Original Article

Hydro-methanol leaf extract of lemon grass is friendly with the histology of albino Wistar rats' kidneys

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Abstract

Introduction: Lemon grass (*Cymbopogon citratus*) is an aromatic perennial tall grass with rhizomes densely tufted fibrous root. The aim of the study was to determine the histological effect(s) of the hydro- methanol leaf extract of lemon grass (HLELG) on Albino Wistar rats kidney. The objectives were to: (a) Determine the LD50 of HLELG. (b) Determine if there is any effect(s) of HLELG on the kidney. (c) Determine whether the histological effects of the HLELG is dose-dependent (d) Determine whether the histological effects of the HLELG is time-dependent. Twelve (12) male albino wistar rats were used to determine the LD50 of HLELG. **Materials and Methods:** Thirty-five albino wistar rats were recruited for this experiment. After 2 weeks of acclimatization, they were divided into three experimental groups (A, B and C) of 10 rats per group and a control group of 5 rats. Experimental groups (A, B and C) ingested 500 mg/kg, 1000 mg/kg and 2000 mg/kg of HLELG respectively once daily. Two rats from each experimental group were sacrificed at the end of each week for three consecutive weeks. Two rats were sacrificed from the control group at the end of the third week. Kidneys from each rat were collected and fixed in 10% formal saline. They were processed for routine staining with Heamatoxylin and Eosin (H and E). **Results:** The LD50 "intraperitoneal" of HLELG was found to be higher than 10,000 mg/kg. Obliteration of the Bowman's capsule was seen in; experimental group C at the end of week one, experimental groups A-C at the end of week two and experimental group C at the end of week three. **Discussion:** The obliteration of Bowman's capsule is as a result of hypertrophy of the glomerular tufts and it is likely to be due to essential oil of lemon grass which has been reported to be toxic against mice. **Conclusion:** The index study concluded that: (a) The LD50 of the HLELG is above 10,000 mg/kg. (b) HLELG distorts the histology of albino wistar rats' kidneys. (c) The effects of HLELG on albino wistar rats' kidneys are both time and dose-dependent.

Key words: Albino Wistar, hydro-methanol, lemon grass

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INTRODUCTION

Lemongrass (*Cymbopogon citratus*) is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Carlini *et al*., 2008). Lemongrass has been used by the Brazilian Quilombolas tribe to decrease blood pressure and to calm individuals (Rodrigues and Carlini, 2004).

Lemongrass has been traditionally used to treat gastrointestinal discomforts (Devi *et al*., 2011). Indians have used it as a medicinal plant for more than 2000 years. It is widely used in Asian cuisine for its citrus flavor. The tea from its leaves has been widely used as an antiseptic, febrifuge, carminative, and tranquilizer (Selvi *et al*., 2011). The genus *Cymbopogon* belongs to the grass family, *Poaceae* (syn. Gramineae). The *Poaceae* family has about 700 genera and 11,000 species: Widely distributed in all regions of the world. *Cymbopogon* is a genus comprising about 180 species, subspecies, varieties, and subvarieties (Bertea and Maffei, 2010).

There are two main types of lemongrass: East Indian lemongrass (*Cymbopogon flexuosus* [Nees ex Steud] J. F. Watson) which is considered to have its origin from Southern India. West Indian lemongrass (*C. citratus* [Dc ex Nees]) Stapf which is thought to have its origin in Malaysia and is mainly cultivated in Central and South America and parts of Africa, South East Asian, and the India Ocean Islands. Both species produce an essential oil rich in citral.

The chemical composition of the essential oil of *C. citratus* varies according to the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, and mainly aldehydes have constantly been registered (Abegaz and Yohanne, 1999).The essential oil (0.2–0.5%, West Indian lemon grass) consists of mainly citral (Ming *et al*., 1996). Citral and myrcene have been shown to induce maternal toxicity in pregnant rats in high dosages (Fandohan *et al*., 2008). Topical application of lemon grass has rarely led to an allergic reaction (Fandohan *et al*., 2008). On the contrary (Leite *et al*., 1986) conducted a study, which showed that lemon grass has no potential toxic properties. Therefore, it is better to say that Lemon grass is not generally safe.

Lemon grass leaves has many uses, ranging from aromatherapy to medicinal therapy. An infusion of lemongrass given orally to male and pregnant female rats for 2 months in doses up to 20 times the corresponding human dose did not induce any toxic effects. No external malformations were noted in the pups (Souza *et al*., 1986). Lorenzetti *et al*. (1991) worked on peripheral analgesic effect of myrcene which was confirmed by testing a standard commercial preparation of myrcene on the hyperalgesia induced by prostaglandin in the rat paw test and the contortions induced by intraperitoneal (ip) injection of iloprost in mice. The study showed that in contrast to the central analgesic effect of morphine, myrcene did not cause tolerance on repeated injection in rats. The fresh leaf essential oil of lemon grass has a larvicidal activity (Cavalcanti *et al*., 2000)

Dubey (1997) have demonstrated toxicity and apoptosis-inducing action of the essential oil and extracts against mouse and human leukemia cells respectively. Vinitketkumnuen *et al*., (1999) in a study on induced hepatocellular carcinoma in rats showed that ethanol leaf extracts of lemon grass reduced the number but not the size of lesions in rat livers.

Arhoghro and Kpomah (2013), carried out a research aimed at evaluating the dose-dependent (5% and 10%) and time course curative potential of aqueous leaf extract of *C. citratus* on cisplatin-induced renal oxidative damage in rats using biochemical and histopathological approaches. The result of the study indicated that aqueous leaf extracts of *C. citratus* has an antinephrotoxic action against cisplatin-induced renal toxicity in rats, which might be ascribed to its antioxidant and free radical scavenging property.

The human kidney is made up of 10–18 lobes. In the adult, the cortical components of the lobes are fused so that the cortex forms a continuous smooth outer zone, which extends down between the pyramids. The renal medulla is made up of multiple medullary pyramids separated by medullary extensions of the cortex. Each renal papilla is surrounded by a branch of the renal pelvis called a calyx; the whole urinary collecting system within the kidney being described as the pelvicalyceal system. The space between the branches of the pelvicalyceal system is filled with fatty supporting tissue and is known as the renal sinus (Barbara *et al*., 2006).

The aim of the study was to determine the histological effect(s) of the hydro-methanol leaf extract of lemon grass (HLELG) on albino Wistar rats kidney. The objectives were to: (a) Determine the LD_{50} of hydro-methanol extract of lemon grass leaves, (b) Determine if there is any effect(s) of lemon grass on the kidney, (c) Determine whether the histological effect(s) of the hydro-methanol extract is dose dependent, (d) Determine whether the histological effect(s) of the hydro-methanol extract is time dependent.

MATERIALS AND METHODS

Ethical clearance for this study was obtained from Ethical Committee of the College of Health Sciences, University

of Port Harcourt, and the WHO committee guidelines for care and use of animals for research was strictly adhered to.

Lemon grass leaves were collected from Aluu town in Ikwerre Local Government Area of Rivers State and identified by the Curator/plant taxonomist, Department of Plant Science and Biotechnology, University of Port Harcourt. The leaves were dried for 13 days and ground to a fine powder using an electrical blender, after which the powder was dissolved in hydro-methanol and allowed for 72 h to allow for proper dissolution plant material. It was filtered and the filtrate evaporated to dryness using rotatory evaporator.

Pilot Study (Acute Toxicity "Ld_{50} 'Intraperitoneal' Study")

Stage 1: Nine male albino Wistar rats with an average weight of 200 g were divided into three groups (A, B, and C) of three animals per group. The groups had the intra-peritoneal administration of the plant extract at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg, respectively. All animals had nonrestricted access to water and animal feed and were observed for 24 h and no death was recorded.

Stage 2: Three rats were given 10,000 mg/kg of plant extract per rat intraperitoneally, using 2 ml syringes and observed for 24 h, none of the rats died.

Experimental design

Thirty-five albino Wistar rats were recruited for this experiment. After 2 weeks of acclimatization, they were divided into three experimental groups (A, B, and C) of 10 rats per group and a control group of 5 rats.

Wistar rats were weighed at the onset of the experiment, and subsequently on weekly basis for 3 weeks.

Experimental groups (A, B, and C) had 500 mg/kg, 1000 mg/kg, and 2000 mg/kg of HLELG respectively. Oral administration of extracts was carried out once daily for 3 weeks. Two rats from each experimental group were sacrificed at the end of each week for 3 consecutive weeks. Two rats were sacrificed from the control group at the end of the 3rd week. Kidneys from each rat were collected and fixed in 10% formal saline for further tissue processing. The kidneys were collected on 8th, 15th, and 22nd days.

Tissue Preparation

The tissues were processed histologically for paraffin wax embedding, sectioned, stained with Hematoxylin and Eosin, mounted on slides (Disbrey and Rack, 1974; Drury and Wellington, 1968) and viewed with light microscope for micro histological alteration, after which the photomicrographs were prepared.

RESULTS

The LD_{50} "ip" of HLELG was found to be higher than 10,000 mg/kg. The control group served as reference for comparing with the experimental groups: Micrographs from the control group showed the presence of renal corpuscles with visceral and parietal layer of Bowman's capsule, and renal tubules in good condition.

Micrographs in Figure 1 represent experimental and control groups at the end of week 1. Experimental groups A and B were observed to be similar to the control group. However, experimental group C had renal glomeruli with obliterated Bowman's capsule.

Micrographs in Figure 2 represent the end of experimental week 2; all micrographs from the experimental groups A-C revealed obliteration of the Bowman's capsule.

Micrographs in Figure 3 represent the end of experimental week 3; micrographs from experimental groups A and B were similar to the control group. However, micrographs from experimental group C showed obliteration of the Bowman's capsule.

DISCUSSION

Obliteration of the Bowman's capsule was the

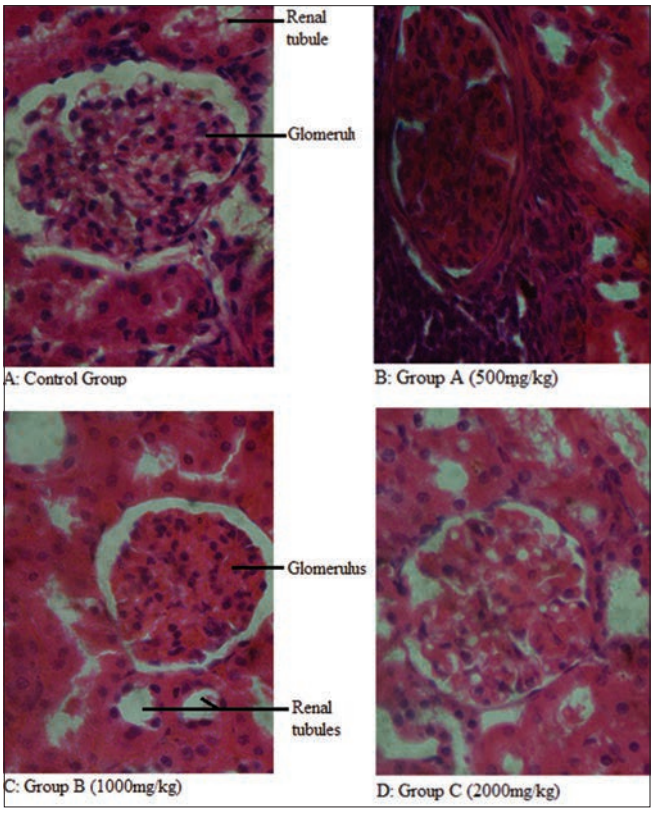

Figure 1: Micrographs of albino Wistar rats kidney treated with hydro-methanol leaf extract of lemon grass for 1 week (H and E, ×1000)

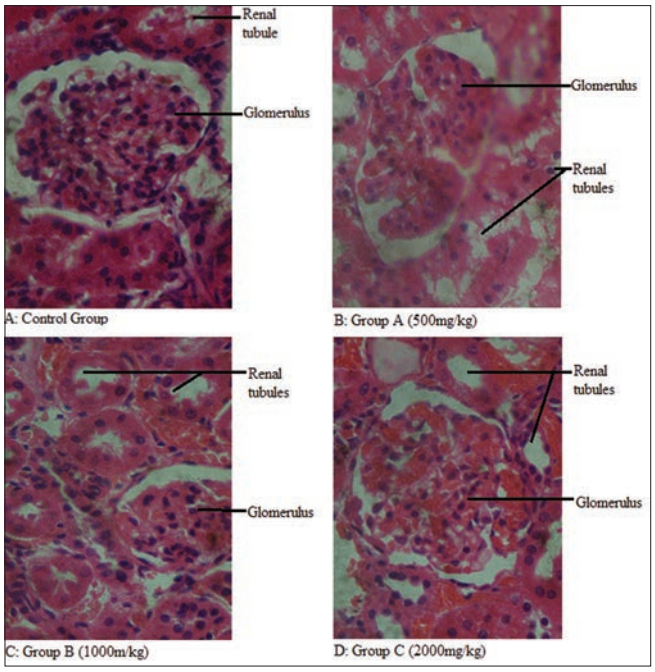

Figure 2: Micrographs of albino Wistar rats kidney treated with hydro-methanol leaf extract of lemon grass for 2 weeks (H and E, ×1000)

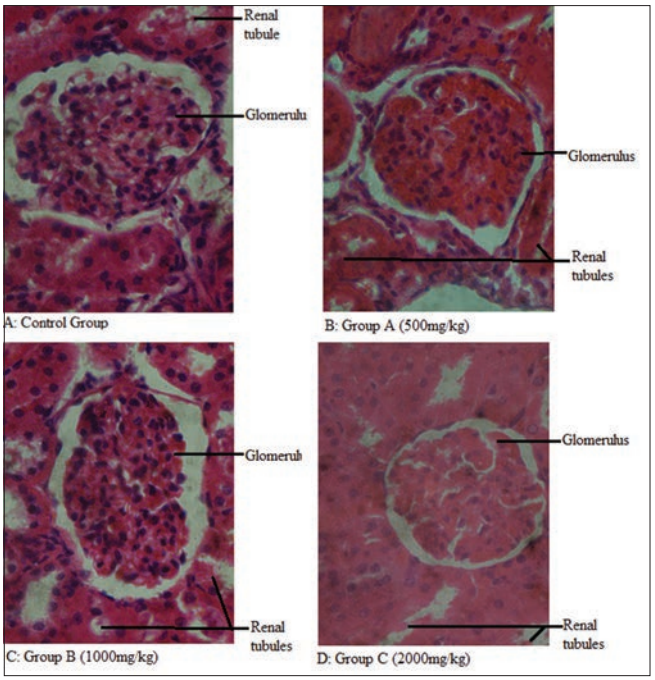

Figure 3: Micrographs of albino Wistar rats' kidney treated with hydro-methanol leaf extract of lemon grass for 3 weeks

major abnormal histologic feature observed in the micrographs. The obliteration of Bowman's capsule is as a result of hypertrophy of the glomerular tufts. The index study agrees with a previous study (Dubey, 1997) and the effect is likely to be due to the essential oil of lemon grass which has been reported to be toxic against mice. However, the index study disagrees with Souza e*t al.* (1986) and Leite *et al*. (1986). At the end of experimental week 1, only micrographs from the group C (exposed to the highest dose HLELG) had obliteration of the Bowman's capsule. This picture is suggestive of the fact that the effect of this plant extract is dose-dependent.

All micrographs from experimental groups at the end of the 2nd week of the experimental period presented with obliteration of the Bowman's capsule. When compared with the finding at the end of week 1, it suggests the fact that the effects the HLELG is time-dependent. At low doses, the distortion of histo-architecture of the urinary space is not noticeable initially, but it becomes noticeable as time progresses. Only micrographs from experimental group C at the end of the third week had features of obliteration of the Bowman's capsule. This implies that the effect of HLELG on the histology of albino Wistar rats' kidneys is transient at low doses. In addition, it implies that the effects of HLELG are noticed faster at higher doses and last longer. By extrapolation, HLELG is likely to have similar effects on human kidneys, but this may not be the case, since we know that certain substances that are toxic to these rodents are not toxic to humans.

CONCLUSION

In conclusion, this study has: (a) Established that the LD_{50} of the HLELG harvested in June from Aluu town near Port Harcourt is above 10,000 mg/kg, (b) Revealed that HLELG distorts the histology of albino Wistar rats' kidneys, (c) Established that the effects of HLELG on albino Wistar rats kidney are both time and dose-dependent.

We recommend that caution should be applied in the consumption high doses of HLELG in humans because there is a possibility of renal toxicity.

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Conflicts of Interest
There are no conflicts of interest.

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