CAT) and glutathione peroxidase enzyme in liver tissue of guinea pig.

It is known that DOX causes weight loss. Thus their prolonged use and over dosage cause death. Thus, drug cause disruption in basal metabolism by showing toxic effects especially in liver and heart tissues.

2. Materials and Methods

2.1. Plant Collection

The leaves of *Emilia coccinea* were obtained from the vegetation in Akpagher in Gboko local government area of Benue state. The plant was authenticated by a botanist in the department of botany, faculty of Sciences University of Agriculture Markurdi. The leaves were washed with tap water and dried at room temperature for about four days, away from sunlight to avoid possible damage to the phytochemical constituents.

2.2. Preparation Of The Plant Extract

The dry leaves were crushed and weighed. 240g of the dried leaves was macerated in 1.5litre of 80% ethanol, and then it was shook for 10mins and then allowed to stand for 72hours. The extract was first filtered with cotton wool, then with Whatman No 1 filter paper. The filtrate was evaporated to dryness by air drying at room temperature. The filtrate was later weighed and reconstituted in normal saline to the required dosage for administration.

2.3. Phytochemical Screening

Standard qualitative phytochemical screening was performed to identify bioactive chemicals (tannins, saponins, steroids, alkaloids, flavonoids and anthraquinones in the leaf extracts of *Emilia coccinea* using

standard procedures.

2.4. Test For Tannins

To 2.0ml of each extract, a few drop of 0.1% (w/v) ferric chloride were added; the appearance of blue precipitate indicates the presence of tannins.

2.5. Test For Saponins

To 2.0ml of each extract taken in a test tube, 5.0ml of distilled water was added and shook vigorously for 15 seconds and the extracts were allowed to stand for 10minutes. Formation of a stable foam (1cm) shows the presence of saponins.

2.6. Test For Steroids

Chloroform was added to 2.0ml of all plant extract. To this extract, 1.0ml of acetic anhydride was added, and 2.0ml of concentrated sulphuric acid was added along the side of the test tubes. Color formation at the junction was noted (from violet to blue). Appearance of blue-green color indicates the presence of steroids.

2.7. Test For Alkaloids

2.0ml of the extract was acidified with few drops of dilute hydrochloric acid and the 1.0ml of Dragendroff reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

2.8. Test For Flavonoids

1.0ml of the extract was acidified with few drops of concentrated sulphuric acid and then 2.0ml of dilute ammonia was added. The appearance of yellow color indicates the presence of flavonoids.

2.9. Test For Anthraquinones

To 1.0ml of the plant extract, 5.0ml of chloroform was added and shaken for 5minutes. The extract was filtered and the filtrate shaken with equal volume of 100% ammonia solution. Pink or violet red color indicates the presence of anthraquinones.

2.10. Acute Toxicity Study (Ld50)

The toxicity study was done using a dose-escalation method. Sixteen mice grouped into four groups of four mice per group. The mice of each group were administered with ethanol leaf extract of *Emilia coccinea* at varying doses of 1200, 1600, 1800 and 2000mg/kg body weight and monitored for signs and symptoms of toxicity.

Table 1: Experimental design for acute toxicity of *E.coccinea* using mice.

2.11. Experimental Animals

Twenty one rats (males and females) of albino wistar strain weighing 65-95g were used for study. The animals were divided into three groups of seven rats per group. They were obtained from the small animal unit, Central Diagnostic Division of National Veterinary Research Institute VOM, Plateau State. The animals were allowed two weeks acclimatization in the laboratory before commencement of the experiment. They were maintained in standard wooden cages and given standard animal feeds throughout the experimental period which lasted for 21 days. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). Animal care followed institutional animal-care guidelines. Fourteen animals were induced with doxorubicin (xmg/kg body weight) intraperitoneally to cause hepatic injury and were treated according to table 2 below. Treatment was done once daily for 21 days.

TABLE 2: Experimental design and Treatment Schedule for in-vivo Examination of E. coccinea extract in rat model.

2.12. The Collection Of Blood And Organs For Biochemical And Histological Analysis

At the end of the extract administration the rats were kept overnight fasting and then sacrificed after they were anaesthetized with chloroform and blood was collected. Serum was separated by the centrifugation and used to estimate the biochemical parameters which are the biomarkers of liver function tests. The livers were excised immediately from the animals, washed with cold normal saline and placed in sterile sample bottles containing 10% formalin at the end of the experiment. The tissue specimen was embedded in paraffin after being dehydrated in alcohol and subsequently cleared in xylene. Five micrometer thick serial histological sections were obtained from the paraffin block and stained with hematoxylin and eosin. The sections were examined under light microscope to evaluate pathological changes and photomicrographs were taken.

2.13. Determination Of Serum Enzyme Assay And Other Biochemical Parameters Assessing Liver Integrity

Serum samples from each group were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, total bilirubin, and albumin using Randox kits (Cypress Diagnostics, USA).

2.14. Statistical Analysis

Data obtained were expressed as means ± standard error of means and analysed using the statistical package for social scientists (SPSS version 21) where applicable. Values at p<0.05 will be regarded as significant in comparison with appropriate controls.

Table 1. Acute toxicity study design.

GROUP	NUMBER OF MICE	TREATMENT DOSES
A	4	1200mg/kg
B	4	1600mg/kg
C	4	1800mg/kg
D	4	2000mg/kg

Table 2. Animal groups and treatment schedule.

GROUPS	DESCRIPTION	NO OF RAT
A	Positive control	7
B	Negative control(30mg/kg Dox)	7
C	Dox induced with immediate treatment(Dox + 1000mg/kg extract)	7

3. Results

The preliminary phytochemicals of the extract revealed the presence of alkaloids, saponins, tannins, steroids and flavonoids as shown in table 3.

The acute toxicity (LD50) of ethanol seed extract of *E. coccinea* at doses of 1200mg/kg, 1600mg/kg, 1800mg/kg and 2000mg/kg body weight recorded no mortality in lethal dose.

Table 3. Qualitative phytochemical screening of the ethanolic leaf extract of *Emilia coccinea*.

Phytochemical Constituents	<i>E.coccinea</i>
Alkaloids	+
Flavonoids	+
Anthraquinone Glycosides	-
Saponins	+
Tannins	+
Steroids	+

The results of the ethanol extract of *E. coccinea* on the enzyme and biochemical assays of wistar rats are present in table four and five below respectively.

Table 4. Serum liver enzyme activities in the study groups.

Group	AST (U/L)	ALT (U/L)	ALP (U/L)
A (normal control)	15.14 ± 0.67	12.17 ± 0.36	146.94 ± 7.60
B (negative control)	27.57 ± 1.21*	18.14 ± 0.26*	179.00 ± 2.41*
C (treatment)	14.14 ± 0.77 $\square$	14.29 ± 0.71 $\square$	169.57 ± 3.00 $\square$

Results are expressed in mean $\pm$ SEM (n=7); *significant (p<0.05) compared with normal control group, b significant (p<0.05) compared to negative control group, c not significant (p<0.05) when compared to negative control. In this work, ALT, AST, ALP activity were used to determine the protective effect of E.coccinea on DOX induced hepatotoxicity in Wistar rats and were interpreted as follows; Statistical evaluation revealed that, for ALP levels, the extract recorded a significant (p<0.05) increase in the negative control group (B) when compared with the normal control group. The treatment group does not show significance when compared with the negative group. For AST and ALT levels, the extract recorded a significant (p<0.05) decrease in treatment group when compared with the negative control group (B).

Table 5. Serum biochemical parameters in the study groups.

Group	Total bilirubin (source unit: g/dL)	Total protein (source unit: g/dL)	Albumin (source unit: g/dL)
A (normal control)	0.99 ± 0.06	61.85 ± 3.82	36.61 ± 0.25
B (negative control)	2.15 ± 0.21 $\square$	52.09 ± 2.42 $\square$	38.10 ± 1.96 $\square$
C (treatment)	1.72 ± 0.30 $\square$	55.13 ± 1.32 $\square$	37.99 ± 1.08 $\square$

Results are expressed in mean $\pm$ SEM (n=7); a significant (p<0.05) compared to normal control group, c not significant p<0.05 compared to negative control.

Total bilirubin, total protein and albumin were also examined in wistar rats and were interpreted as follows;

Statistical evaluation revealed that both the protein and total bilirubin showed a significant difference (p<0.05) increase in for the negative group when compared with the normal control group but the treatment group showed no significant differences.

3.1. Histopathology

GROUP: RESULT

A: Normal control: The morphology is normal with hepatocytes surrounded by intact nuclear membranes.

B: Negative control: There is severe haemorrhage and severe hepatic cord atrophy evident by the presence of red blood cells in the interstitial spaces and the sinusoids, with loss of cellular integrity (necrosis).

C: Treatment: Tissue morphology is normal evident by the presentation of intact hepatocytes surrounded by intact cell membrane. The hepatic sinusoids harbour few red blood cells.

4. Discussion

The presence of alkaloids, tannins, saponins, steroids and flavonoids from the phytochemical screening suggests the rational for its use in various medicinal purposes in folk medicine. The antioxidant potential of *E. coccinea* may be related to the flavonoids detected in the extract. DOX in the form of semiquinone has been suggested to play a major role in hepatotoxic action. Hepatocytes are major targets for reactive oxygen species causing injury thus, alters their transport function, permeability and leading to leakage of enzymes from the cells. This leakage causes decrease in levels of ALT and AST in hepatic cells but increases their levels in the serum. These serum transaminases have long been used as sensitive indicators of hepatic injury.

Administration of DOX to rats significantly increased serum ALT, ALP and AST levels as shown in table 4. ALT is a more liver specific enzyme and its increase indicates liver injury. Increase in AST activity is also roughly proportional to the extent of cardiac damage. However it was observed that treatment of wistar rats with ethanol leaf extract of *E.coccinea* after DOX administration caused a significant decrease in their serum activity ($p<0.05$). It was also observed that there was an increase in total bilirubin levels on DOX administration and a decrease after treatment with the leaf extract of *E.coccinea*, however it was not statistically significant. This finding was backed up the histopathology examination of the hepatic tissues and the result revealed that treatment with *E.coccinea* showed some degree of amelioration from severe hemorrhage induced by DOX.

4.1. Limitations and Future Work

Further work on this plant should be done on dose depended design.

The researcher should be concerned with the effect of the extract only in the biochemical indices in rats.

The plant should be characterized using HPLC/ MS to identify its active compounds in `in-vivo study.

5. Conclusions

In conclusion, the leaf extract of *E.coccinea* could have antioxidative property by scavenging free radicals in the body thus protecting the liver from injury.

Abbreviations

The following abbreviations are used in this manuscript: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DOX, doxorubicin; LD₅₀, median lethal dose.

Declarations

Author Contributions: Conceptualization, [S.D.J. and Q.O.E.]; methodology, [S.D.J.]; investigation, [S.D.J. and Q.O.E.]; formal analysis, [initials]; writing—original draft preparation, [S.D.J.]; writing—review and editing, [Q.O.E.]; supervision, [S.D.J. and Q.O.E.]. Both authors have read and agreed to the submitted version of the manuscript.

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Institutional Review Board Statement: animal protocol was approved by an **Institutional Animal Ethics Committee (IAEC)**. Animal care followed the **European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123)**, citing “European Treaty Series, 2005.”

Informed Consent Statement: Not applicable. The study involved animals and no human participants.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors have none.

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Figures

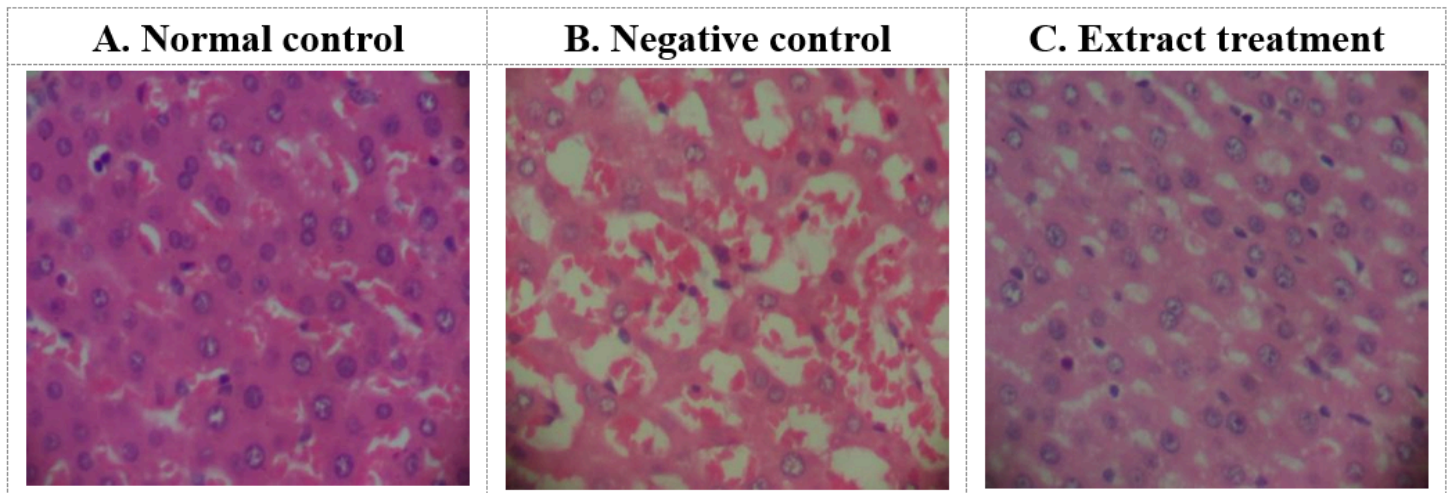


Figure 1

Representative liver histology in the study groups (hematoxylin and eosin, ×400). A, normal control; B, doxorubicin negative control, showing haemorrhage, hepatic cord atrophy, and necrosis; C, doxorubicin plus *Emilia coccinea* extract, showing preserved tissue morphology. Images reproduced from the source study.