Journal of Medicinal Plants Research Vol. 2(10), pp. 261-267, October, 2008
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2008 Academic Journals

Full Length Research Paper

Gastroprotective and antioxidant activities of *Phyllanthus amarus* extracts on absolute ethanol-induced ulcer in albino rats

O. S. Shokunbi[1]* and A. A. Odetola[2]

[1]Biochemistry Unit, Chemical and Environmental Sciences Department, Babcock University, Nigeria.
[2]Nutritional and Industrial Biochemistry Unit, Department of Biochemistry, University of Ibadan, Nigeria.

Accepted 6 October 2008

This study was designed to evaluate the gastroprotective and antioxidant effects of aqueous and acetone extracts of *Phyllanthus amarus* leaves in albino rats. *P. amarus* extracts (500 and 1000 mg/Kg) as well as cimetidine (100 mg/Kg) was administered orally once a day for two weeks before challenge with absolute ethanol (1 ml/ 200 g body wt). Pretreatment with *P. amarus* aqueous extract (500 mg/Kg) and cimetidine inhibited the ulceration damage of absolute ethanol by 59.3 and 41.2% and decreased the serum alanine aminotransferase (ALT) by 35%, 24% and aspartate aminotransferase (AST) by 7 and 6% respectively. The acetone extract (1000 mg/Kg) also significantly increased (P < 0.01) the absolute ethanol mediated decrease in the activities of gastric mucosal catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transferase (GST) by 53, 8 and 33% respectively. Cimetidine respectively caused 52, 14 and 38% significant (P< 0.01) increase on the absolute ethanol-induced decrease in the activities of CAT, SOD and GST. Furthermore, *P. amarus* aqueous extract (500 mg/Kg) and cimetidine were noted to increase the activities of liver CAT by 18 and 20%, SOD by 25 and 19% and GST by 122 and 54% respectively. However, the liver thiobarbituric acid reacting substances (TBARS) values of all the groups pretreated with *P. amarus* extracts and cimetidine were not significantly different (P < 0.05) from the ethanol group. In this study, *P. amarus* extracts appears to act as an *in vivo* natural antioxidant and an effective gastroprotective agent that is as effective as cimetidine. *P. amarus* may also offer protection against toxic effects of alcohol to the liver.

Key words: *Phyllanthus amarus*, gastroprotective activity, antioxidant activity, thiobarbituric acid reacting substances (TBARS), acute toxicity.

INTRODUCTION

Peptic ulceration is one of the common diseases affecting millions of people. It is now considered to be one of the modern age epidemics affecting about 10% of world population (Shah et al., 2006).

Mucosal damage can easily be produced by the generation of exogenous and endogenous active oxygen and free radicals. Ethanol increases super oxide anion and hydroxyl radical production and lipid peroxidation in the gastric mucosa (Bagchi et al., 1998). These and other reactive metabolites react with most of the cell components, changing their structures and functions, or contributing to other mechanisms that finally promote enhanced oxidative damage. Ethanol-induced gastric mucosal injury is associated with extensive damage to mucosal capillaries and increased vascular permeability (Kato et al., 1990; Nordmann, 1994).

Plants provide an alternative strategy in search for new drugs. There is a rich abundance of plants reputed in traditional medicine known to possess antiulcer properties. It is likely that plants will continue to be a valueble source of new molecules which may, after possible chemical manipulation, provide new and improved antiulcer drugs (Shah et al., 2006).

Phyllanthus amarus Schum. and Thonn. (Euphorbiaceae) is an annual, many branched herb, 10 to 70 cm high. It is erect and has numerous small leaves on lateral branches of the stem that give the plant the appearance of having pinnate leaves. *P. amarus* is a plant that has

*Corresponding author. E-mail: osshokunbi@gmail.com. Tel.: +2348030689633.

long been used as a medicinal agent in cultures around the world. It is indigenous to the rain forest of the Amazon and other tropical areas throughout the world (Raintree Nutrition Inc, 1996; Akobundu and Agyakwa, 1998).

P. amarus is a rich source of phytochemicals such as alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitannins, gallocatechins, *geraniin*, hypophyllanthin, phyllanthin, lignans, lintetralins, lupeols, methyl salicylate, phyllanthine, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal and tricontanol (Khanna et al., 2002).

P. amarus has been reported to be gastroprotective (Odetola and Akojenu, 2000), hepatoprotective and antiviral (Sane et al., 1995). It elicits a wide spectrum of pharmacological activities (Calixto et al., 1998). Some of the earlier studies show that it possesses antioxidant and antidiabetic properties (Regi et al., 2002), antitumors (Rajeshkumar and Kuttan, 2000) and that it suppresses the expression of hepatitis B viral mRNA in cell lines. Traditionally, *P. amarus* has been used to treat jaundice, gonorrhea, uncontrolled menstruation, dysentery and diabetes (Srividya and Periwal, 1995). It has also been used topically as a treatment for skin ulcers, sores, swelling and itchiness (Khanna et al., 2002; Kumar and Kuttan, 2004).

Considering the myriads of phytochemicals in *P. amarus*, the aim of this research is to study and evaluate the antioxidant and the gastroprotective properties of the plant acetone and aqueous extracts on ethanol-induced ulcer in rats.

MATERIALS AND METHODS

Plant materials

Phyllanthus amarus Schum. and Thonn. (Euphorbiaceae) leaves were collected at Babcock University (Ilisan Remo, Ogun State, Nigeria) environs by March 2007. They were identified and authenticated by Dr. A.A. Adebisi (an Ethnobotanist) in the Department of Forestry, University of Ibadan, Nigeria.

Some leaves were oven-dried at 55^{o}C in a Gellen Kamp drying oven; shortly after collection. This was then pulverized and stored in an air-tight container till it was needed for acetone extraction (Hagerman and Klucher, 1986).

Another batch of leaves were collected fresh and used for aqueous extraction.

Preparation of extracts

Powdered *P. amarus* leaves were extracted with 70% acetone by soaking for 72 h and filtering (Hagerman and Klucher, 1986; Hagerman, 1988). The filtrate was evaporated under reduced pressure at <40^{o}C. This resulted in a very dark brown jelly-like acetone extract, which was stored at 4^{o}C till it was required. Its yield was 29.33% (w/w) of the total weight of dry powder.

Fresh *P. amarus* leaves were also extracted with de-ionized water by soaking at 25 – 30^{o}C for 9 h and filtering. The filtrate was freeze-dried and stored at 4^{o}C as well. Its yield was 5.3% (w/w) of the total weight of wet leaves.

Acute toxicity test

The test was carried out with a modified method of Seth et al. (1972). Swiss male albino mice weighing 20 – 25 g were divided into seven groups of five animals each. The control group received deionized water (2 ml/kg, PO). The other groups received 250, 500, 1000, 2000, 4000 and 8000 mg of the acetone extract (dissolved in de-ionized water) per Kg body weight respectively. Immediately after administration, the animals were observed for the first 4 h and twice daily for 7 days for signs of any behavioral changes and mortality.

Animals

45 male albino rats (140 – 185 g) were obtained from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan. They were maintained in a standard bioclean animal room, in well ventilated cages with raised floors of wide wire mesh to prevent coprophagy. They were housed in an ambient temperature of 22 ± 3^{o}C in a 12 h light-dark cycle. They were fed with balanced laboratory chow purchased from Ladokun Feeds (Ibadan, Nigeria) and were given free access to water *ad labitium*. They were also allowed to acclimatize for one week before extract administration. All procedures were performed in sterilized conditions. All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Treatment

The rats were divided into 9 groups of 5 animals each. All treatments were administered orally by a cannula for 14 days (once daily) to each group. Groups 3 (Normal Control) and 4 received the vehicle (de-ionized water, 4 ml/Kg body weight). Group 5 received cimetidine (100 mg/Kg body weight). Groups 1, 6 and 7 received 1000, 500 and 1000 mg aqueous extract per Kg body weight respectively. Groups 2, 8 and 9 received 1000, 500 and 1000 mg acetone extract per Kg body weight respectively. On the 15th day, animals in groups 4, 5, 6, 7, 8 and 9 were challenged with absolute ethanol (1 ml/ 200 g body weight) orally. The animals were deprived of food for 24 h before the challenge with absolute ethanol but had free access to water until 1hr before ethanol administration (Rodriguez et al., 2007).

Ulceration index

One hour after absolute ethanol challenge to the animals, they were sacrificed by cervical dislocation. Their livers were removed for subsequent biochemical assays. Their stomachs were also removed and opened along the greater curvature and their lesions were examined and scored (mm^2) macroscopically, using a hand lens (Nwafor et al., 2000).

The gastric mucosa was scrapped with glass slides and stored at 4°C for subsequent biochemical assays. Ulcer index (UI) and percentage inhibition were calculated thus:

$$UI = \frac{\text{Total ulcer score}}{\text{Number of animals ulcerated}}$$

$$\text{Percentage Inhibition} = \frac{\text{UI of ethanol treated group} - \text{UI of pretreated groups}}{\text{UI of ethanol treated group}} \times 100$$

Preparation of tissue homogenate

The liver and gastric mucosa scrapings were weighed and homogenized in 4 volume of ice cold isotonic phosphate buffer, pH 7.4 and centrifuged at 10,000 g for 15 min at 4°C using cold centrifuge. The resultant supernatants were used for subsequent biochemical assays (Agnihotri et al., 2007).

Preparation of serum

After the treatment period, the animals of all groups were sacrificed by cervical dislocation. Blood sample was collected and allowed to coagulate. This was centrifuge at 3000 g for 10 min to obtain serum.

Biochemical assays

Catalase (CAT) activity of the liver and the gastric mucosa homogenate were determined according to the method of Sinha (1972). The levels of their total super oxide dismutase (SOD) activity were determined by the method of Mistra and Fridovich (1972). The activity of glutathione-s-transferase (GST) was measured according to the method of Habig et al. (1974). The levels of thiobarbituric acid reacting substances (TBARS) in the liver, taken as lipid peroxide index, were also measured according to the method of Varshney and Kale (1990). Protein content was determined by the Biuret method as described by Gornall et al. (1949) with slight modification. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also determined. ALT and AST RANDOX KITS were used- Randox Laboratories Ltd, UK.

Statistical analysis

The results are given as mean ± standard deviation (SD) and analyzed by a two-tailed student's t-test. Differences were considered statistically significant at $P < 0.05$; $P < 0.01$; or $P < 0.001$.

RESULTS

In acute toxicity study, there was no mortality at any of the tested doses by the end of 7 days observation.

Oral administration of absolute ethanol (1 ml/ 200 g body wt.) induced multiple, elongated, reddish bands of hemorrhagic erosions in rat gastric mucosa. The ethanol control group had the highest ulcer index of 45.20 ± 2.39 (Table 1). Pretreatment with *P. amarus* aqueous extract (500 mg/Kg) and cimetidine (100 mg/kg) significantly inhibited ($P < 0.001$) ulceration by 59.3 and 41.2% respectively. The acetone extract yielded a dose dependent percentage ulcer inhibition and the lower dosage of the aqueous extract had a higher ulcer inhibition.

Table 2 shows that absolute ethanol significantly increased ($P < 0.05$) the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Cimetidine and *P. amarus* extracts attenuated this effect on ALT. The effects of cimetidine and most *P. amarus* extracts on AST activities were non significant.

The effect of pretreatment with *P. amarus* extracts on antioxidant enzymes in gastric mucosa homogenate is presented in Table 3. Significant reductions ($P < 0.05$) in the gastric mucosa catalase (CAT), super oxide dismutase (SOD) and glutathione-s-transferase (GST) activities were observed in the ethanol group compared with the normal control group. Pretreatment with *P. amarus* acetone extracts (1000 mg/Kg) and cimetidine (100 mg/Kg) caused an elevation by 53 and 52% for CAT, 8 and 14% for SOD and 33 and 38% for GST respectively when compared with the ethanol group. Acetone extract produced a dose-dependent increase whereas 500 mg/Kg of the aqueous extract seems more effective.

In the liver homogenate, only the GST activity of the ethanol control group was significantly depressed (Table 4). This effect was however minimized in all the groups pretreated with *P. amarus* extracts or cimetidine. Pretreatment with *P. amarus* extracts and cimetidine elicited increased activities of CAT and SOD. The lipid peroxidation (as indicated by TBARS values) of the liver homogenate of all the pretreated groups was not significantly different from the ethanol control group. Groups that were not induced with ethanol had similar TBARS values compared with the normal control group.

DISCUSSION

Our study demonstrates the *in vivo* gastroprotective and antioxidant activities of *P. amarus* acetone and aqueous gastric ulcer (Oluwole et al., 2002). More so, Odetola and Akojenu (2000) reported the gastroprotective property of *P. amarus* aqueous extract in diarrhea-induced rats.

P. amarus acetone extract did not cause any death,

Table 1. Effect of *Phyllanthus amarus* extracts on absolute ethanol-induced gastric ulcer.

Experimental Group	Ulcer Index (mm^2)	Inhibition (%)
PA Aqueous extract (1000 mg/ Kg)	0.00 ± 0.00^{NSc}	-
PA Acetone extract (1000 mg/ Kg)	0.00 ± 0.00^{NSc}	-
Normal Control	0.00 ± 0.00	-
Ethanol (1 ml/ 200 g)	$45.20 \pm 2.39^{\#\#a}$	-
Cimetidine (100 mg/ Kg) + ethanol	$26.60 \pm 1.52^{\#\#b}$	41.2
PA Aqueous extract (500 mg/ Kg) + ethanol	$18.40 \pm 2.70^{\#\#b}$	59.3
PA Aqueous extract (1000 mg/ Kg) + ethanol	$21.00 \pm 2.74^{\#\#b}$	53.5
PA Acetone extract (500 mg/ Kg) + ethanol	$27.00 \pm 3.08^{\#\#b}$	40.3
PA Acetone extract (1000 mg/ Kg) + ethanol	$25.40 \pm 3.21^{\#\#b}$	43.8

Results are expressed as mean ± SD, n = 5. PA – *Phyllanthus amarus*
* - P < 0.05, # - P < 0.01, ## - P < 0.001, NS – Not Significant at P < 0.05.
a – Normal Control group Vs Ethanol (1 ml/200 g) group; b – Ethanol group Vs Cimetidine (100 mg/kg) + ethanol, PA aqueous extract (500 mg/Kg) + ethanol, PA aqueous extract (1000 mg/Kg) + ethanol, PA acetone extract (500 mg/Kg) + ethanol, and PA acetone extract (1000 mg/Kg) + ethanol groups; c - Normal Control group Vs PA aqueous extract (1000 mg/Kg), and PA acetone extract (1000 mg/Kg) groups.

Table 2. Effect of *P. amarus* extracts on the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities.

Experimental Group	Serum ALT Activity(U/L)	Serum AST Activity(U/L)
PA Aqueous extract (1000 mg/ Kg)	3.38 ± 0.16^{NSc}	$5.80 \pm 0.35^{*c}$
PA Acetone extract (1000 mg/ Kg)	3.18 ± 0.25^{NSc}	6.90 ± 0.56^{NSc}
Normal Control	3.48 ± 0.19	6.40 ± 0.27
Ethanol (1 ml/ 200 g)	$5.33 \pm 0.45^{\#\#a}$	$7.00 \pm 0.41^{*a}$
Cimetidine (100 mg/ Kg) + ethanol	$4.05 \pm 0.41^{\#b}$	6.60 ± 0.36^{NSb}
PA Aqueous extract (500 mg/ Kg) + ethanol	$3.48 \pm 0.20^{\#\#b}$	$6.48 \pm 0.21^{*b}$
PA Aqueous extract (1000 mg/ Kg) + ethanol	$4.10 \pm 0.52^{\#b}$	6.62 ± 0.39^{NSb}
PA Acetone extract (500 mg/ Kg) + ethanol	$4.17 \pm 0.39^{\#b}$	6.80 ± 0.45^{NSb}
PA Acetone extract (1000 mg/ Kg) + ethanol	$3.80 \pm 0.44^{\#\#b}$	7.52 ± 0.48^{NSb}

Results are expressed as mean ± SD, n = 5.PA – *Phyllanthus amarus*
* - P < 0.05, # - P < 0.01, ## - P < 0.001, NS – Not Significant at P < 0.05 a – Normal Control group Vs Ethanol (1 ml/200 g) group; b – Ethanol group Vs Cimetidine (100 mg/kg) + ethanol, PA aqueous extract (500 mg/Kg) + ethanol, PA aqueous extract (1000 mg/Kg) + ethanol, PA acetone extract (500 mg/Kg) + ethanol, and PA acetone extract (1000 mg/Kg) + ethanol groups; c - Normal Control group Vs PA aqueous extract (1000 mg/Kg), and PA acetone extract (1000 mg/Kg) groups

even at a dose of 8 g/Kg body weight; which clearly suggests the non-toxic nature of the plant extract. Toxicologists agree that any test substance that is not lethal on acute administration at a concentration of 5 g/Kg body weight is essentially non toxic (OECD, 1981; Brock et al., 1995). The non-toxic nature of *P. amarus* aqueous extract has also been reported at 5 g/Kg body weight have also reported the gastroprotective property of *P. amarus* aqueous extract in indomethacine-induced extracts in ethanol induced ulcer. Previous investigators (Sirajudeen et al., 2006).

In this study, absolute ethanol (1 ml/200 g body wt.) induced gastric ulcer in all treated animals as reported in previous studies (Coskun et al., 2004; Zullyt et al., 2007). The significant percentage inhibition of ulceration shown by the groups pretreated with cimetidine and *P. amarus* extracts suggests that these treatment agents have gastroprotective properties. 500 mg/Kg aqueous extract seems to be most potent of all the extract dosages. *P. amarus* had been reported to contain secondary metabolites like flavonoids, alkaloids, major lignans and polyphenols (Foo, 1993; Houghton et al., 1996; Chevallier, 2000); some of which are extractible by water. These phytochemicals may help to justify the gastroprotective property of *P. amarus* aqueous extract.

Furthermore, acetone extract is likely to contain a myriad of crude tannins; since plant tannins are extractible with 70% acetone (Hagerman, 1988; Khennouf et al., 2003). Previous studies have in fact speculated that *geraniin* (hydrolysable tannin) from *P. amarus* may pos-

Table 3. Effect of *P. amarus* extracts on the gastric mucosa catalase (CAT), superoxide dismutase (SOD) and glutathione -s-transferase (GST) activities.

Experimental Group	Gastric Mucosal CAT Activity (nmol of H_2O_2 decomposed/ min/mg protein)	Gastric Mucosal SOD Activity (U/min/mg protein)	Gastric Mucosal GST Activity (nmol/min/ mg protein)
PA Aqueous extract (1000 mg/ Kg)	497.84±13.05^{NSc}	277.78 ± 11.94 ^{NSc}	7.50 ± 1.22 ^{NSc}
PA Acetone extract (1000 mg/ Kg)	528.03 ± 4.82$^{\#\#c}$	284.37 ± 16.93^{NSc}	9.72 ± 0.69 ^{NSc}
Normal Control	511.36 ± 3.51	279.90 ± 16.82	9.26 ± 1.38
Ethanol (1 ml/ 200 g)	301.20 ± 3.61$^{\#\#a}$	170.63 ± 9.35$^{\#\#a}$	7.20 ± 0.47^{*a}
Cimetidine (100 mg/ Kg) + ethanol	457.85 ± 3.80$^{\#\#b}$	194.07 ± 6.43$^{\#b}$	9.95 ± 1.36$^{\#b}$
PA Aqueous extract (500 mg/ Kg) + ethanol	327.00 ± 5.18$^{\#\#b}$	194.65 ± 12.19$^{\#b}$	8.87 ± 0.56$^{\#\#b}$
PA Aqueous extract (1000 mg/Kg) + ethanol	334.18 ± 4.94$^{\#\#b}$	160.75 ± 19.34 ^{NSb}	7.33 ± 0.65^{NSb}
PA Acetone extract (500 mg/ Kg) + ethanol	373.83 ± 2.21$^{\#\#b}$	154.22 ± 16.03 ^{NSb}	8.80 ± 0.90$^{\#b}$
PA Acetone extract (1000 mg/ Kg) + ethanol	460.33 ± 5.79$^{\#\#b}$	183.74 ± 13.04 ^{NSb}	9.36 ± 1.03$^{\#b}$

Results are expressed as mean ± SD, n = 5. PA – *Phyllanthus amarus*
* - P < 0.05, # - P < 0.01, ## - P < 0.001, NS – Not Significant at P < 0.05
a – Normal Control group Vs Ethanol (1 ml/200 g) group; b – Ethanol group Vs Cimetidine (100 mg/kg) + ethanol, PA aqueous extract (500 mg/Kg) + ethanol, PA aqueous extract (1000 mg/Kg) + ethanol, PA acetone extract (500 mg/Kg) + ethanol, and PA acetone extract (1000 mg/Kg) + ethanol groups; c - Normal Control group Vs PA aqueous extract (1000 mg/Kg), and PA acetone extract (1000 mg/Kg) groups

Table 4. Effect of *P. amarus* extracts on the liver catalase (CAT), superoxide dismutase (SOD), glutathione -s-transferase (GST) activities and lipid peroxidation.

Experimental Group	Liver CAT Activity (nmol of H_2O_2 decomposed/ min/mg protein)	Liver SOD Activity(U/min/ mg protein)	Liver GST Activity (nmol/min/ mg protein)	Liver TBARS (nmol MDA/ mg protein)
PA Aqueous extract(1000 mg/Kg)	52.97 ± 0.47$^{\#\#c}$	10.76 ± 0.95$^{\#\#c}$	5.51 ± 0.68$^{\#\#c}$	581.95 ± 38.06 ^{NSc}
PA Acetone extract (1000 mg/ Kg)	53.06 ± 0.24$^{\#\#c}$	11.85 ± 1.20$^{\#\#c}$	4.63 ± 0.70$^{\#\#c}$	569.27 ± 31.65 ^{NSc}
Normal Control	45.32 ± 0.40	7.94 ± 0.72	2.84 ± 0.13	601.83 ± 34.13
Ethanol (1 ml/ 200 g)	45.20 ± 0.23 ^{NSa}	7.83 ± 0.37 ^{NSa}	1.81 ± 0.24$^{\#\#a}$	632.13 ± 37.87 ^{NSa}
Cimetidine (100 mg/ Kg) +Ethanol	54.02 ± 0.37$^{\#\#b}$	9.31 ± 0.75$^{\#\#b}$	2.79 ± 0.33$^{\#\#b}$	619.22 ± 36.25 ^{NSb}
PA Aqueous extract(500 mg/ Kg) + ethanol	53.58 ± 0.82$^{\#\#b}$	9.79 ± 047$^{\#\#be}$	4.02 ± 0.63$^{\#\#b}$	604.23 ± 41.51 ^{NSb}
PA Aqueous extract(1000 mg/ Kg) + ethanol	45.30 ± 0.32 ^{NSb}	8.46 ± 0.19$^{\#b}$	3.12 ± 0.48$^{\#\#b}$	605.41 ± 40.68 ^{NSb}
PA Acetone extract(500 mg/ Kg) + ethanol	46.04 ± 0.35 $^{\#b}$	8.27 ± 0.12^{*b}	1.92 ±0.38 ^{NSb}	587.00 ± 37.36 ^{NSb}
PA Acetone extract(1000 mg/ Kg) + ethanol	61.37 ± 0.68$^{\#\#b}$	10.81 ± 0.67$^{\#\#b}$	4.21 ± 0.61$^{\#\#b}$	636.95 ± 48.19 ^{NSb}

Results are expressed as mean ± SD, n = 5. PA – *Phyllanthus amarus*
* - P < 0.05, # - P<0.01, ## - P < 0.001, NS – Not Significant at P < 0.05
a – Normal Control group Vs Ethanol (1 ml/200 g) group; b – Ethanol group Vs Cimetidine (100 mg/kg) + ethanol, PA aqueous extract (500 mg/Kg) + ethanol, PA aqueous extract (1000 mg/Kg) + ethanol, PA acetone extract (500 mg/Kg) + ethanol, and PA acetone extract (1000 mg/Kg) + ethanol groups; c - Normal Control group Vs PA aqueous extract (1000 mg/Kg), and PA acetone extract (1000 mg/Kg) groups

sess antiulcer property, in addition to its pain relieving effect (Raintree Nurtrition Inc., 1996). Thus, the gastro-protective effect of the acetone extract may be due to its crude tannins content, as similarly reported by Khennouf et al. (1999). Our results also corroborate previous reports on the gastroprotective properties of cimetidine (an analogue of ranitidine), which was used as a positive control drug (Olaleye and Farombi, 2006; Shah et al., 2006).

P. amarus extracts significantly decreased (P < 0.05) the absolute ethanol induced increase in the activities of serum AST and ALT, which serve as the biochemical

markers of liver damage (Moss and Ralph Handerson, 1999). This suggests a hepatoprotective activity of the extracts against acute ethanol toxicity. Hepatotoxic drugs cause damage to the liver cell membranes which makes enzymes like AST and ALT leak out into serum and show increased activities (Sturgill and Lambert, 1997; Kumar et al., 2004).

The significantly decreased ($P < 0.05$) activities of the ROS scavenging enzymes, viz CAT, SOD and GST, by acute exposure to ethanol, conform to previous work of Coskun et al. (2004). These decreased activities may be due to damaging effects of free radicals or alternatively, could be due to a direct effect of aldehyde, formed from oxidation of ethanol on these enzymes (Sandhir and Gill, 1999). Our results revealed that *P. amarus* extracts could induce the activities of these antioxidant enzymes and perhaps reduce the free radicals generation and gastric mucosa damage. This induction will enhance the free radical scavenging property of the mucosa.

The liver homogenate showed increased activities of CAT, SOD and GST in the groups pretreated with *P. amarus* extracts and cimetidine. This suggests that, as *P. amarus* extracts potentiate gastroprotective and antioxidant status of the mucosa, they simultaneously improve the antioxidant status of the liver, likely by the induction of these enzymes.

Conclusion

In conclusion, our results suggest that *P. amarus* aqueous and acetone extracts are gastroprotective against acute ethanol-induced ulcer. Their gastroprotective activities might be mediated, at least partially, by their inductive effect on antioxidant enzymes such as CAT, SOD and GST that constitute endogenous scavengers of ROS. *P. amarus* simultaneously improved the antioxidant status of the liver through increased activities of CAT, SOD and GST. So, we suggest that natural antioxidants and gastroprotective agents in *P. amarus* may be effective as plant gastroprotector and thus may have some obvious therapeutic implications.

ACKNOWLEDGEMENTS

The technical assistance of Mr. Ekor Martins of Department of Pharmacology, Olabisi Onabanjo University, Nigeria is gratefully acknowledged. The authors appreciate the efforts of Miss Chiaka of Institute of Advanced Medical Research and Training (IAMRAT), University of Ibadan, Nigeria, for making available some indispensable materials. The support of Mr. Abolanle Kayode, Miss Dorcas Oisamoje, Mr. Isaac Akanmu, Miss Sharon Nwichi and Mr. Kayode Ezekiel of Biochemistry Department, University of Ibadan, Nigeria are also highly appreciated.

REFERENCES

Agnihotri N, Kaur H, Kaur N, Sarotra P (2007). Role of oxidative stress in lansoprasole- mediated gastric and hepatic protection in Wistar rats. Ind. J. Gastroenterol. 26:118-121.

Akobundu IO, Agyakwa CW (1998). A handbook of West African Weeds, 2nd Edn. International Institute of Tropical Agriculture (IITA), Ibadan, pp. 270, 271.

Bagchi D, Carryl O, Tran M, Krohn R, Bagchi DJ, Garg A, Bagchi M, Mitra S, Stohs S (1998). Stress, diet and alcohol- induced oxidative gastrointestinal mucosal injury in rats and protection by bismuth subsalicylate. J. Appl. Toxicol. 18(Suppl.1):3-13.

Brock WD, Trochimowicz HJ, Millischer RJH, Farr C, Kawano T, Rusch GM (1995). Acute and subchronic toxicity of 1,1-dichloro-1-fluroethane (HCFC-141 b). Food. Chem. Toxicol. 33:483-490.

Calixto JB, Santos ARS, Filho VC, Yunes RA (1998). A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. Med. Res. Rev. 18(4):225-258.

Chevallier A (2000). Encyclopedia of Herbal Medicine: Natural Health, 2nd edn. Dorling Kindersley Book. USA. p.336.

Coskun O, Kanter M, Armutcu F, Cetin K, Kaybolmaz B, Yazgan O (2004). Protective effect of quercetin, a flavonoid antioxidant, in absolute ethanol-induced acute gastric ulcer. Euro. J. Gen. Med. 1(3):37-42.

Foo LY (1993). Amarulone, a novel cyclic hydrolysable tannin from *Phyllanthus amarus*. Nat. Prod. Lett. 3:45-52.

Gornall AG, Barawill JC, David MM (1949). Determination of serum protein by means of biuret reaction. J. Biol. Chem. 177:751-761.

Habig WH, Pabst MJ, Jakoby, WB (1974). Glutathione -s-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130-7139.

Hagerman AE (1988). Extraction of tannin from fresh and preserved leaves. J. Chem. Ecol. 14(2):453-461.

Hagerman AE, Klucher KM (1986). Plant Flavonoids in biology and Medicine: biochemical, pharmacological, and structure-activity relationships. New York: Ed. Cody V., Middleton E. Jr., Harborne J. – Alan R. Liss, pp 67-76.

Houghton PJ, Woldemariam TZ, O'Shea S, Thyagarajan SP (1996). Two securinega-type alkaloids from *Phyllanthus amarus*. Phytochemistry 43:715-717.

Kato S, Kawase T, Alderman J, Inatomi N, Lieber CS (1990). Role of Xanthine oxidase in ethanol-induced lipid peroxidation in rats. Gastroenterology 98:203-210.

Khanna AK, Rizvi F, Chander R (2002). Lipid lowering activity of *Phyllanthus niruri* in hyperlipenic rats. J. Ethnopharmacol. 82(1):19-22.

Khennouf S, Benabdallah H, Gharzouli K, Amira S, Ito H, Yoshida T, Gharzouli A (2003). Effect of tannis from *Quercus suber* and *Quercus coccifera* leaves on ethanol-induced gastric lesions in mice. J. Agric. Food Chem. 51(5):1469-1473.

Khennouf S, Gharzouli K, Amira S, Ghazouli A (1999). Effects of *Quercus ilex* L. and *Punica granatum* L. polyphenols against ethanol-induced gastric damage in rats. Pharmazie 54:75-76.

Kumar G, Sharmila Banu G, Vanitha Pappa P, Sundararajan M, Rajasekara Pandian M (2004). Hepatoprotective activity of *Triantherma portulacastrum L.* against paracetamol and thioacetamide intoxication in albino rats. J. Ethnopharmacol. 92:37-40.

Kumar HKB, Kuttan R (2004). Protective effect of an extract of *Phyllanthus amarus* against radiation-induced damage in mice. J. Radiat. Res. 45:133-139.

Mistra HP, Fridovich I (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for super oxide dismutase. J. Biol. Chem. 247(10):3170-3175.

Moss DW, Ralph Handerson A (1999). Clinical Enzymology, Burtis, C.A. and Ashword, E.R. (Eds.), Tietz text book of clinical chemistry, WB Saunders Company, 3rd edn., Philadelphia, pp 651-683.

Nordmann R (1994). Alcohol and antioxidant systems. Alcohol 29:513-522.

Nwafor A, Paul F, Okwasaba K, Binda LG (2000). Antidiarrhoeal and antoulcerogenic effect of methanolic extract of *Asparagus pubescens* root in rats. J. Ethnopharmacol. 72:421-427.

Odetola AA, Akojenu SM (2000). Anti-diarrhoea and gastro-intestinal potentials of aqueous extract of *Phyllanthus amarus* (Euphorbiaceae). Afr. J. Med. Med. Sci. 29(2):119-122.

OECD (1981). Guidelines for the Testing of Chemicals. Organization for Economic Cooperation and Development, Paris.

Olaleye SB, Farombi EO (2006). Attenuation of Indomethaein-and HCl/ethanol-induced oxidative gastric mucosa damage of garcinia kola seed. Phytother. Res. 20(1):14- 20.

Oluwole FS, Maduabuchi NO, Odetola AA (2002). Anti-ulcerogenic effects of *Phyllanthus amarus* in rats. Nig. J. Physiol. Sci. 17(1-2):52-56.

Raintree Nutrition Inc. (1996). Chanca Piedra (*Phyllanthus niruri*). Retrieved June 12, 2007, from http://www.rain-tree.com/chanca.htm.

Rajeshkumar NV, Kuttan R (2000). *Phyllanthus amarus* extract administration increases the lifespan of rats with hepatocellular carcinoma. J. Ethnopharmocol. 73(1-2):215-219.

Regi RK, Subu MC, Kuttan R (2002). Hypoglycemic effect of methanol extract of *Phyllantus amarus* schum and Thonn on alloxan induced diabetes mellitus in rats and its relation with antioxidant potential. Ind. J. Exp. Biol. 40:905-909.

Rodriguez ZBZ, Alvarez RG, Guanche D, Merino N, Rosales FH, Cepero SM, Gonzalez YA, Schuluz S (2007). Antioxidant mechanism is involved in the gastroprotective effects of ozonized sunflower oil in ethanol- induced ulcer in rats. Mediators of Inflammation. Article ID: 65873,pp. 1-6.

Sandhir R, Gill KD (1999). Hepatoprotective effects of Liv-52 on ethanol-induced liver damage in rats. Ind. J. Exp. Biol. 37:762-766.

Sane RT, Kuber VV, Menon S (1995). Hepatoprptectionby *Phyllanthus amarus* and *Phyllanthus debris* in CCl_4 induced liver dysfunction. Curr. Sci. 20:1243-1246.

Seth UK, Dadkar NK, Kamat UG (1972). Selected topics in experimental pharmacology, 1st edn. Kothari Book Depot, Bombay, p 126.

Shah JS, Shah MB, Goswami SS, Santani DD (2006). Mechanism of action of antiulcer activity of bark extracts of *Manikara hexandra* against experimentally induced gastric ulcers in rats. Phcog. Mag. 2(5):40-45.

Sinha KA (1972). Colorimetric assay of catalase. Anal. Biochem. 47:389-394.

Sirajudeen KNS, Suliaman SA, Madhava M, Ismail Z, Swamy M, Ismail MdL, Yaacob M (2006). Safety evaluation of aqueous extract of leaves of a plant *Phyllanthus amarus* in rat liver. Afr. J. Trad. CAM 3(4):78-93.

Srividya C, Periwal S (1995). Diuretic, hypotensive and hypoglycermic effect of *Phyllanthus amarus*. Ind. J. Exp. Biol. 33(11):861-864.

Sturgill MG, Lambert GH (1997). Xenobiotics-induced hepatotoxicity; Mechanism of liver injury and method of monitoring hepatic function. Clin. Chem. 43:1512-1526.

Varshney R, Kale RK (1990). Effect of calmodulin antagonist on radiation induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 58(5):733-743.

Zullyt BZR, Ricardo GA, Dailen G, Nelson M, Frank HR, Silvia MC, Yaima AG, Siegfried S (2007). Antioxidant mechanism is involved in the gastroprotective effects of ozonied sunflower oil in ethanol-induced ulcer in Rats. Mediators of Inflammation, Hindawi Publishing Corporation,6: 6-8