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Hepatoprotective activity of *Ocimum americanum* L leaves against paracetamol – induced liver damage in rats

B. T. Aluko[1, 2], O. I. Oloyede[1], A. J. Afolayan[2,*]

[1]Department of Biochemistry, Ekiti State University, Ado Ekiti, Nigeria
[2]Department of Botany, University of Fort Hare, Alice, South Africa

E mail address:

aafolayan@ufh.ac.za(A. J. Afolayan)

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Abstract: This study was designed to investigate the hepatoprotective activity of aqueous extract of *Ocimum americanum* leaves against paracetamol – induced liver damage in rats. Hepatic damage was induced by paracetamol. Thereafter, the levels of some serum biochemical parameters such as alanine trasaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin, total bilirubin (TBIL) and total protein (TP) were investigated. The activities of ALP, AST, ALT and histological changes in the liver of rats were also determined. Silymarin was used as the standard hepatoprotective drug. The pre – treatment of rats with aqueous extract of *O. americanum* leaves caused a significant increase in the serum levels of TP and albumin. There was a significant decrease in the serum levels of ALP, AST, ALT and TBIL with a corresponding increase in the activities of ALP, AST and ALT in the liver of extract treated rats. The hepatoprotection was confirmed by histological examinations of liver sections of normal and treated rats. Furthermore, rats intoxicated with paracetamol alone had their serum ALP, AST, ALT and TBIL levels significantly increased, while TP and albumin concentrations decreased when compared with the normal rats. The aqueous extract of *Ocimum americanum* leaves at doses of 200 and 400 mg /kg p.o. have significant hepatoprotective ability against paracetamol – induced hepatic damage in rats.

Keywords: *Ocimum americanum*, Hepatoprotective Activity, Paracetamol, Biochemical Parameters

1. Introduction

The liver is the chemical factory which regulates, synthesizes, stores and secretes important macromolecules in the body. It has a strategic anatomical location and large capacity for metabolic transformation of drugs and other toxins entering from the gastrointestinal tract. As a result of this, the healthy functioning of the liver determines the health status of an individual [1]. Liver diseases are a global problem and the synthetic drugs available for the treatment of liver disorders are believed to have serious adverse effects on biological systems [2]. Due to these facts, attention has been given to finding suitable curative agents for the treatment of liver diseases from natural product of plant origin [3]. Acetaminophen – induced liver injury in living systems has been well documented. Hepatotoxicity of acetaminophen has been attributed to the formation of N – acetyl – p – benzo quinoneimine (NAPQI) a toxic metabolite that causes glutathione depletion and oxidative stress. Acetaminophen (Paracetamol) is an analgesic and antipyretic drug that is known to be safe at recommended doses. However, it produces acute liver damage at higher doses [4].

Ocimum americanum L commonly known as African basil belongs to the genus *Lamiaceae*. It is a wild herb with a distinct mint flavor, hairy leaves and scented flowers that is native to tropical Africa [5]. It is popularly called "Efinrin elewe dudu" in south-western Nigeria. The leaf has been used in traditional folk medicine in Ghana to treat diabetes [6]. It is used by traditional healers in Nigeria for the treatment of constipation, diarrhoea, piles, dysentery and as insect repellent. The leaf is rich in essential oils of therapeutic importance and mostly used as a condiment for the preparation of delicious local soup because of its aromatic properties [7]. The acetone extract of this plant has been reported to inhibit neurotoxins - induced brain damage in rats [8]. Our survey of literature reveals that the hepatoprotective activity of *Ocimum americanum* leaves has not been scientifically investigated. The aim of this research therefore, was to assess the protective effects of aqueous extract

O. americanum leaves in rat model of acetaminophen – induced liver injury.

2. Materials and Methods

2.1. Collection and Identification of Plant Material

The leaves of *Ocimum americanum* were obtained from a local farmland near Orin Ekiti South-Western Nigeria in the month of May, 2011. The plant was identified and authenticated by Mr Omotayo (herbarium curator) at the Department of Plant Science, University of Ado Ekiti, Nigeria where the voucher specimen (Aluko 09) was deposited.

2.2. Reagents

Silymarin used in this study was purchased from Sigma-Aldrich Gmbh, Sternheim, Germany. Assay kits for the determination of ALT, AST and ALP were purchased from Randox laboratories Ltd. Ardmore, United Kingdom. All other chemicals used were of high analytical grade.

2.3. Sample Extraction

The leaves of *O. americanum* were air dried for 10 days and then ground into fine powder using an electric blender. Fifty grams of the powdered sample was extracted in 1 liter of cold sterile distilled water maintained on a mechanical shaker (Stuart Scientific Orbital Shaker SO1, Essex, UK) for 24 h. The extract was filtered using a Buchner funnel with Whatman's No 1 filter paper. The filtrate was frozen at -40^{o}C and dried for 72 h using a freeze drier (Savant Refrigerated vapor Trap, RVT 41404, USA) to give a percentage yield of 14.2% w/w. The resulting extract was reconstituted in cold distilled water to give doses of 200 and 400 mg/kg body weight.

2.4. Experimental Animals

Male Wistar rats (*Rattus norvegicus*) weighing 140 – 180 g were obtained from the animal house of the laboratory of School of Biological Sciences, University of Fort Hare Alice 5700 South Africa. They were kept in clean cages placed in a well ventilated house condition (temperature: 22 ± 2^{o}C; photoperiod: 12 h light and 12 h dark cycle; humidity: 40 – 45%). The animals were fed *ad libtum* with rat pellets and tap water freed of contaminants. The experiment was carried out in line with the guidelines of Ethics Committee on the use and care of Experimental Animals of the University of Fort Hare Alice, South Africa.

2.5. Acute Toxicity Studies (LD50)

Aqueous extract of *O. americanum* leaves was studied for acute oral toxicity as per Organization for Economic Co-operation and Development (OECD) guidelines number 423 [9]. The modified method of Nirmala et al [10] was used to assess the acute oral toxicity of *O. americanum* leaves. Healthy Wistar rats weighing (140 – 180 g) were used for this purpose. Four doses of 500, 1000, 2000 and 4000 mg/kg, p.o. of the extract were given to 4 groups containing 4 animals in each group. Single dose of the extract was administered orally to each animal. The animals were observed individually during the first 30 min and thereafter 24 hourly for a period of 14 days. Signs of toxicity, body weight, feed and water intake for each group was observed everyday for 14 days. The extract was devoid of toxicity even at a dose of 4000 mg/kg by oral route in rats. Hence, 200 and 400 mg/kg doses of the extract were selected for further experiment.

2.6 . Acetaminophen – Induced Hepatotoxicity in Rats

The method of Nirmala et al [10] was used to evaluate the hepatoprotective activity of aqueous extract of O. americanum leaves with some modifications. The animals were divided into five groups of six animals each. Animals were treated for a period of seven days as follows:

Group 1 (normal control) received 1ml of distilled water p.o. for 7 days

Group 2 (toxic group) received 1ml of distilled water p.o. for 7 days and paracetamol (2 g/kg b.w., p.o.) on the 4th and 5th day.

Group 3 received aqueous extract (200 mg/kg b.w. p.o.) for 7 days and paracetamol (2 g/kg b.w., p.o.) on the 4th and 5th day, 30 min after extract administration.

Group 4 received aqueous extract (400 mg/kg b.w. p.o.) for 7 days and paracetamol (2 g/kg b.w., p.o.) on the 4th and 5th day, 30 min after extract administration.

Group 5 (standard group) received silymarin (100 mg/kg b.w. p.o.) for 7 days and paracetamol (2 g/kg b.w., p.o.) on the 4th and 5th day 30 min after the administration of silymarin.

No mortality was observed at the end of the experimental period (7 days). Four animals per group were sacrificed under light ether anesthesia 24 h after their respective doses. Blood samples were collected into EDTA bottles by cardiac puncture while the liver was quickly removed into ice cold 0.25 M sucrose solution. Thereafter, the liver was blotted with clean tissue paper and homogenized in Tris Hcl buffer (0.1 M pH 7.4 1: 10 w/v). The homogenates were kept frozen overnight before being used for various enzyme assays.

2.7. Determination of Biochemical Parameters

The biochemical parameters such as alanine trasaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin, total bilirubin (TBIL) and total protein (TP) in the serum were analyzed using liver panel plus reagent discs on an automated chemistry analyzer (Piccolo Blood Chemistry Analyzer, Abaxis, Union City, CA, USA. [11] The liver homogenate was assayed for ALT, AST and ALP according to the manufacturer's instructions on the assay kits.

2.8. Histopathological Studies

The liver specimen from the control and test groups were fixed in 10% buffered formalin for 48 h. The formalin fixed

samples were stained with haematoxylin – eosin. The sections were examined microscopically for changes in histopathological architecture.

2.9. Statistical Analysis

Results were expressed as mean ± SD and analyzed by one – way analysis of variance (ANOVA) followed by Duncan multiple range test. The results obtained from both the extract and standard drug were compared with that of the normal rats. P values < 05 were considered significant.

Table 1. *Effect of aqueous extract of O. americanum leaves on some serum biochemical parameters of paracetamol intoxicated rats.*

Treatment	Albumin (g/dl)	ALP (U/L)	ALT (U/L)	AST (U/L)	TP (g/dl)	TBIL (mg/dl)
Normal rats	4.07 ± 0.42[a]	264.67 ± 8.08 [a]	45.00 ± 4.58 [a]	116.33± 5.13 [a]	6.37 ± 0.05 [a]	0.27 ± 0.06 [a]
PARA (2 g/kg)	1.80 ±0.20 [c]	323.66± 14.50[c]	92.31 ± 5.86 [c]	188.00 ± 9.00 [c]	3.40 ±0.20 [c]	1.27 ± 0.35 [c]
PARA + AOA (200 mg /kg)	2.40 ±0.20 [b]	280.33 ± 6.51 [b]	65.00 ± 2.65 [b]	138.33 ± 3.51 [b]	5.37 ±0.25 [b]	0.67 ± 0.05 [b]
PARA + AOA (400 mg /kg)	2.83 ±0.45 [b]	259.67 ± 9.71 [a]	44.30 ± 6.03 [a]	120.67 ± 5.86 [a]	6.13 ± 0.06 [a]	0.30 ± 0.10 [a]
PARA + SILY (100 mg /kg)	3.73 ±0.31 [a]	258.00 ± 4.36 [a]	45.33 ± 2.89 [a]	117.00 ± 1.73 [a]	6.30 ± 0.10 [a]	0.33 ± 0.06 [a]

Data represent mean ± SD values of triplicate determination for each animal. Test values carrying superscripts ([b - c]) are significantly different (P < 05) from the normal control ([a]) for each parameter. PARA: paracetamol ; AOA: aqueous extract of O. americanum leaves; SILY: silymarin.

3. Results

3.1. Acute Toxicity

The aqueous extract of *O. americanum* leaves was found to be safe. No mortality was observed for 14 days of treatment with a limit dose of 4000 mg/kg body weight. All the rats tolerated the extract with no signs of toxicity.

3.2. Effect of Aqueous Extract of O. Americanum Leaves Against Paracetamol – Induced Hepatotoxicity in Rats

The effect of aqueous extract of *O. americanum* leaves on some serum biochemical parameters is presented in Table 1 The administration of paracetamol (2000 mg/kg b.w.) induced a marked significant increase in the levels of blood ALT, AST, ALP and TBIL as compared to normal control. However, the pretreatment of rats with aqueous extract of *O. americanum* leaves prior to paracetamol administration caused a significant reduction in the levels of these markers in a dose dependent manner. The administration of the extract and silymarin (400 and 100 mg/kg b. w. respectively) restored the levels of these parameters to normal levels in the blood. In the case of albumin and total protein there was a remarkable reduction in their levels in the blood of paracetamol intoxicated rats indicating hepatocellular injury. Treatment with *O. americanum* extract caused a significant rise in their levels to values that were comparable with those of silymarin treated group and normal control.

Also, there was a significant decrease in the levels of ALT, AST and ALP in the liver of paracetamol treated. This was an indication of liver damage due to leakage of these enzymes from hepatic cells into the extracellular compartment. However, a dose dependent increase in the levels of these enzymes was noticed in the rats pretreated with *O. americanum* extract and silymarin which was reversed to the level of normal control (Table 2).

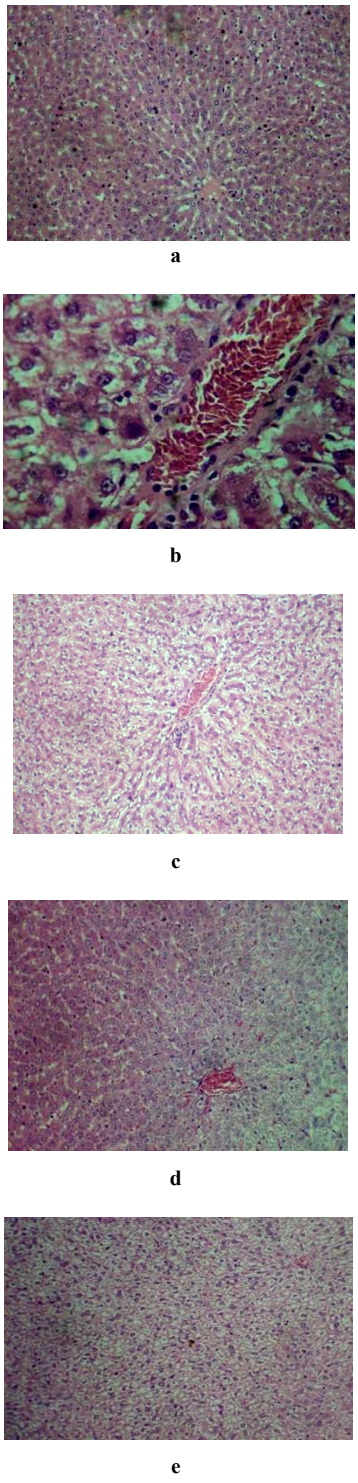
Table 2. *Effect of aqueous extract of O. americanum leaves on the activity of some enzymes in the liver of paracetamol intoxicated rats.*

Treatment	ALP (U/L)	ALT (U/L)	AST (U/L)
Normal rats	365.03 ± 13.51[a]	127.83 ± 6.22 [a]	141.83± 8.47 [a]
PARA (2 g/kg)	201.60 ± 6.68 c	73.90 ± 6.18[c]	76.56 ± 5.86 [d]
PARA + AOA (200 mg /kg)	224.83 ± 12.48[c]	78.86 ± 4.09 [bc]	100.47 ± 10.11 [c]
PARA + AOA (400 mg /kg)	317.19 ± 12.35[b]	126.19± 8.22 [a]	130.41 ± 11.42 [b]
PARA +SILY (100 mg /kg)	367.30 ± 25.95[a]	127.92 ± 5.66 [a]	137.04 ± 3.04 [b]

Data are mean ± SD values of triplicate determination for each animal. Test values carrying superscripts ([b - c]) are significantly different (P < 05) from the normal control ([a]) for each parameter. PARA: paracetamol ; AOA: aqueous extract of O. americanum leaves; SILY: silymarin.

3.3. Histopathological Studies

The hepatoprotective potential of O. americanum leaf extract was confirmed by histological examination of normal and treated rats. The histological profile of normal rats showed normal cellular architecture (Fig 1 a). In paracetamol intoxicated animals, there were drastic alterations in the histological architecture of the liver. Histological examination showed distende hepatocytes, fatty degeneration and area of necrosis (Fig 1 b). The administration of O. americanum extract and silymarin brought about significant recovery. There was less degeneration of hepatocytes as a result of marked regeneration activity. (Fig 1 c - e).



a

b

c

d

e

Figure 1. *Histological micrograph of liver section excised from the normal rat (a, X 40) showed normal structure of the hepatocytes. Histological section of paracetamol intoxicated rat (b, X40) showed damage to the liver structure as indicated by infiltration of monocytes and the presence of necrotic cells. Treatment with 200 mg/kg b. w.of aqueous extract of O. americanum leaves normalized the damaged hepatocytes with a great reduction in the quantity of necrotic cells (c, X 40). When the concentration of the extract was increased to 400 mg/kg b. w. the liver micrograph showed recovery of hepatocytes into normal with very mild monocyte infiltration (d, X 40). Upon treatment with silymarin, the structure of the hepatocytes reverted to normal (e, X 40).*

4. Discussion

The liver plays a pivotal role of detoxification of xenobiotics and drugs in biological systems. Hepatic damage has been reported to upset the normal metabolic processes in the body [12]. Paracetamol is metabolized in the liver to excretable glucuronide conjugates [13]. However the overdose of this drug results in the accumulation of a toxic metabolite known as N – acetyl – p – benzo quinoneimine (NAPQI) [14]. This metabolite induces toxicity by binding to proteins and DNA to produce protein adducts which are responsible for the dysfunction and death of hepatocytes leading to liver necrosis [15]. Protection against paracetamol – induced liver damage has been used to assess the hepatoprotective potential of plant extracts [16, 17, 18].

In this study, the evidence of paracetamol – induced liver injury was the elevation of blood levels of cellular markers (AST, ALT, ALP and TBIL) and decreased levels of albumin and total proteins. Increased levels of enzyme in the blood have been attributed to disintegration and turnover of tissues [19]. Therefore, the increased levels of these markers in the blood of intoxicated animals imply that damage has been inflicted on the plasma membrane of the hepatic cells leading to their leakage into the extracellular fluid [20]. This is confirmed by the reduction in the level of marker enzymes in the liver of paracetamol – induced rats. Bilirubin on the other hand, is a product of enzymatic breakdown of heme within the reticuloendothelia system. Its elevation in the blood stream can be adduced to over production, increased hemolysis, decreased conjugation or impaired bilirubin transport [21]. Bilirubin is an index that is used to assess the normal functioning of the liver instead of the extent of hepatocellular injury. The increase in the level of TBIL in the blood is a pointer to the impaired function of the liver. Our findings revealed that the hepatoprorection exhibited by the extract *of O. americanum* leaves was dose dependent. The highest dose of the extract (400 mg/kg b.w.) and the standard drug (silymarin 100 mg/kg b.w.) caused the reduction of elevated blood levels of AST, ALT, ALP and TBIL to normal values. There was also an increase in the levels of albumin and total protein in extract and silymarin pretreated groups. Therefore, it could be deduced that the aqueous extract of *O. americanum* leaves provided protection to the liver against the toxic effect of paracetamol by preserving the functionality of this organ. Consequently, the beneficial effect of the extract and standard was supported by significant increase in the levels of marker enzymes of the liver (AST, ALT and ALP) of treated groups in an attempt to recover from the assault inflicted on the tissue by paracetamol. Reduction in the concentrations of these enzymes in the liver implies compromised plasma membrane integrity which resulted into the leakage of these markers into the blood circulation [22].

The extent of damage by paracetamol was further supported by histological changes in the liver of experimental animals. Histological examination of the liver sections showed that the normal liver architecture was disturbed by

hepatotoxin intoxication [23]. There were signs of necrosis and derangement of hepatic cells in the liver section of rats treated with paracetamol alone. Whereas in sections obtained from rats pretreated with *O. americanum* leaf extract or silymarin and intoxicated with paracetamol, the normal cellular architecture was retained as compared to those of the control rats.

The findings of this study elucidate the protective effect of *O. americanum* leaf extract on the liver. The possible mechanism of hepatoprotection may be attributed to presence of phytoconstituents in the leaves of this plant. However, further investigation is required to isolate and identify the compounds present the leaves that is responsible for its hepatoprotective property.

5. Conflict of Interest Statement

The authors declare that we have no conflict of interest.

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