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Effect of Methanolic Extract of Emilia Praetemissa on Paracetamol Induced Liver Damage in Albino Wistar Rats

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Abstract: *The present investigation shows the hepato-protective role of Emilia praetermissa in paracetamol-induced liver toxicity. The study was conducted using forty Albino rats randomly divided into four groups of ten rats each. Group A, the control group received tap water and animal feeds only. Group B received 2g/kg body weight of Paracetamol. Group C was administered with 500mg/kg body weight of the extract while group D received co-administration of the same concentrations of the extract and Paracetamol. All the treatment lasted for a period of 7days. Blood sample was collected from the rats through cardiac puncture under diethyl ether anaesthesia for analysis of AST, ALT, ALP, Albumin, Total protein, direct bilirubin and conjugated bilirubin. The results showed that paracetamol increased the biochemical parameters (AST, ALT) studied when compared to control while Emilia praetermissa decreased all the biochemical parameters tested. However, the extract significantly ($p<0.05$) reduced blood proteins such as ALB and Total proteins. Histopathological observations of liver tissues corroborated these findings. The results therefore suggest that Emilia praetermissa has protective effects on liver toxicity. Therefore, the use of E. praetermissa as vegetables and other medicinal effects could be encouraged.*

Keywords: *Emilia praetermisa, hepatoprotective, hepatotoxicity, medicinal plants, paracetamol.*

I. Introduction

Emilia praetermissa milne-Redh belongs to the family of Asteraceae of West tropical Africa. It was originally described from Sierra Leone and Nigeria [1] and was subsequently found in other West African countries, including Cameroun, Cote d' Ivoire, Ghana, Guinea and Liberia [2,3,4]. It is used as food (Leaves - cooked and eaten as spinach) and medicine for general healing [5]. *E. praetermissa* has been established as an anti-ulcerogenic plant producing complete mucosal cytoprotection at a dose of 500mg/kg [6]. It has also been suggested be an effective supplement in hyperlipidemic patients [7,8] as well as its haemopoietic activities even in the presence of ulceration [9].

Hepatic cells are involved in a variety of metabolic events; therefore the establishment of liver protective/therapeutic agents is of paramount importance in the protection from liver damage. Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity. Some previous studies [10,11,12,13,14] have shown that hepatoprotective effects are associated with phytoextracts/ phytocompounds rich in natural antioxidants. Many bioactive compounds and extracts from plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxin-induced liver damage [15,16].

Paracetamol (Acetaminophen, APAP, 4-hydroxyacetanilide) is a well-known antipyretic and analgesic agent, which is harmless in therapeutic doses but can produce fatal hepatic necrosis in experimental animals as well as in humans [17]. It is employed as a well-established experimental hepatotoxic agent for pre-clinical research [18]. At limited therapeutic doses, paracetamol is metabolized by cytochrome P_{450} (CYP) to form the highly reactive species, N-acetyl-p-benzoquinone imine (NAPQI), which under normal conditions is readily detoxified by conjugation, with glutathione (GSH) [18]. However, in human and experimental animals, high doses of paracetamol saturate detoxification pathways, leading to hepatic glutathione depletion and excessive production of NAPQI, which freely binds to cellular metabolites/molecules [19] thereby producing cell necrosis in the liver [20].

There has not been any scientific report or whatsoever on the ability of E. praetermissa leaf extract on liver damage caused by drugs or xenobiotics. We therefore designed this work to determine its effect on paracetamol-induced liver damage.

II. Materials And Method

The study took place at the laboratory of the Department of Human Physiology, Nnamdi Azikiwe University, Nnewi Campus. A total of forty (40) male albino wistar rats were used for the study.

2.1 Plant Material and Extraction

The leaves of *E. praetermissa* were collected from Nnamdi Azikiwe University, Awka. The botanical identification and authentification was done at the Botany Department, UNIZIK, Awka, with the Voucher number of the specimen **N.A.U.H. NO. 148**.

The leaves were washed thoroughly with clean water to remove all forms of dirt and were air dried under ambient temperature. The dried leaves were milled to fine powder using an electric grinder (Saisho Magic Blender, S-999) and then extracted using methanol. The extracted residue was dried in air oven of $40^{O}C$. The dried extract was preserved in a bottle and stored in a refrigerator at $4^{O}C$ until required for use. Preliminary phytochemical screening was performed [21]. The LD_{50} determination of E. praetermissa leaf extract was carried out according to the method of Lorke [22].

2.2 Animal Handling/Selection

Experiments were performed using 40 adult male albino rats (120 – 200g). They were kept in well ventilated cages with suitable temperature and relative humidity. Rat feed (Grower vital feed) and drinking water was given ad libitium. The animals were kept for 14 days to acclimatize before proceeding with the experiment.

2.3 Experimental Procedure

The animals were randomly grouped into 4 groups of 10 animals each. Group A, the control and received tap water and animal feeds only. Group B received paracetamol 2g/kg body weight for 7 days [23,24]. Group C received 500mg/kg body weight of the plant extract of *E. praetermissa* for 7 days. Group D received co-administration of paracetamol 2g/kg and *E. praetermissa* 500mg/kg body weights for 7days.

2.4 Assessment of Liver Function

Blood sample was collected via cardiac puncture on the 8^{th} day under deep ether anesthesia. The blood was put into sterile bottles and centrifuged at 7000rpm for 10mins. The estimations of serum activities of AST, ALT, ALP, total bilirubin and blood proteins were carried out according to the methods of Reitman, Kind, Mally and Lyne using Randox test kits [25,26,27,28].

2.4 Histopathology

The liver samples were excised and washed with normal saline and were fixed immediately in 10% formal saline solution. A paraffin embedding technique was carried out and sections were taken at 5-mm thickness, stained with hematoxylin and eosin and examined microscopically for histopathological changes [29] and photographs were taken at x400 objective.

2.5 Data Analysis

Data was expressed as means ± standard deviations. Comparative analysis between two groups was done using independent sample t-test, while one-way ANOVA was used to compare multiple groups. Statistical significance was set at $p < 0.05$. All analysis was done using statistical Package for Social Science (SPSS) software (version 20).

III. Results and Discussion

The liver is a major target organ for toxicity of xenobiotics and drugs, because most orally ingested xenobiotics and drugs pass through the liver and some chemicals are metabolized into toxic intermediates in the liver [30]. Paracetamol, when used at high doses, could cause acute liver injury most probably via formation of N-acetyl-p-benzoquinone imine (NAPQ1), a toxic metabolite, by cytochrome P4502E1 (CYP2E1). N-acetyl-p-benzoquinone imine is usually inactivated by hepatic glutathione. When produced excessively, they covalently bind to centrilobular hepatic proteins, contributing to hepatic toxicity [31,32]. Paracetamol is being used extensively to investigate hepatoprotective activity of different treatments on various experimental animals [33].

Phytochemical products including plant herbs and extracts have been used for centuries to promote liver health. Although the exact mechanisms behind this protection are uncertain, many theories have been proposed. Flavonoids have been reported to possess inherent antioxidant effect and scavenge free radicals liberated during the process of lipid peroxidation [34]. The hepatoprotective effect of flavonoid has been indicated by the actions of Silymarin; a known hepatoprotective drug. Silymarin is a flavonoid – complex containing silybin, silydianin and silychristin – that is derived from milk thirtle plant (Silybum marianum) [35].

Amongst so many uses of this drug is its use in the protection of the liver against pharmaceuticals that stress the liver such as paracetamol.

Phytochemical screening of the leaf of this *Emilia praetermissa* showed the presence of tannins, favonoids and steroids in appreciable amounts; minute amounts of cardiac glycosides and terponoids; while Saponins and alkaloids were not detected (Table 1).

Table 1: - Pytochemical Analysis of *Emilia praetermissa* leaf extract

Constituents	Observation
Carbohydrates	+
Saponins	—
Tannins	++
Alkaloids	—
Cardiac Glycosides	+
Flavonoids	++
Terpenoids	+
Reducing Sugar	+
Steroids	++

++ = appreciable amount; + = minute amount; **—** = not detected

The acute oral toxicity of the methanol leaf extract of *Emilia praetermissa* in albino rats up to the dose of 5000mg/kg body weight using Lorke's method [22] produced no mortality (Table 2). Thus, the medial lethal dose (LD_{50}) of the extract is greater than 5000mg/kg body weight. This indicates that the extract is relatively safe. It was reported that any substance whose oral LD_{50} value is above 1000mg/kg body weight is regarded as being of low toxicity or relatively safe [36].

Table 2: Acute oral toxicity study of methanol leaf extract of *Emilia praetermissa* on albino Wistar rats.

PHASE	NUMBER OF RATS	DOSE (mg/kg)	CLINICAL SIGNS	MORTALITY
1	3	10	None	Zero
1	3	100	None	Zero
1	3	500	None	Zero
2	1	1500	None	Zero
2	1	2500	None	Zero
2	1	5000	None	Zero

Present results using the model of paracetamol-induced hepatotoxicity in the rats demonstrated that *Emilia praetermissa* caused non-significant increase in body weights in the rats (Table 3).

Table 3: Effect of aqueous extract of *E. praetermissa* against paracetamol-induced hepatotoxicity on percentage weight change.

GROUPS	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)	BODY WEIGHT DIFFERENCE (g)	% WEIGHT CHANGE
A	158.00±23.47	171.00±23.30	13.00±3.83	8.48±3.76
B	143.00±23.59	130.00±25.38*	-13.00±4.83*	-9.40±4.07*
C	135.00±19.00	145.00±19.00	10.00±0.00*	7.52±0.95
D	146.00±17.76	139.00±15.95*	-10.00±0.00*	-4.66±3.27*

Data represent mean±SEM. * P< 0.05 when compared to Group A.

The increase in weight indicates that the leaves of *Emilia praetermissa* were not toxic to the animals and could be attributed to their content of nutrients such as proteins, carbohydrates, lipids, minerals and vitamins which are needed for growth, body repair and maintenance as shown in Table 1. Thus, the vegetable could be a valuable and viable source of bioactive nutrients and non-nutrient substances with potential hepatoprotective properties. There was a significant decrease ($p<0.05$) in the body weights of rats in group B when compared to the normal. The decrease in the body weight of the rats administered with the extract and paracetamol may be due to the loss of appetite observed in the cause of study.

Acute exposure to various hepatotoxins is associated with the structural damage to organs and tissues along with pathological and inflammatory changes which results into altered morphology of affected liver organ [37]. The liver weight to body weight ratio was calculated and was found to be substantially increased ($p<0.05$) in all the groups when compared to the normal group rats (Table 4). The increase is more in the paracetamol treated group as recorded by Zargar, [38] and could be attributed to the inflammation caused by the toxic potentials of paracetamol.

Table 4: Effect of methanol leaf extract of *E. praetermissa* against paracetamol-induced hepatotoxicity on mean liver weights

GROUPS	LIVER WEIGHT (g)	BODY WEIGHT (g)	LIVER/BODY WEIGHT RATIO (%)
A	3.14±0.48	171.00±23.30	1.86±0.33
B	4.82±0.32*	130.00±25.38*	3.81±0.68*
C	4.16±0.32*	145.00±19.00	2.91±0.48*
D	4.32±0.72*	139.00±15.95*	3.16±0.73*

Data represent mean±SEM. * P< 0.05 when compared to Group A.

Liver transaminases such as AST (aspartate transaminase) or SGOT (serum glutamic oxaloacetic transaminase), and ALT (alanine transaminase) or SGPT (serum glutamic pyruvic transaminase) have still remained the gold standards for the assessment of liver injury, and have been used as biomarkers of choice for decades [39]. ALT is predominantly found in liver unlike AST which is also abundantly present in other organs namely, cardiac muscle and kidneys. For this reason, ALT is more specific indicator of liver inflammation than AST [40]. Increase in activities of liver enzymes such as alanine transaminase and aspartate transaminase are roughly proportional to the extent of liver tissue damage [41]. Alkaline phosphatase levels usually increases remarkably in diseases that impair bile formation and to lesser extent in hepatocellular diseases [42].

As observed in the study, the administration of paracetamol to the hepatotoxic group showed an increase in the levels of ALT, AST and ALP. This is in line with the fact that paracetamol is a known model for both hepatic injury and GSH depletion. The treatment with leaf extract of *E. praetermissa* did not significantly ($p>0.05$) change the levels of ALT and AST in the animals when compared with the control rats. This result is closely related to the earlier findings of Ebunlomo *et al.,* [9] where administration of the leaf extract caused a decrease in the levels of AST, ALT and ALP. This showed that E. praetermissa does not have any noticeable or apparent toxic effects on the liver.

There were significant ($p<0.05$) increase in AST and ALT levels in the animals given a co-administration of paracetamol and *E. praetermissa* leaf extract. This increase could be caused by the toxicity caused by the by-product of paracetamol metabolism [43] (Table 5).

Table 5: Mean values of biochemical parameters compared between the normal control group A and negative control group B, positive control group C and treatment group D of rats.

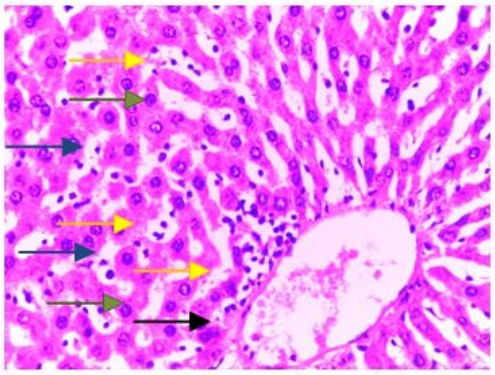
GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)	TOTAL BILIRUBIN (mg/dl)	CONJUGATED BILIRUBIN (mg/dl)	ALBUMIN (g/L)	TOTAL PROTEIN (g/L)
GROUP A (N = 10)	42.71±8.34	24.42 ± 5.22	246.7±25.24	0.35 ± 0.11	0.12± 0.07	33.28 ± 3.90	66.28±11.17
GROUP B (N = 10)	86.42±10.01*	57.28±16.67*	308.1±29.81	0.65 ± 0.25*	0.08 ± 0.02	22.85 ± 4.74*	44.71 ± 4.75*
GROUP C (N = 10)	62.0 ±24.48	40.85 ±5.27	136.5±74.07*	0.55 ± 0.26	0.14 ± 0.02	33.14 ± 2.11	64.01 ± 7.85
GROUP D (N = 10)	73.14±10.63*	56.42±18.12*	233.14±64.26	0.33 ± 0.12	0.08 ± 0.03	27.42 ± 5.09	50.71 ± 5.64*

Data are expressed as means ± standard deviations. *Significantly different ($p<0.05$) from group A.

Total protein levels are rough measures of protein status but reflect major functional changes in liver functions [44]. The increase in serum Total protein is a function of the increased Albumin. The functional role of ALB makes it a reliable marker for diagnosis of liver disease [45]. This increase could result from a concomitant increase in the number of cells responsible for ALB synthesis, or a direct enhancement of Albumin-synthesizing mechanism in the liver as obtainable in mammalian cells or a combination of both [46]. In this research there was a significant decrease ($p<0.05$) in the protein levels of all the hepatotoxic animals which could indicate liver dysfunction (Table 5). This is in accordance with the result of Orhue *et al.,* [47]**.** The non-significant decrease in the total protein levels in the E. praetermissa treated group indicates the hepatoprotective effects of the extract.

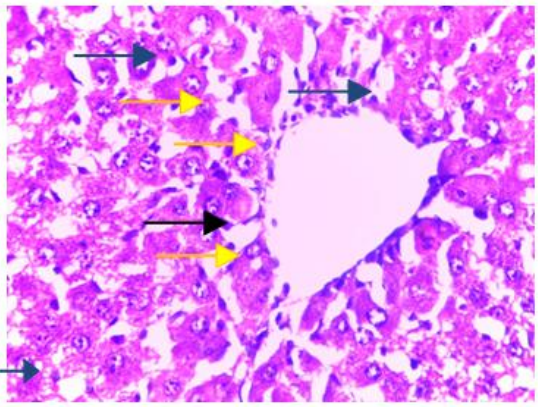
Bilirubin is one of the most clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte [48]. In the present study group B rats showed a significant increase in the level of serum total bilirubin when compared with control rats. Oral administration of aqueous leaves extract of *E. praertermissa* significantly restored serum total bilirubin level near normal value (Table 5). This indicates the effectiveness of these extracts in normal functional status of the liver. The ability of the extract to decrease the serum concentrations of total and conjugated bilirubin shows that the extract could be useful in the treatment of hyperbilirubinemia. Again, this effect could also be mediated by any of the active biological principles contained in the extract. The non-significant changes ($p>0.05$) seen in the extract treated

group is supported by the previous study of Ebunlomo *et al.*, [9] which suggested that *E. praetermissa* improves haemopoiesis even in the presence of stress-induced ulceration in rats.



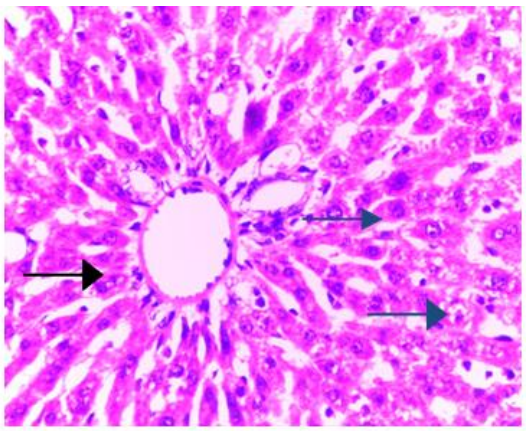
A.

Figure 1 - Group A (given water and animal feeds only): photomicrograph of a liver section with no obvious pathological architectural change. Central vein (marked by black arrow), hepathocytes with normochromic nuclei (marked by blue arrows) and sinusoids (marked by green arrows). However, a few lymphocytic infiltrates are evident around the central vein and in the liver sinusoids (marked by yellow arrows). Stained by Haematoxylin (Erhlich's) and eosin staining technique. X400 Objective.



B.

Figure 2 - Group B (administered 2g/kg of Paracetamol): photomicrograph of a liver section with severely necrotic hepatocytes containing hypochromatic nucleus (Karyolysis; marked by blue arrows), central vein is dilated with necrotic vascular wall (marked by black arrow), moderate microvasicular steatosis (marked by green arrows), few inflammatory cells (marked by yellow arrows) and disoriented liver sinusoids. Stained by Haematoxylin (Erhlich's) and eosin technique. X400 Objective.



C.

Figure 3 - Group C (administered 500 mg/kg of *Emilia praetemissa* leaf extract): photomicrograph of a liver section with minimal change in tissue architecture. It features normal central vein (marked by black arrow) and mildly affected hepatocytes with normochromic nucleus (marked by blue arrows). Stained by Haematoxylin (Erhlich's) and eosin technique. X400 Objective.

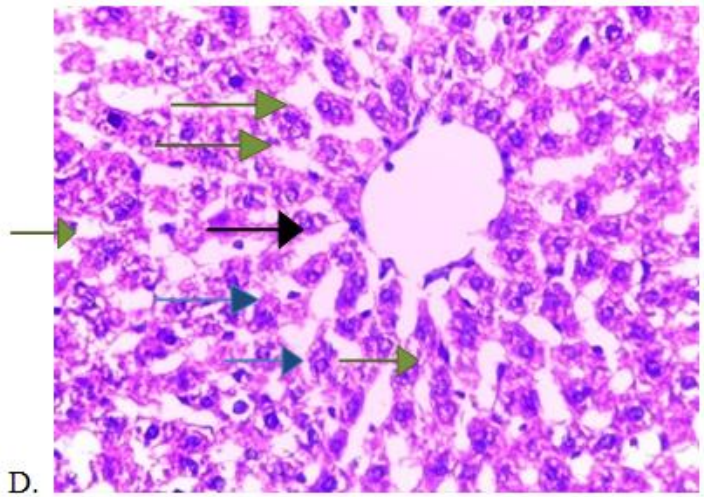


Figure 4 - Group D (administered of 2 g/kg of paracetamol followed by the administration of 500 mg/kg of *Emilia praetemissa* leaf extract): photomicrograph of a liver section with mildly distorted vascular wall; central vein (marked by black arrow) and moderately necrotized hepatocytes with normochromic nucleus (marked by blue arrows). More so, some of the nuclei appear regenerative (marked by green arrows). Stained by Haematoxylin (Erhlich's) and eosin technique. X400 Objective

The liver of control animals showed normal histological features (Fig. 1). The necrotic effect of paracetamol seen in the abnormal histological changes in the liver of the hepatotoxic animals (Fig. 2) is similar to that gotten by Garba *et al.*, [42] where aqueous extract of Kohautia grandiflora plant was investigated against paracetamol-induced hepatotoxicity. However, the consumption of *E. praetermissa* reduced this necrosis (Fig. 3) indicating some level of hepatoprotective and regeneratve properties. This result shows that the consumption of *E. praetermissa* does not have any apparent toxicity on the liver of rats. Tropical green leafy vegetables are usually associated with hepatoprotective properties [49]. The animals with co-adminitration of paracetamol and the extract revealed appreciable regeneration of hepatic tissue (Fig. 4) which is in line with the findings of Mesole *et al.*, [50]. This could be linked to the potency of *E. praetemissa* leaf extract to facilitate rapid tissue repair; replacing necrotized hepatocytes.

IV. Conclusion

In conclusion, the results of this study demonstrate that the aqueous extract of *Emilia praetermissa* has hepatoprotective potentials against paracetamol-induced liver damage in albino Wistar rats. This effect is probably due to its ability to preserve the structural integrity of hepatocytes when challenged with hepatotoxins; inhibition of Cytochrome P-450 enzymes or enhancing the antioxidant activities. It can therefore be concluded that the aqueous extract of *Emilia praetermissa* could be used in the treatment of any form of illness without imposing an effect on the liver. Further study is suggested to be carried out at a longer period to determine the potency of this extract with time.

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