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Comparative effect of aqueous and crude leaf extracts of Albizia zygia on gastric acid secretion in male wistar strain albino rats

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ABSTRACT: Albizia zygia leaves have been used in folklore medicine to treat gastrointestinal disorders. This study aimed to authenticate the effects of aqueous and crude leaves extracts (ALEAZ and CLEAZ) of Albizia zygia respectively on gastric acid secretion and compare same effect produced by the two extracts using in animal model.A total of 35 male wistar strain albino rats weighing 120-250g were randomly separated into seven groups and administered with Normal saline, 200, 400 and 600mg/kg ALEAZ and 200, 400 and 600mg/kg CLEAZ via the oral routes respectively. Trachea and pyloro-duodenal junction were cannulated. Gastric acid output was measured by the continuous perfusion of rat's stomach under anesthesia with normal saline. Phytochemical screening of the extracts of Albizia zygia was also carried out.Both the aqueous and crude leaf extracts of Albizia zygia contained flavonoids (+), carbohydrates (+) and cardiac glycosides (+). However, only the crude extract contained terpenes (++) at a higher amount. Rats pretreated with 200mg/kg CEAZ showed a significant reduction in the concentration of acid(0.003±0.0008µEq/L/10mins) compared tocontrol(0.008±0.002µEq/L/10mins). There was also a reduction in the concentration of acid produced by 400mg/kg of CEAZ (0.004±0.001µEq/L/10mins) compared to 400mg/kg of AEAZ(0.009±0.002µEq/L/10mins).200mg/kg crude extract of Albizia zygia significantly reduced gastric acid secretion and may have acted by inhibiting H_2 receptors or H^+,K^+-ATPase. Sequel to this, Albizia zygia could be beneficial in the treatment of peptic ulcer disease.Further studies directed at isolating the responsible active compound(s) and elucidating its mechanism of action is however recommended.

KEYWORDS:Albizia zygia, Crude extract, Aqueous extract, Gastric acid secretion, Phytochemicals

I. INTRODUCTION

[1]Peptic ulcer is an imbalance between the gastric offensive factors like acid and pepsin and defensive mucosal factors like environmental and host factors.[2]It is one of the world's major gastrointestinal disorders and affecting 10% of the world population.[3]In clinical evaluation, many of the existing anti-ulcer drugs showed that there are incidences of relapses,adverse effects and danger of drug reactions during ulcer therapy.[4]Gastric acid has an important pathophysiological role in human beings. Numerous methods have been evaluated over the years in an attempt to measure gastric acid and stomach acidity, to study the role of gastric acid in gastrointestinal diseases in humans and to evaluate the effects of acid suppressing drugs.[5]In spite of the domination of synthetic drugs in managing most of human diseases including gastric ulcer, extensive proportion worldwide now directed to traditional medicine.

[6]Albizia zygia(DC.) J.F. Macbr. Leguminosaesubfamily Mimosoideae is a fast-growing medium sized deciduous tree widely found in tropical Africa wherein Nigeria, different tribal groups have their indigenous names as: Nyie avu (Igbo); Ayin rela(Yoruba);Madobiyar rafi(Hausa).[7] Previous phytochemical studies have shown that lupen-20(30)-en-3β-ol,14α-stigmast-5-en-3β-ol and 5α-stigmast-7,22-dien-3β-ol were isolated from the bark of Albizia zygia) and same compounds were isolated from the leaves including a glycoside of lupen-20(30) 3β-ol as well

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as four other compounds. [7]; [8]Albiziaprenol and phytol as well as three flavonoids(4',7-dihydroxy flavanone; 3'4'7-trihydroxy-3methoxyflavone;3-o-methylfisetin(3',4',7-trihydroxy-3methoxyflavone)) have also been isolated from the bark.

Some pharmacological studies have been carried out on Albizia zygia. [8]; [9] Traditionally, the roots bark of A.zygia are used against cough, while its stem bark is used as a purgative antiseptic, aphrodisiac, to treat gastritis, fever, conjunctivitis, as well as to fight worms and overcome female sterility. [10]The methanolic stem bark of A. zygia is very active against Plasmodium falciparum K1 strain and Trypanosoma brucei rhodesciense, with respective IC50 values of 1.0µg/ml and 0.2µg/ml and also [11]possess analgesic effects. [12]The aqueous and hydroethanolic root extracts possess anticancer activity with high selective toxicity against Jurkat cells.

Following the claim on the use of Albizia zygia in folklore medicine to treat gastrointestinal disorders, this study aimed to authenticate the effects of aqueous and crude leaves extracts (ALEAZ and CLEAZ) of Albizia zygia respectively on gastric acid secretion and compare same effect produced by the two extracts using animal model.

Figure 1: Albizia zygia plant [13]

II. MATERIALS AND METHODS

Collection and identification of plant materials

Fresh plant sample of Albizia zygia was collected from Mazah village, Jos North, Plateau State, Nigeria. It was identified, classified and vouchered at the herbarium of the College of Forestry, Jos, Nigeria.

Extraction and preparation of aqueous and crude extracts

The mature leaves of Albizia zygia were washed with clean water, air dried under ambient temperature condition and blended into fine powder after which 490g of same was weighed and extracted via a Soxhlet apparatus using distilled water as solvent. The extract was evaporated and later dried via a desiccator giving a yield of 6.1g. It was later reconstituted in distilled water to give the required doses of 200,400 and 600mg/kg. The crude extract was prepared by adding 10ml of distilled water to 1.5g of the powdered leaves with shaking and mixing.

Experimental animal procurement, preparation and ethics

47 male albino rats of Wistar strain weighing between 120-250g were obtained from the Central Animal House, University of Jos, Plateau State, Nigeria. They were housed in plastic cages under standard laboratory conditions and were fed with standard commercial pelleted grower feed (Vital Feed, Nigeria) and allowed access to clean water ad libitum. The animals were acclimatized for two weeks prior to the study. The experimental procedures and techniques used were in accordance with accepted principle for the 1996 Guide for the Care and Use of Laboratory Animals: all protocols and procedures approved by Animal Ethics Committee of the University with reference number (UJ/FPS/F17-00379).

Phytochemical screening

The aqueous leaf extract and powder of Albizia zygia were subjected to various qualitative phytochemical screening tests for the presence of secondary metabolites (alkaloids, saponins, tannins, flavonoids, carbohydrates, terpenes, steroids, anthraquinones and cardiac glycosides using standard procedures as described by [14] Builders et al., (2016).

Acute toxicity test

Twelve rats were used (Nine for phase 1 and three for phase 2) to determine the lethal dose (LD_{50}) of the aqueous and crude leaf extract of Albizia zygia by way of Lorke's method as described by [15] Ezenwali et al., (2010).

Gastric scid secretory study with ALEAZ and CLEAZ

A total of 35 rats were divided into seven groups (II, III, IV, V, VI, VII) with five animals each. The animals were fasted for 24hours but with free access to clean water after which they were anaesthetized with 25%(w/v) urethane (1.5mg/kg body weight). The trachea and pyloro-duodenal junction were cannulated. Each group of animals was administered with normal saline and extracts via the oral route, post-surgery as follows:

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Group I (Control) - 1ml normal saline, Group II (200mg/kg ALEAZ), Group III (400mg/kg ALEAZ), Group IV (600mg/kg ALEAZ), Group V (200mg/kg CLEAZ), Group VI -400mg/kg CLEAZ) and Group VII – (600mg/kg CLEAZ). Fifteen minutes post treatment, the stomach was perfused with normal saline (37^0C) and gastric effluent was collected at a constant rate of 10ml/min. The effluent was assayed for its acid content by titrating against 0.01M NaOH with phenolphthalein as indicator. These were done by way of modified continuous perfusion technique described by [16] Yinusa et al., (2004).

The acid concentration was calculated using: Ne × Ve = Nb × Vb

where: Ne=Concentration of effluent, Ve = Volume of effluent, Nb = Concentration of base, Vb =Volume of base.

Statistical analysis

Results were expressed as mean ± S.E.M. One way ANOVA was used for analysis followed by Tukey's multiple comparison test were used to compare means between treatment and control groups and between treatment (AEAZ) and treatment (CEAZ) groups. Differences between means were considered significantly different ($p< 0.05$) using Graph Pad Prism version 9.0 for Windows (Graph Pad Software, San Diego, California, USA).

III. RESULTS

Phytochemical screening

Our study showed that both the aqueous and crude leaf extracts of Albizia zygia contained flavonoids (+), carbohydrates (+) and cardiac glycosides (+). However, only the crude extract contained terpenes even at a higher amount (++) – Table 1.

Table 1: Phytochemical constituents of aqueous and crude leaves extracts of Albizia zygia

Phytochemical constituents	Aqueous Extract	Crude Extract
Alkaloids	-	-
Saponins	-	-
Tannins	-	-
Flavonoids	+	+
Carbohydrates	+	+
Terpenes	-	++
Steroids	-	-
Anthraquinones	-	-
Cardiac glycosides	+	+

+ = Present, + + = Highly present, - = Absent

Acute toxicity test

Oral administration of ALEAZ and CLEAZ up to 5000mg/kg did not produce any sign of toxicity nor cause mortality in rats, thus LD_{50} ≥5000mg/kg.

Effect of aqueous leaves extract of Albizia zygia leaves on gastric acid secretion

The concentration of basal acid output of rats for various pretreatment was 200mg/kg (0.005±0.0009µEq/L/10mins), 400mg/kg (0.009±0.002µEq/L/10mins) and 600mg/kg 0.005±0.0007µEq/L/10mins of ALEAZ respectively and control (0.008±0.002µEq/L/10mins). The 200mg/kg and 400mg/kg had lower concentration of basal gastric output than the control but the values were not significant.

Effect of Crude leaves Extract of Albizia zygia on Gastric Acid Secretion

For CLEAZ, the concentration of basal acid output of rats for various pretreatment was 200mg/kg(0.003±0.0008µEq/L/10mins), 400mg/kg(0.004±0.001µEq/L/10mins), 600mg/kg (0.004±0.0008µEq/L/10mins) respectively. Administration of 200mg/kg produced a significantly($P<0.05$) lower concentration of basal gastric output compared to the control(0.008±0.002µEq/L/10mins). Also, there was a significant difference($P<0.05$) in the concentration of acid produced by 400mg/kg of crude extract compared to 400mg/kg of aqueous extract.

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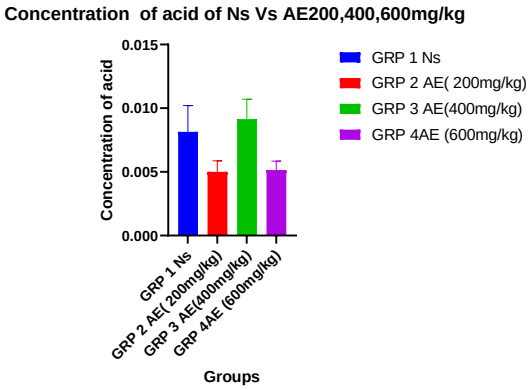

Fig 1: Concentration of acid of Normal saline (Control) versus ALEAZ 200mg/kg, 400mg/kg and 600mg/kg

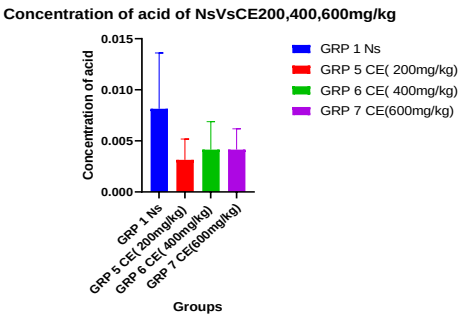

Fig 2: Concentration of acid of Normal Saline (Control) versus CLEAZ 200mg/kg, 400mg/kg and 600mg/kg

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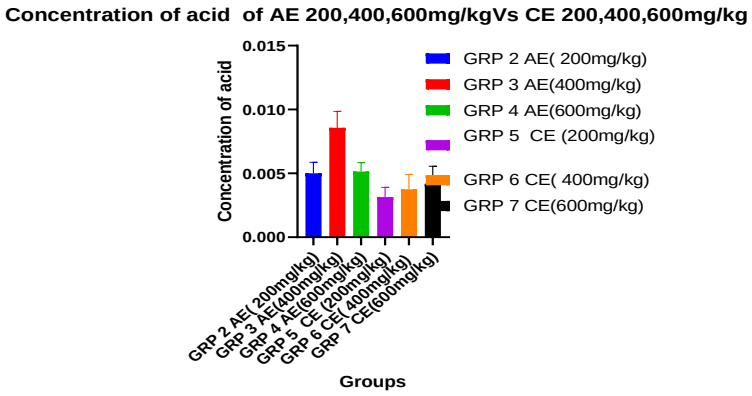

Fig 3: Concentration of acid of ALEAZ 200mg/kg, 400mg/kg, 600mg/kg versus CLEAZ 200mg/kg, 400mg/kg, 600mg/kg

IV. DISCUSSION

The result of the toxicity study showed that the extracts at a high dose of 5000mg/kg caused no observable adverse effect nor death suggesting that the LD_{50} of the extracts is above 5000mg/kg and thus has a wide margin of safety and administration as it is used in folk medicine may not have any immediate adverse effects. The experiment demonstrated that the crude extract at a dose of 200mg/kg significantly inhibited basal gastric acid secretion compared to the control. Also, the crude extract at lesser dose of 200mg/kg produced a greater inhibition than higher doses of the aqueous and crude extract(400mg/kg and 600mg/kg)– Figure 1 and 2.Thisobservation could be due to 'therapeutic windows" effect as suggested by [17] Tripathi, (2013). In other words, it could be that the extract has reached its maximum effect at a dose of 200mg/kg. The crude extract also at a dose of 400mg/kg significantly inhibited basal gastric secretion compared to400mg/kg of the aqueous extract (Figure 3)[18] inferring that there may have been a phytochemical loss in the aqueous extract as a result of heat.

The stimulation of gastric acid secretion is regulated at different levels by neural, hormonal and paracrine mechanisms. Gastric H^+,K^+-ATPase is an important enzyme involved in acid secretion. [19]; [20] This enzyme catalyzes H^+ transport at the expense of ATP hydrolysis in the final step of gastric acid secretion. [21] Histamine is a central and potent stimulus of acid secretion and acts through specific H_2 receptors present on mammalian parietal cells. [22]Several mechanisms have been proposed to explain the gastroprotective effect of flavonoids: these include increase of mucosal prostaglandin content, decrease of histamine secretion, and [23] inhibition of gastric H^+,K^+-ATPase.

The phytochemical analysis of the and crude extract revealed the presence of flavonoids and terpenes. This is in consonance with Abdallah andLaatsch, (2012) [8]who Confirmed the presence of flavonoids from the stem bark extract of same plant in which one of the two showed high antimalarial activity (IC_{50} 0.078 µg/ml) against Plasmodium falciparum. This inhibitory action of the extract on basal acid secretion may be due to these constituents and may have acted by inhibiting H2 receptor or H^+,K^+-ATPase.

V. CONCLUSION

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200mg/kg of the crude extract of Albizia zygia significantly($p<0.05$) reduced gastric acid secretion and may have acted by inhibiting H_2 receptors or H^+,K^+-ATPase. This however authenticates the folkloric practice of using it in the management of peptic ulcer, thus, the crude extract of Albizia zygiacould be beneficial in the treatment of peptic ulcer disease.

Further studies directed at isolating the responsible active compound(s) and elucidating its mechanism of action are therefore suggested.

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CONFLICT OF INTEREST

Authors declare no conflict of interest regarding this study.

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