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Effect of pulverized leaves of *Ocimum gratissimum* (scent leaf) and *Pterocarpus santalinoides* (*utrukpa* leaf) on liver function enzymes of indomethacin-induced ulcerated adult male Wistar rats

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Abstract:

This study investigated the effect of pulverized leaves of *Ocimum gratissimum* (scent leaf) and *Pterocarpus santalinoides* (*utrukpa* leaf) on the liver function enzymes activities of indomethacin-induced adult male Wistar rats. Fresh leaves of the samples were harvested, weighed and washed with tap water. One hundred grams of each leaf was blended with 200 ml of water to produce the pulverized samples used for the rat study. The results of the acute toxicity (LD_{50}) test of the two leaf samples showed that the leaves were safe at high doses. Forty-nine adult male rats used for the study were grouped into 7 (A-G) based on body weight. The groups B-G were induced with 30 mg/kg/body weight of indomethacin after a 3-day acclimatization. Two rats were randomly selected from each induced group and sacrificed to ascertain the presence of ulcer. The group A which was not induced served as the control; the Group B was the negative control (induced but not treated);Group C the positive (induced and treated with standard drugs); the Groups D and E were treated with 300 and 600 mg/kg/body weight of pulverized *Ocimum gratissimum* sample, respectively while the Groups F and G were treated with 300 and 600 mg/kg/body weight of pulverized *Pterocarpus santalinoides* sample, respectively. All the groups were fed rat chow and water for 14 days. Liver function enzyme activities of the rats were analyzed using standard methods for baseline and endline. Data collected were statistically analyzed using the statistical product for service solution (SPSS) version 24.0. T-test

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was used to compare the baseline and endline data within the groups. Analysis of variance and Duncan's new multiple range test were used to separate the means. The results were presented as means and standard deviation and a significant level was set at $p<0.05$. The leaf samples significantly ($p<0.05$) reduced the liver enzymes (ALT, AST and ALP) of the treated rats. The 600 mg/kg/body weight of *O. gratissimum* had the highest effect on the ALP with 53.46% (60.60±13.01 to 28.20±4.97) difference between the baseline and endline. The 300 mg/kg/body weight of *P. santalinoide* had the highest effect on the AST and the ALT with 50.86% (46.72±2.73 to 22.96±3.71) and 54.65% (66.60±2.07 to 30.20±2.86) difference between the base and endlines values, respectively. The pulverized leaves of *Ocimum gratissimum* and *Pterocarpus santalinoides* reduced the elevated liver enzymes caused by induction with indomethacin to normal.

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Introduction

The importance of plant to man's existence cannot be over emphasized. Plants are used as sources of foods and medicines since the dawn of civilization (1). According to Nwogu, Ojiako and Ujowundu (2) many indigenous plantsare taken as food, spices or used for medicinal purposes in Nigeria. Phytotherapy is rapidly gaining grounds in sustaining human health and in the prevention of certain diseases such as peptic ulcer. Plants as natural products are valuable source of bioactive compounds and have been used for medicinal purposes all over the world. Some tender leaves of some plants are used as vegetable and extracts of the leaves are used in ethno – medicine for treatment of various ailments (3). Recently, there has been increasing interest in plants- derived compounds as raw food and medicinal agents. Current estimates suggest that, in many developing countries like Nigeria, a large proportion of the population relies heavily on medicinal plants and crops to meet their primary health care needs (4).

Liver function enzymes are proteins produced by the liver that facilitate chemical reactions in the body. They play crucial roles in various bodily functions, including detoxification, protein synthesis and the production of digestive enzymes. Common liver enzymes include alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP). They are important because they can show if there is any tissue damage or inflammation in the liver (5). Elevated liver enzymes are generally a sign of a problem. This is because an inflamed liver leak higher than normal amounts of liver enzymes into the bloodstream, resulting in elevated liver enzymes. There can be variety of reasons for elevated liver enzymes and consumption of Indomethacin a non-steroidal anti-inflammatory drug (NSAID) is one of them. Indomethacin administered to the experimental animals caused a marked elevation in the levels of serum ALP, ALT and AST (6).

Many nutrients in food promote health and protect the body from disease. Plants such as *Ocimum gratissimum* (*O. gratissimum*) and *Pterocarpus santalinoides* (*P. santalinoidess*) are

widely used for the treatment of various ailments due to their extensive medicinal and nutritional properties (7). *Ocimum gratissimum* commonly called African basil and belongs to the family of Lamiaceae is a herbaceous perennial flowering plant which is woody at its base. The leaf is called scent leaf because it possesses a pleasant aroma which is responsible for its use as spice and condiments in cooking (8). The leafy vegetable is used in traditional medicine for treatment and management of diseases that have their etiology and pathophysiology in free radical generation and oxidative stress like peptic ulcer and hepatotoxicity (9). According to study done by Akah, John-Africa and Nworu (10), *Ocimum gratissimum* has gastro-protective effect on experimental ulcer rats. In addition, Scent leaf has antioxidant properties that can help to reduce oxidative stress, which is a key factor in chronic inflammation (11).

Pterocarpus santalinoides is a small – to – medium – sized deciduous tree belonging to the fabaceae family. It is locally known as *uturukpa.* Bioactive compounds found in the plant have been shown to have a wide range of biological activities (7) suggesting the potential of *Pterocarpus santalinoides* for the treatment of various diseases. A wide array of biological activities and potential health benefits of *Pterocarpus santalinoides* have been reported, including antioxidative, antidiabetic, antimicrobial, anticancer and anti-inflammatory properties and protective effects on the liver, gastric mucosa and nervous system (7)
Despite the beneficial roles and medicinal values of *Pterocarpus santalinoides* and *Ocimum gratissimum* leaves there is paucity of information on its micro anatomical effects on liver enzyme activities and on the role of these leaves in the management of drug induced elevated liver enzymes. This study is, therefore, aimed at evaluating the effect of *Pterocarpus santalinoides* and *Ocimum gratissimum* leaves in indomethacin- induced peptic ulcer in rat models.

Methods

Study design

Experimental design which entails subjecting the subjects to different treatments and then observing effect(s) of the treatment on the outcome of variables was used for the study.

Procurement and preparation of the samples

Fresh leaves of *Ocimum gratissimum and Pterocarpus. santalinoides* were plucked from the trees and plant shrubs in Orba in Udenu L.G.A. of Enugu State, Nigeria. These leaves were authenticated by a Taxonomist at Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The leaves were sorted, weighed using electronic weighing scale (SKU: RENG4032) and washed under running water before 100 g was blended with 200 ml of water into pulp using an electric blender (Mainstays 6- speed blender, T7199 model) and stored in an air-tight jar in a refrigerator at 3 ^{o}C for 3 days. Fresh samples were prepared every 3 days.

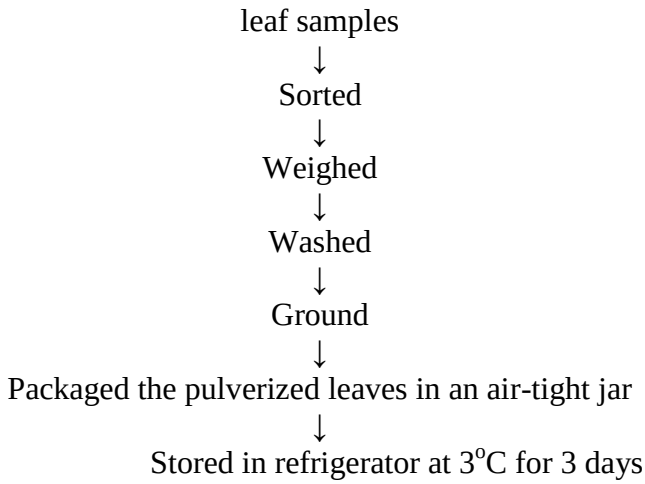


Fig. 1 Flow chart for the processing of the *Ocimum gratissimum and Pterocarpus santalinoides* leaves.

Acute toxicity and lethality test of pulverized leaves of *Ocimum gratissimum* and *Pterocarpus santalinoides*

The acute toxicity and lethality (LD_{50}) of pulverized leaves of *Ocimum gratissimum* and *Pterocarpus santalinoides* on the rats were determined using the methods described by Lorke (1983). Thirty-six albino rats obtained from Department of Veterinary Pathology, University of Nigeria Nsukka, Nigeria were used for the 2-stage tests. In stage one, the animals were grouped into 3 of 3 rats and were given 10, 100, 1000 mg/kg body weight of pulverized leaves of *Ocimum gratissimum* and *Pterocarpus santalinoide*., respectively. They were then observed for 24 hours for death and at the expiration of 24 hours, the doses were increased to 1600, 2900 and 5000 mg/kg body weight, respectively (stage 2). The administration of the leaves was done orally with canular. They were observed for 24 hours for behavioural changes or death. In absence of any behavioural changes or death at the expiration of 24 hours, these levels were accepted as safe levels of *Ocimum gratissimum* and *Pterocarpus santalinoides* for this study.

Animal study

Sourcing of Rats, Indomethacin, Omeprazole and Animal Feed

A total of 49 adult male wistar rats with no prior drug treatment, weighing between 150-250 g were used for this study. The rats were purchased from the Department of Veterinary Pathology, University of Nigeria Nsukka, Nigeria. The indomethacin and omeprazole (standard drugs) that were used in this study were purchased from Dera Pharmacy store in Nsukka, Nigeria while the animal feed - Vital feed growers mash of grand cereals, Jos was purchased from Ogige market Nsukka, Enugu State, Nigeria.

Housing and grouping of the rats

The rats (49) were divided into 7 groups of 7 rats based on their body weights with differences not greater than 5 g and housed in the animal house of the Department of Nutrition and Dietetics, University of Nigeria, Nsukka. The groups were tagged A to G.

Ulcer Induction

The rats were left to acclimatize for 3 days before commencement of the experiment. All the rats were fed poultry growers mash and water *ad libitum* throughout the period of acclimatization. The rats in groups B - G were deprived of food but had free access to water for 24 hours prior to ulcer induction. The ulcer induction solution was prepared by dissolving 100 mg of indomethacin in 10 ml of water to form concentration of 10 mg/ml. Thirty kilogramme/body weight of the prepared indomethacin solution was administered orally with a canular to rats in groups B -G (13). Two rats were randomly selected from each group and sacrificed according to the modified method of Brzozowski *et al*. (1998) to ascertain the presence of ulcer 4 hours after induction. Blood to determine the baseline data of the selected liver marker enzymes and antioxidant biochemical markers were collected. The Group A which served as a control did not receive indomethacin.

Feeding of the study animals

The animals in group C were given omeprazole the common ulcer drug served as the positive control while animals in groups D to G were fed orally with 300 and 600 mg/kg/body weights of the pulverized leaves of *Ocimum gratissimum* (PLOG*)* and *Pterocarpus snatalinoides* (PLPS*)* using canular for a period of 14 days. Table 3.1 shows the summary of the treatment given to rats in each group.

$$\text{Quantity of pulverized leaves of each rat (mg)} = \frac{Dose \times body\ weight\ of\ rat\ (g)}{1000}$$

Table 1: Summary of the experimental design

Group	Treatment	Dose (mg/kg BW)	Number of rats	Duration (Days)
A	No induction + no treatment		5	14
B	Ulcer induced but no treatment (negative control)		5	14
C	Ulcer induced + treatment with Omeprazole (positive control)	30	5	14
D	PLOG	300	5	14
E	PLOG	600	5	14
F	PLPS	300	5	14
	PLPS	600	5	14

PLPS= Pulverized leaves of *Ptroccarpus santalinoides*, PLOG = Pulverized leaves of *Ocimum grastissimum*, BW = Body weight

Collection of blood samples

Blood samples were collected from the retrobulbar plexus of the eyes of all the rats in each group using a micro capillary tube into the sample bottles. The blood was allowed to clot to obtain serum for the determination of alanine amino transferase, alkaline phosphatase, aspartate amino transferase, according to the method described by Parasuraman et al. (2010). The values were compared with the standards.

Determination of liver function enzymes

Determination of alkaline phosphatase (ALP)

Principle: Liver alkaline phosphatase was measured using the method of Tietz (1976). The principle of this method was based on the reaction involving serum alkaline phosphatase and a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turn pink that can be determined spectrophotometrically.

P- nitrophenyl phosphate + H_2O $\longrightarrow$ ALP $\longrightarrow$ PO_4^{2-} + P-nitrophenol (pink at pH = 9.8)

Method: Blank and sample test tubes were set up in duplicates and 0.05 ml of serum was pipetted into the sample test tubes while 0.05 ml of distilled water was pipetted into the blank tube. Three milliliters (3.0 ml) of phenolphthalein monophosphate was pipetted into each tube and mixed, the initial absorbance taken at 405mm. A stopwatch was started, and the absorbance of the test sample and the blank which served as the standard was read again three more times at one-minute intervals using spectrophotometry.

Alkaline phosphatase activity was calculated as:

$$Activity\ of\ ALP(\frac{U}{L}) = \frac{absorbance\ of\ sample}{absorbance\ of\ standard} \times 3300$$

Determination of Alanine transferase (ALT)

Principle: The method of Henry, Chiamori, Golub and Berkman (1960) was used. The alanine transferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4- dinitrophenyihydrazine. The colour intensity was measured against the blank at 540 m

Method: One blank and one sample test tubes were set up in duplicates and 0.1 ml of serum sample pipetted into the sample test tubes. To these was added 0.5 ml buffer solution containing phosphate buffer, L- alanine and α-oxoglutarate, mixed thoroughly and incubated for 30 minutes at 37°C and pH 7.4. A 0.5 ml of pyruvate hydrazone containing 2, 4-dinitrophenylhydrazine was later added to the blank and sample tubes while 0.l ml of the sample was added to sample blank tube. The contents of the tubes were mixed thoroughly and incubated for 20 minutes at 25°C. Five millilitres of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank using spectrophotometry after 5 minutes at 540 nm.

Determination of Aspartate transferase (AST)

Principle: The method of Henry et al. (1960) was used. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity was measured against the blank at 546 nm.

Method: The blank arid sample test tubes were set up in duplicates. A volume, 0.1 ml of serum was pipetted into the sample tubes and 0.5 ml of oxaloacetate hydrazone was pipetted into both sample and blank tubes. The solutions were thoroughly mixed and incubated for exactly 30 minutes at 37°C and pH 7.4 and 0.5 ml of Reagent 2 containing 2, 4-dinitrophenylhydrazine was added into all the test tubes followed by 0.l ml of sample into the blank tubes. The content of the tubes was mixed thoroughly and incubated for exactly 20 minutes at 25°C and 5.0 ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 546 nm.

Data analysis

Data were analyzed using the Statistical Product and Service Solutions (SPSS), version 24.0 and the results were expressed as means ± standard deviations. Analysis of variance (ANOVA) was used to generate the means while Duncans Multiple Rang test and t-test were used to separate and compare the means. Statistical significance was set at $p < 0.05$ for all the analyses.

Results

Table 4.2 delineates the effect of PLOG and PLPS on the alkaline phosphate (ALP) levels of the experimental rats. At baseline, there was no significant ($p<0.05$) difference among all the groups except for the group A which had the lowest (37.40±5.98 IU/l) value., followed by the Group F (54.80±16.02 IU/L). The endline values were similar but for the group B had significantly different high (71.20±6.98 IU/I) value.

Table 4.3 shows the effect of PLOG and PLPS on alanine transferase (ALT). Baseline values of all the groups were similar except for the group A which had the 30.20 IU/L of blood while the other groups were within the range of 57.40 IU/L to 66.60 IU/L. The endline value showed that there was no significant ($p<0.05$) difference in all the groups except for group B but there was significant difference between the baseline and endline values except the group A.

Table 4.4 displays the effect of PLOG and PLPS on aspartate transferase (AST) of the experimental rats. There were no significant differences in the baseline values of all the groups except for the group A (28.15 IU/L). The AST value of groups B - G ranged from 46.66 to 51. 96 IU/L while group A was. The endline values showed no significant ($p<0.05$) differences in all the groups except for G

Table 2: Effect of PLOG and PLPS on alkaline phosphate (ALP) of experimental rats (IU/L)

Group	Baseline	End value	p-value	t-value	% Difference
A	37.40±5.98[a]	36.00±5.10[a]	0.296	1.20	-3.74
B	61.40±6.54[bc]	71.20±6.98[c]	0.014	-4.16	15.96
C	62.80±12.28[bc]	29.20±5.89[a]	0.012	4.39	-53.50
D	57.20±7.63[b]	35.20±7.01[a]	0.019	3.78	-38.46
E	60.60±13.01[bc]	28.20±4.97[a]	0.005	5.76	-53.46
F	54.80±16.02[b]	35.00±5.24[a]	0.077	2.36	-36.13
G	64.80±11.48[bc]	31.40±6.19[a]	0.001	7.86	-51.54

Values **are means± standard deviation of duplicate determinations. Means with different superscripts in the same column are significantly different (p<0.05) PLOG=Pulverized Leaves of *Ocimum gratissimum*, PLPS-=Pulverized Leaves of *Ptroccarpus santalinoides***

Group A= Normal rats not induced, not treated)
Group B = Negative **control,** ulcer **induced** rats**, not treated;**
Group C =Positive control, u**lcer induced rats, treated with standard ulcer drug;**
Group D = Ulcer induced rats treated with 300 mg PLOG/kg body weight
Group E = Ulcer induced rats treated with 600 mg PLOG/kg body weight
Group F = Ulcer induced rats treated with 300 mg PLPS /kg body weight
Group G = Ulcer induced rats treated with 600 mg PLPS /kg body weight

Table 3: Effect of PLOG and PLPS on ALT of the experimental rats (IU/L)

Group	Baseline)	Endline	p-value	t-value	% Difference
A	34.20±4.87[a]	32.80±4.60[a]	0.439	0.86	-4.09
B	66.60±10.09[bc]	74.20±8.41[c]	0.003	-6.52	11.41
C	65.80±7.36[bc]	35.80±9.15[a]	0.003	6.40	-45.59
D	61.00±6.44[b]	33.80±5.72[a]	0.001	9.65	-44.59
E	57.40±8.02[b]	30.60±44.51[a]	0.000	11.94	-46.69

F	66.60±2.07^{bc}	30.20±2.86^{a}	0.000	18.53	-54.65
G	63.20±7.89^{b}	32.80±4.32^{a}	0.002	7.30	-48.10

Values **are means± standard deviation of duplicate determinations. Means with different superscripts in the same column are significantly different (p<0.05) PLOG=Pulverized Leaves of *Ocimum gratissimum*, PLPS-=Pulverized Leaves of *Ptroccarpus santalinoides***

Group A= Negative control (Normal rats not induced, not treated);

Group B = Positive control, rats induced, not treated;
Group C = Ulcer induced rats, treated with standard ulcer drug;
Group D = Ulcer induced rats treated with 300 mg PLOG/kg body weight
Group E = Ulcer induced rats treated with 600 mg PLOG/kg body weight
Group F = Ulcer induced rats treated with 300 mg PLPS /kg body weight
Group G = Ulcer induced rats treated with 600 mg PLPS /kg body weight

Table 4: Effect of PLOG and PLPS on AST of the experimental rats (IU/L)

Groups	Baseline	Endline	p-value	t-value	% Difference
A	28.15±2.87^{ab}	27.10±2.10^{ab}	0.438	0.86	-3.73
B	51.15±5.21^{c}	66.74±4.67^{d}	0.007	-5.04	30.48
C	51.96±5.87^{c}	29.94±1.80^{b}	0.001	7.92	-42.38
D	46.66±5.26^{c}	27.04±2.31^{ab}	0.001	9.11	-42.05
E	47.96±1.50^{c}	27.40±2.35^{ab}	0.000	13.97	-42.86
F	46.72±2.73^{c}	22.96±3.71^{a}	0.000	14.67	-50.86
G	47.16±6.31^{c}	26.06±1.51^{ab}	0.003	6.42	-44.74

Values are means± standard deviation of duplicate determinations. Means with different superscripts in the same column are significantly different (p<0.05) PLOG=Pulverized Leaves of *Ocimum gratissimum*, PLPS-=Pulverized Leaves of *Pterocarpus santalinoides*
Group A= Normal rats not induced, not treated;
Group B = Negative **control,** ulcer **induced** rats**, not treated;**
Group C =Positive control, u**lcer induced rats, treated with standard ulcer drug;**
Group D = Ulcer induced rats treated with 300 mg PLOG/kg body weight
Group E = Ulcer induced rats treated with 600 mg PLOG/kg body weight
Group F = Ulcer induced rats

Discussion

In the study, the indomethacin administered to the experimental animals caused a markable elevation in the levels of serum ALP, ALT and AST. Elevated liver enzymes are generally a sign of a problem because they can show if there is any tissue damage or inflammation in the liver (5). The finding agreed with the result of a study (6) on biochemical and histological evaluation of indomethacin-induced hepatotoxicity in rats which showed that indomethacin can cause elevated liver enzymes. It was observed in this study that pulverized leaves of *Ocimum gratissimum* and *Pterocarpus. santalinoides* reduced the elevated liver enzymes to normal values when compared with control groups (Tables 2, 3 and 4). Study by (18) on the effects of extracts of *Ocimum gratissimum* on hepatic profile of rats supported this finding. Also, a study on hepatoprotective and antioxidant activities of *Pterocarpus santalinoide* methanol leaf extract (19) recorded that the leaf has hepatoprotective effect. The reduction in the serum liver enzymes could be attributed to the bioactive compounds contained in the leaves. This was in line with the findings of a study that established that vegetables and fruits are major sources of antioxidants (20).

Conclusion

Indomethacin treatment in adult male Wistar rats elevates the activities of liver functions enzymes and pulverized leaves of *Ocimun gratissimum* and *Pterocarpus santalinoides* reduced the elevated liver enzymes to normal values.

Recommendations

There is need for further experimental studies on the effect of pulverized leaves of *Ocimum gratissimum* and *Pterocarpus santalinoides* on liver enzymes in man. Also, there should be nutrition education to encourage people to consume more *Ocimum gratissimum* and *Pterocarpus santalinoides* as they have many health benefits and agricultural extension workers should encourage people to cultivate the vegetables more to avoid their extinction

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