REPORT

Phytochemical screening, and assessment of ameliorating effect of aqueous and ethanolic extracts of *Gmelina arborea* on drug induced hepatic and renal insufficiency in rats

Ogbonnaya Enyinnaya Anthony*[1], Awah Francis Mbuh[2] & Mounmbegna Philippe Emmanuel[2]
[1]Biochemistry Department, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria
[2]Biochemistry Department, Faculty of Science, Madonna University, Elele, Rivers State, Nigeria

Abstract: Phytochemical screening of stem bark and leaves of *Gmelina arborea*; and effect of aqueous and ethanolic extracts of *Gmelina arborea* stembark on hepatic and renal insufficiency in rats was assessed in this study. Phytochemical screening was carried out on the air-dried leaf, oven-dried leaf, air-dried stembark and oven-dried stembark samples. Sixty five (65) wister albino rats, (50.7-117.5g) were divided into thirteen groups of five animals each. Three groups serve as Controls and were administered Cisplatin (5mg/kg b.w; i.p), Paracetamol (200mg/kg b.w; i.p) and Normal saline (0.002ml/kg b.w; oral). Other groups were administered, either, cisplatin and extracts (1g/kg b.w; oral); Paracetamol and extracts (1g/kg b.w; oral); extracts alone; or drugs and combination of extracts. Animals were starved, 24 hours prior to sacrifice and sacrificed on the 9th day after commencement of treatment. Phytochemical screening results show the presence of alkaloid, flavonoid, tannin, saponin, cyanogenic glycoside, phytate, and carbohydrate. Saponin and carbohydrate were shown to be much higher in concentration than other phytochemicals. The percentage composition of cyanogenic glycoside and phytate were highest in air-dried stembark and oven-dried leaf samples, respectively. All the *Gmelina arborea* extracts and extract mixture administered to both paracetamol and cisplatin treated animals, significantly, lowers both the activities of the SGOT and SGPT, and the levels of serum creatinine and urea. When administered alone, the aqueous and ethanolic extracts show little or no sign of toxicity. Thus *Gmelina arborea* extracts may have ameliorating effect on hepatic and renal insufficiency caused by paracetamol and cisplatin respectively, and any inherent toxicity may be reduced or eliminated through adequate heat treatment.

Keywords: *Gmelina arborea* ,cisplatin, paracetamol, hepatic, renal insufficiency.

INTRODUCTION

Cisplatin is a platinum containing complex known to exhibit great activity against neoplastic cells by interfering with the multiplication of cancer cells. It is usually used in treatment of cancer of the ovaries, cervix, testicles, endometrium, bladder, head, and neck (Brady, 2000). Cisplatin is rapidly and well absorbed systematically following intravenous (i.v) administration and it is not effective when administered orally. About 90% of the drug binds to plasma proteins and are concentrated in tissues such as kidney, liver, large and small intestine, but not in the brain. Cisplatin is mainly excreted by glomerular filteration in the urine (Akubue, 2006).

Being a cytotoxic agent, cisplatin affects a wide variety of cells. Its toxic effects can be generally grouped into gastrointestinal, audiological, hematological and renal.

The kidney is the principal target organ for cisplatin toxicity, as it affects the renal proximal tissues (Gonzalez-Vitale *et al*., 1999).

**Corresponding author:* e-mail: ogboenyi@yahoo.com

Paracetamol (acetaminophen), the active metabolite of Phenacetin, is one of the most commonly used non-narcotic analgesic and antipyretic agents. At normal doses paracetamol is extensively metabolized by liver microsomal enzyme to yield glucuronide (about 60%) or sulphate (about 37%). Large doses of paracetamol are metabolized to toxic intermediates, leading to serious hepatic or renal side effects (Bradley, 1999). Acute over dosage (10-15g) results in hepatic toxicity due to the formation of the reactive metabolite, N-acetyl-Beta benzoquinone imine.

Gmelina arborea (Family: Verbenaceae) is among the plants known to have therapeutic effects. The root and bark are stomachic, galactogogue, laxative and antihelminthic. It improves appetite, useful in hallucination, piles, abdominal pains, burning sensations, fevers and urinary discharge (Hartwell, 1995).

Leaf paste is applied to relieve headache and juice used as wash for ulcers. Flowers are sweet, cooling, bitter, acrid and astringent. They are useful in leprosy and blood diseases (Duke, 1985). The plant in combination with other drugs is used for treatment of snake bite and

scorpion sting. In snake bite a decoction of the root is given internally (Morataya, 1999). Boiled leaves are used for inflamed gums while infusion from fruit is used as eye lotion. So far there is no information on the effect of *Gmelina arborea* extracts on the liver and kidney, and this is the subject of this study.

MATERIALS AND METHODS

All the chemicals used in this study were of highest grade commercially available (from Sigma-Aldrich). Assay Kits were either Randox (Creatinine, Urea, and GPT) or QCA (GOT).

Plant samples

Gmelina arborea stem bark and leaves were collected from the wild in Imo State, S.E- Nigeria and identified at Plant Science and Biotechnology Department, University of Port Harcourt.

Part of the leaf samples were divided into two equal portions; one portion air-dried for 10 days and the other oven-dried at 60^{o}C, for two days, in an electric oven. Part of the stem bark were also divided into two portions, and treated similarly. All dried samples were collected and reduced to fine powder using a mechanical grinder to obtain air-dried leaf sample (**G1**), Oven-dried leaf sample (**G2**), air-dried stem bark (**G3**) and oven-dried stem bark (**G4**).

The remaining portion of the freshly collected stem bark sample was divided into two parts; one part was air-dried for 10 days and the other portion used for hot aqueous extraction (decoction).

Phytochemical analysis

Four plant samples; **G1**, **G2**, **G3**, and **G4** were individually subjected to phytochemical screening. The presence of alkaloid was determined by Dragendorfs, Wagner, and Harger's test as described by Iweala, (2009). Also the presence of flavonoid was determined by forrestal, BAW, and ammonium test as described by Iweala (2009). Tannins (Ferric chloride test), saponin (frothing test), Cyanogenic glycoside, Phytate and Carbohydrate were quantitatively determined by standard procedure as described by Edoga *et al.* (2005) and Sofowora (1992) .

Extraction of plant samples

Equal weights (350g) of powdered air-dried *Gmelina arborea* stem bark were extracted by 24- hour maceration, separately, in 80% ethanol and distilled water. Extracts were filtered in vacuo and filtrate concentrated using a rotary evaporator and then reduced to a constant weight.

Also fresh *Gmelina arborea* stem bark (500g) was placed in appropriate amount of distilled water and heated to boiling. Boiling was allowed for about 70mins, followed by cooling to room temperature. The mixture was filtered in vacuo and filtrate concentrated using rotary evaporator, and then reduced to a constant weight.

Animal treatment

Sixty five (65) wister albino rats, 3-4 weeks old (50.7-117.5g) were obtained from the animal house of Faculty of Verterinary Medicine, University of Nigeria, Nsukka.

The animals were housed in wire cages in a ventilated animal house at 27^{o}C and had unlimited access to standard rat chow and water.

The animals were divided into thirteen groups of five animals each. Three groups serve as Controls and were administered Cisplatin (5mg/kg b.w; i.p), Paracetamol (200mg/kg b.w; i.p) and Normal saline (0.002ml/kg b.w; oral). Other groups were administered, either, cisplatin and extracts (1g/kg b.w; oral); Paracetamol and extracts (1g/kg b.w; oral); extracts alone; or drugs and combination of extracts.

Animals were starved, 24 hours prior to sacrifice and sacrificed on the 9th day after commencement of treatment. Animals were sacrificed under mild chloroform anesthesia and blood collected by cardiac puncture. Blood were allowed to stand for 10-15 minutes and centrifuged at 4000 rev/min for 15 minutes. Serum was used for further analysis.

Serum assays

Serum Glutamate-oxaloacetate transaminase (SGOT) and Glutamate-pyruvate transaminase (SGPT) assays were carried out on all Paracetamol and Normal saline treated groups. Both SGOT and SGPT were assayed by colourimetric method (Reitman and Frankel, 1957). Serum Creatinine and Urea assays were carried out for all Cisplatin and Normal saline treated groups. Creatinine was assayed essentially by modified Jaffe method as described by Blass *et al.* (1974). Serum Urea was assayed by urease-Berthelot method as described by Weatherburn (1967).

STATISTICAL ANALYSIS

The data obtained from the study were analyzed statistically using Analysis of Variance (ANOVA). Post-hoc comparisons were made using the Bonferron's test. A $P< 0.05$ was considered statistically significant.

The phytochemical screening result (table 1) shows the presence of alkaloid, flavonoid, tannin, saponin, cyanogenic glycoside, phytate, and carbohydrate. Saponin

RESULTS

Table 1: Phytochemical screening result of *Gmelina arborea*

S.No.	Test	Air- Dried Leaf Sample **G1**	Oven Dried Leaf Sample **G2**	Air dried Stem bark **G3**	Oven dried Stem bark **G4**
1	Alkaloid	+	+	+	+
2	Flavonoid	+	+	+	+
3	Tannin (%)	0.25±0.01	0.38±0.03	0.22±0.01	0.27±0.02
4	Saponin (%)	8.95±0.21	8.81±0.13	14.90±0.14	19.73±0.11
5	Cyanogenic glycoside	0.53±0.02	0.54±.01	1.10±0.02	0.57±0.04
6	Phytate (%)	0.23±0.01	0.69±.04	0.23±0.01	0.42±0.03
7	Carbohydrate (±)	10.37±0.23	12.99±0.25	15.45±0.49	10.15±0.21

Value is % Mean± SD of duplicate determination. + means qualitatively, present.

Table 2: Serum Glutamate-oxaloacetate transaminase (SGOT) and Glutamate-pyruvate transaminase (SGPT) assay result.

Group n=5	Treatment	SGOT Mean ± SD (U/L)	SGPT Mean ± SD (U/L)
I	Normal saline alone	1.98 ± 0.69	27.64 ± 6.02
II	Paracetamol alone	13.21 ± 2.33[a]	59.60 ± 10.69[a]
III	EtOH Extract alone	7.50 ± 1.57[a]	24.30 ± 1.92
IV	Aqueous Extract alone	7.51 ± 1.59[a]	24.06 ± 1.92
V	Paracetamol + EtOH Extract	7.16 ± 1.57[ab]	20.58 ± 4.81[b]
VI	Paracetamol + Aqueous (Fresh) Extract	6.74 ± 1.11[ab]	18.30 ± 0.96[b]
VII	Paracetamol + Aqueous (Dry) Extract	7.28 ± 1.14[ab]	20.42 ± 4.81[b]
VIII	Paracetamol + EtOH Extract + Aqueous (Fresh) Extract	7.09 ± 1.32[ab]	18.32 ± 1.17[b]

[a]Significant difference (P< 0.05) compared to Group 1; [b]Significant difference (P< 0.05) compared to Group 2.

Table 3: Serum creatinine and urea assay result

Group n=5	Treatment	Creatinine Mean ± SD (mg/dl)	Urea Mean ± SD (mg/dl)
I	Normal saline alone	5.83 ± 0.34	26.66 ± 4.10
II	Cisplatin alone	8.46 ± 0.20[a]	33.97 ± 3.80[a]
III	Cisplatin + EtOH Extract	2.50 ± 0.12[ab]	21.30 ± 0.34[b]
IV	Cisplatin + Aqueous (Fresh) Extract	2.93 ± 0.15[ab]	24.87 ± 1.61[b]
V	Cisplatin + Aqueous (Dry) Extract	2.73 ± 0.10[ab]	25.85 ± 2.33[b]
VI	Cisplatin + EtOH Extract + Aqueous (Fresh) Extract	2.40 ± 0.11[ab]	20.38 ± 2.18[ab]

[a]Significant difference (P< 0.05) compared to Group 1; [b]Significant difference (P< 0.05) compared to Group 2.

and carbohydrate were shown to be much higher in concentration than other phytochemicals. The percentage composition of cyanogenic glycoside and phytate were highest in G3 and G2 respectively. Also G4 and G3 show the highest composition of saponin and carbohydrates respectively of all the samples screened.

Table 2 shows the result of SGOT and SGPT assays for the various animal treatments. Group II animals show significant increase in the activity of the enzymes compared to control (Group I). For SGOT assay, there were significant changes in the activities of the enzyme for other groups compared to groups I and II. In SGPT assay, while groups III and IV showed insignificant difference when compared to Group I, the enzyme activities of all the groups are significantly different from Group II.

In creatinine and Urea assay results (table 3), there were significant difference in the levels of these parameters in Group II, compared to Group I. There were significant changes in the level of creatinine for all other groups compared to groups I and II. The changes in the level of urea for groups III, IV, V and VI were all significant compared to Group II.

DISCUSSION

There were relative increases in the percentage compositions of tannin, saponin, phytate and carbohydrate in the oven-dried leaf samples (G2). This is may be relative to the moisture content of the sample which is expected to be less compared to the air-dried sample (G1). Except for carbohydrate, this trend was also observed for oven-dried stem bark (G4), compared to the air-dried sample (G3). The reduction in the levels of cyanogenic glycosides and carbohydrates in this sample (G4) may be an indication of the heat lability of the glycosides which is also part of the observed carbohydrate level.

The phytochemicals; alkaloids, flavonoids, tannins, saponins, and cyanogenic glycosides have shown diverse pharmacological, nutritional and medicinal actions when ingested by animals or man (Amadi *et al*., 2006).

Alkaloids are known for their bitter and sweet sensations. Their resemblance with neurotransmitters had made possible their use as powerful analgesics (pain-killer medications). They also have anti-malarial and anti-leukemic properties (Angela and Graham, 1991). The presence of this phytochemical supports the use of *Gmelina arborea* in the treatment of abdominal pains, burning sensation, gum inflammation, and blood diseases. Tannins reduce digestibility of proteins by binding to, and precipitating proteins (Bryant *et al*., 1992). They can be used to extract poisons. They also have antihelminthic (Molan *et al*., 2000a, b, 2002; Elgamal *et al*., 1995) and analgesic properties. This supports the plant's use as anti-poison, anthelminthic and in treatment of headache. Saponins break down membranes of red blood cells (Ezeagu *et al*., 2003) and also have anti-ulcer activities (Zhang and Hu, 1985; Aguwa and Okonji, 1986; Marhuenda *et al*., 1993).

Both paracetamol (200mg/kg bw) and cisplatin (5mg/kg bw) were able to induce liver and kidney dysfunctions respectively, at the dosages administered, as shown by the increases in the activities of the serum enzymes(SGOT and SGPT) and the levels of the kidney function parameters (creatinine and urea). These increases were ameliorated by administration of *Gmelina arborea* extracts, with no significant difference in the values for aqueous compared with ethanolic extract. As shown in Table 2, treatment with fresh aqueous extract (Group VI) seems to have similar effect as treatment with dried aqueous extract (Group VII). Also both aqueous and ethanolic extracts have similar effect on the animals.

All the extracts and extract mixture administered to the cisplatin treated animals, have similar effects on the animals, and were able to lower the creatinine and urea levels, significantly. When administered alone, the aqueous and ethanolic extracts show little or no sign of toxicity. Any toxicity may, in addition to other factors, be attributed to cyanogenic glycoside content. These, on hydrolysis yield toxic hydrocyanic acid (HCN), which ions inhibits several enzyme systems; depress growth through interference with some essential amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death (Osuntokun, 1972; Fernando, 1987).

Thus *Gmelina arborea* extracts may have ameliorating effect on hepatic and renal insufficiency caused by paracetamol and cisplatin respectively, and any inherent toxicity may be reduced or eliminated through adequate heat treatment.

REFERENCES

Aguwa CN and Okonji CO (1986). Antifungal effects of extracts of some Nigerian herbal plants. *J. Ethnopharmacol.* **15**: 45.

Amadi BA, Ibegbulen CO and Egbebu AC (2006). Assessment of the effect of aqueous extract of pawpaw (*Asimina triloba*) root on organ weights and liver functions of albino rats. *Int. J. Natl. Applied Sci.* **2**: 79-81.

Angela S and Graham W (1991). Plant Cell and Tissue Culture 91, pp.124-131.

Akubue PI (2006). Drugs used for treatment of rheumatoid arthritis, first edition, Africana Publishers Limited, Onisha, pp.165-167.

Blass KG, Thierbert RJ and Lam LK (1974). A study of the mechanism of the Jaffe reaction. *J. Clin. Chem. Clin. Biochem*. **12**: 336-343.

Bradley JD and Brandth KD (1999). Comparison of an inflammatory dose of ibrofen and acetaminophen in the treatment of patients with oestroarthritis of the knee. *New Engl. J. Med*., **25**: 87-91.

Brady HR (2000). Mitochondrial injury: An early event in cisplatin toxicity to renal proximal tubules. *Am. J. Phsiol.* **258**: 1181-1195.

Bryant JP, Reichard PB and Clausen TP (1992). Chemically mediated interactions between woody plants and browsing mammals. *J. Range*, **45**: 18-24

Duke JA (1985). Handbook of Medicinal Herbs. CRC Press, FL, pp.120-121.

Edeoga HO, Olawu DE and Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* **4**(7): 685-688.

Elgamal MHA, Shaker KH, Pollman K and Seifert K (1995). Triterpenoid saponins from *Zygophyllum species*. *Phytochemistry*, **40**(4): 1233-1236.

Ezeagu IE, Maziya-Dixon B and Tarawali G (2003). Seed characteristics, nutrient and anti-nutrient composition of 12 Mucuna accessions from Nigeria. *Tropical and Subtropical Agroecosystems*, **1**: 129-139.

Fernando R (1987). Plant poisoning in Sri Lanka. *In*: Progress in venom and toxin research. Proc. of the 1st Asia-Pacific Congress in Animal, Plant and Microbial Toxins, pp.624-627.

Gonzalez-Vitale JC, Hayes DM, Cvitkovic E and Stemberg SS (1978). Acute renal failure after Cisdichlorodiammineplatinum (II) and gentamicin-cephalothin therapies. *Cancer Treat. Rep.,* **62**: 693.

Hartwell G (1995). Prevention and management of extravagation of cytotoxic drugs. *Drug safety*, **12**: 245-255.

Iweala EEJ (2009). Preliminary qualitative screening for cancer chemopreventive agents in *Telfairia occidentalis* Hook f, *Gnetum africanum* Welw, *Gongronema latifolium* Benth and *Ocimum gratissimum* L. from Nigeria. *Journal of Medicinal Food Plants*, **1**(2): 58-63.

Marhuenda E, Marbin MJ and DelaCastra CA (1993). Anti-ulcerogenicactivity of Aescine in different experimental models. *Phytother. Res.,* **7**: 13.

Molan AL, Duncan A, Barry TN and McNabb WC (2000a). Effects of condensed tannins and sesquiterpen lactones extracted from chicory on the viability of deer lungworm larvae. *Proc. New Zealand Soc. Anim. Prod.,* **60**: 25-29.

Molan AL, Hoskin SO, Barry TN and McNabb WC (2000b). Effect of condensed tannins extracted from four forages on the viability of the deer lungworms and gastrointestinal nematodes. *Vet. Rec.,* **147**: 44-48.

Molan AL, Waghorn GC and McNabb WC (2002). Effect of condensed tannins on egg hatching and larval development of *Trichostrongylus colubriformis in vitro*. *Vet. Rec*., **150**: 65-69.

Morataya RGG *et al*. (1999). Foliage biomass-sapwood relationships of Tecona grandis LF and *Gmelina arborea* Rotbi silv and cultural implications. *Forest Ecology and Management*, **113**: 231-239.

Osuntokun BO (1972). Cassava diet and cyanide metabolism in Wistar rats. *Brit. J. Nutr*., **24**: 797-805.

Reitman S and Frankel S (1957). A colorimetric method for the determination of Serumglutamate - oxaloacetic acid and glutamate – pyruvic acid transaminases. *Amer. J. Clin Path.,* **28**: 56-63.

Sofowora EA (1992). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., John Wiley and Sons, Nigeria, pp.134-156.

Weatherburn MW (1967). Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.,* **39**: 971-974.

Zhang S and Hu Z (1985). Anti-ulcerogenic effects of Ginseng flowersaponins in the rat. *Zhongyao. Tongbao* **10**: 331, *Chem. Abstr.,* **104**: 512.