ANTI-ULCEROGENIC EFFECTS OF *PHYLLANTHUS AMARUS* IN RATS

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SUMMARY The effects of aqueous and methanol extracts of *Phyllanthus amarus* on indomethacin-induced ulceration, gastric acid secretion and on some haematological parameters in male albino rats were studied. The rats for the study of indomethacin- induced ulceration and haematological parameters were divided into five groups of six rats per group. Aqueous and methanol extracts of *Phyllanthus amarus* at both high and low doses of 125mg/ kg and 6.25mg/kg respectively were administered orally to the animals for a period of 15days. These doses significantly reduced indomethacin- induced ulceration in the animals ($P<0.05$). However, the methanolic extract of *P. amarus* (MePa) appears to be more effective than the aqueous extract of *P. amarus*. Some haematological parameters namely Packed Cell Volume (PCV), haemoglobin(Hb) concentration, and Red Blood Cell count (RBC) were investigated. While methanolic extract of P. *amarus* caused significant increase on the investigated haematological parameters, low dose of aqueous extract of *P. amarus* significantly ($P<0.05$) reduced haematological parameters.

Key Words: *Phyllanthus amarus*, gastro-protective, peptic ulceration.

Introduction

Phyllanthus amarus belongs to the family Euphorbiaceae. It is a widely distributed herb in Southern Nigeria where it is often regarded as a weed. It is a highly valued medicinal herb by traditional medicine practitioners.

The plant's preparation is used for the treatment of the hepatitis-B virus infection(Yeh et al, 1993). It has also been reported to cause diuresis, hypertension and hyperglycaemia in non-diabetic hypertensive female subjects(Srividya et al, 1995). The local users in Southern Nigeria claim that the cold decoction of its leaves is effective as a remedy for diarrhea, while another report also have it that the decoction of the plant is used as purgative(Gill, 1992), and to relieve stomach ache(Iwu, 1993). Other studies have demonstrated the anti-diarrhoea efficacy as well as the protective effect on some intestinal mucosal enzymes(Odetola and Akojenu, 2000). The present study investigated the effect of both aqueous and methanol extracts of *Phyllanthus amarus* on experimentally-induced ulceration and some blood parameters in albino rats.

Materials and Methods

Plant Material and Preparation of extracts

Phyllanthus amarus were collected in the vicinity of the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria between July and August 2000. They were authenticated by Prof A. Egunyomi of the Department of Botany and Microbiology, University of Ibadan, Nigeria. Voucher specimen of *P. amarus* (No UIH 22243) were deposited at the University of Ibadan herbarium

Large quantity of the plant's leaves was oven-dried at 40°C to constant weight. The leaves were ground to a powdered form. 50g of the ground substance was taken each time and were soxhlet-extracted with distilled water at 100 °C for 6hours. The filtrate was evaporated to dryness to give 1.68±0.03g of aqueous extract. Another 180g of the dried powdered sample was soxhlet-extracted using methanol as the solvent. The latter extract was concentrated in-vacuo and evaporated to dryness to give a yield of 13.33%.

Animals:

Indomethacin – induced ulceration study

Thirty albino rats of both sexes were divided into five (5) groups, which were given 0.2ml normal saline, low and high doses of the aqueous extract, and low and high doses of methanol extract for 15 days. These animals received a high dose of 125mg /kg (LD_{20} reported by Odetola and Akojenu(2000), and low dose of 6.25mg/kg of both extracts. The extracts were administered orally using oral dosing needle. These animals were used in the study of gastric ulceration and in the determination of blood parameters.

Gastric Acid Secretory Study

In gastric acid secretory study, sixteen adult male rats were used. These were divided into four experimental groups of four animals per group. The control animals received 0.2ml of distilled water daily for 15days while the other three groups received different doses (60, 90 & 120mg/kg BW) of AePa daily for 15 days with volume not exceeding 0.2ml. The distilled water and the extract were given orally.

Induction and Assessment of Ulcer in Rats

After 15 days of extract administration, gastric ulceration was induced in the animals by using the technique described by Djahanguiri(1969). After an overnight fast of the animals, indomethacin (Merck, Sharp and Dohme, Ltd) was administered in its suspension to each animal at a dosage of 40mg / kg body weight via oral route. The animals were later sacrificed after 4 hours by a blow to the head and their abdomen opened. The stomach of each animal was cut opened along the lesser curvature and the gastric contents were gently removed by means of cotton wool moistened with normal saline. Each stomach was examined macroscopically for the presence and scoring of gastric ulceration as described below by Alphin and Ward (1967).

Scoring system

Criteria	**Ulcer Score**
Normal Stomach	0
Punctuate haemorrhagic or pin-point ulcers	0.5
Two or more small haemorrhagic ulcers	1.0
Ulcers greater than 3mm in diameter	2.0

Determination of Blood Parameters

Blood samples were collected from the tail of each rat in all the groups before the induction of gastric ulceration. Standard laboratory methods were used for Red Blood Cell (RBC) counts, Packed Cell Volume (PCV) and for measuring haemoglobin (Hb) concentration.

Preparation of Animals for Gastric Acid Secretory Study

The rats used fasted overnight with free access to water so as to give a reasonably clean stomach before the start of the experiments. Prior to gastric acid assay, these animals were put under general anaethesia induced with an intraperitoneal injection of urethane solution (25% w/v) at 0.6ml per 100g body weight. The animals were surgically prepared for gastric acid secretion study according to the method described by Ghosh and Schild (1958).

The stomachs of the rats were perfused with warm distilled water. The rats' body temperatures were maintained with an electric bulb using a lamp. The flow-rate from the stomach was adjusted to give an effluent volume of 1+ 0.1 ml per minute using Watson Marlow flow-inducer. The gastric effluent was collected at 10 minutes intervals for 1hr and titrated against 0.001N NaOH using phenolphthalein as indicator. Titratable acid in the effluent was expressed in µeq/10min

Statistical Analysis

Results in all the experiments were expressed as Mean ± SEM. These results were compared by the use of the student's t-test to establish the significance of the differences at the level of $P< 0.05$.

Results

Experimental – Ulceration

Results of the Mean Ulcer Score (MUS) are presented in Table 1. In the control group, the MUS recorded was 20.67 ± 0.56, while the MUS for animals treated with aqueous extract of *Phyllanthus amarus* (AePa) at low and high doses were 10.33 ± 0.42 and 6.58 ± 0.61

respectively. In both cases, the marked reduction in MUS were significant (P<0.05). The Mean Ulcer Score for rats treated with low and high doses of methanol extract of *Phyllanthus amarus* (MePa) were 7.17 ± 0.95 and 5.00 ± 1.46 respectively. These values show significant decreases in MUS compared with the rats in the control group (P<0.05).

Haematological Parameters

The results of haematogical tests carried out on Packed Cell Volume, Red Blood Cell counts and Haemoglobin concentration are shown in Table 2. Statistically significant differences were observed in animals treated with methanol extract of *Phyllanthus amarus (MePa)*. Packed Cell Volume, Haemoglobin concentration and Red Blood Cell counts were all increased in both animals treated with low and high doses (P<0.05). A significant reduction in PCV, Hb concentration and Red Blood Cell counts were found with animals given low doses of aqueous extract of *Phyllanthus amarus* (P<0.05). There was however no significant differences in PCV, Hb concentration and Red Blood Cell counts among animals that received high dose of aqueous extract of *Phyllanthus amarus* (P>0.05). Different doses of AePa were tested on gastric acid secretion (GAS).The results are shown in Table 3. The mean basal gastric acid secretion for the control was 2.60 ± 0.28 µeq/10min.There were decreases in the mean values of GAS at all levels of doses used. However, these decreases were not statistically significant (P>0.05).

Table 1: Shows Mean Ulcer Scores in Rats Treated with Aqueous and Methanolic Extracts of *P. amarus*

Group	Mean ulcer score
Control	20.67±0.56
AePa (L.D)	*10.33±0.42
AePa (H.D)	*6.58 ±0.61
MePa (L.D)	*7.17±0.95
MePa (H.D)	*5.00±1.46

H.D= 125mg/kg L.D= 6.25mg/kg P < 0.05 compared to Control

Table 2: Effects of *Phyllanthus amarus* Extracts on Haematological Parameters in Albino Rats

Haematological Parameters	Control Group	Treatment Groups			
		AePa		MePa	
		Low Dose	High Dose	Low Dose	High Dose
PCV(%)	49.20 ±0.58	*23.80±0.37	50.40± 1.03	*53.55± 0.43	*52.43± 1.03
Hb (g/dl)	16.40± 0.20	*7.90 ±0.13	16.74± 0.36	*17.75± 0.19	*17.38± 0.35
Rbc (million/µl)	5.90 ±0.06	*3.52± 0.02	5.96 ±0.07	*9.20 ±0.13	*9.30 ±0.20

*P<0.05 compared with the Control, n (number of animals used per group) = 6 AePa = Aqueous extract of *Phyllanthus amarus* MePa = Methanol extract of *Phyllanthus amarus*

Table 3: Effect of Graded Doses of Aqueous Extract of *Phyllanthus amarus* (AePa) on Gastric Acid Secretion in Albino Rats.

Treatment Groups	Dosage (mg/kg)	GAS Mean ±SEM (µeq/10 min)
Control	-	2.60 ± 0.28
AePa-treated	60	2.50 ± 0.07
AePa-treated	90	2.65± 0.08
AePa-treated	120	2.35 ±0.14

n = (number of animals per group) =4, *P<0.05 compared with control GAS = Gastric Acid Secretion

Discussion

Phyllanthus amarus is traditionally used for the treatment of diseases of widely different physiological or biochemical causes among which are gastrointestinal disorders such as diarrhea(Gill,1992) and stomach ache(Iwu,1993). Earlier work by Odetola and Akojemu(Odetola and Akojenu, 2000) have shown that *P.amarus* confers some degree of protection on gastrointestinal enzymes (dissacharides and ALP) in animals treated with castor oil. Both aqueous (AePa) and methanolic (MePa) extracts of *Phyllanthus amarus* at doses of 6 .25mg/kg and 125mg/kg showed anti-ulcer effect by significantly reducing the mean ulcer scores (MUS) in the treated animals compared to those of the control ($P<0.05$).

However, as shown in Table 3, AePa at different doses showed lack of significance in mean values of GAS recorded when compared to the basal gastric acid secretion ($P<0.05$). The observation suggests the gastroprotective effect of AePa and MePa may not be explained by antisecretory mode of action even though increased gastric acid secretion is an important aggressive factor in the genesis of peptic ulceration(Humphreys et al, 1960). The anti-ulcer property of these extracts could be attributed to other mechanisms, among which might include improved gastric mucosal microcirculation, and increased mucus secretion. The extracts of the whole plant have been reported to show no significant effect on gastric propulsive activity in normal rats(Obasi et al, 1993). However, it is well known that ulcers produced by necrotizing agent like alcohol (ethanol) are not inhibited by anti-secretory agents like cimetidine which increases the rate of ulcer healing by partly causing inhibition of basal gastric acid secretion(Richardson and Fordtran, 1975). They are however inhibited by agents which enhance mucosal defensive factors(Robert et al, 1997). As shown in the result of the present study, AePa and MePa were able to significantly reduce mucosal damage by indomethacin which is a potent inhibitor of prostaglandin biosynthesis. Since it has been documented that increased levels of endogenous prostaglandins enhance gastric mucosal resistance against ulcerogenic agents, the protective property demonstrated by the extracts suggests that AePa and MePa may be mediating their action by a prostaglandin – dependent mechanism.

The effects of AePa and MePa on some hematological parameters; RBC count, Hb concentration and PCV were investigated and reported in Table 2 .Both low and high doses of methanolic extract of *P. amarus* (MePa) significantly increased RBC count, Hb concentration and PCV when compared with control ($P<0.05$).These observation point to the fact that MePa possesses active principle(s) which enhances the formation of red blood cell in addition to its gastroprotective effect.

Our findings equally showed that administration of low dose of AePa to rats pre treated with the extract for 15days significantly reduced the mean values of Red Blood Cell count (RBC), Haemoglobin concentration (Hb) and Packed Cell Volume (PCV) comparable to values recorded for the control rats ($P<0.05$).This might be attributed to a likely antihaemopoietic principle present in the aqueous extract that is not in the MePa. In a preliminary study, AePa at 6mg/ml produced 20.5% level of red blood cell hemolysis using standard laboratory method (data unpublished). Further study on the effects of the various extracts of *P.amarus* on osmotic fragility is in progress in our laboratory.

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