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Research Article

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Evaluation of Pharmacological Potential of Ruzu Herbal Bitter in Experimental Animal Model

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ABSTRACT

Pharmacological potential of ruzu Herbal bitter in an alloxan-induced wistar albino rats was evaluated using standard methods. Group A received 5ml of ruzu herbal bitters, group B received 2.5ml of ruzu herbal bitters, group C received 1.25 ml of ruzu herbal bitters, group D received 0.625ml of ruzu herbal bitters, group E received 250mg of diabenesse which served as the positive control and group F received 1ml of distilled water which served as a normal control. The antimicrobial activity of Ruzu bitters significantly (P<0.05) increased in Nesseria gonorrhea when compared with Ciprofloxacin while Ciprofloxacin showed a significant (P<0.05) increase in Pretous mirabilis and Klebsielle pneumonia when compared with ruzu herbal bitter. The blood glucose level showed a significant (P<0.05) increase in rats administered with 5 ml, 2.5 ml and 0.625 ml of ruzu herbal bitter on day 5 when compared with positive and normal controls where as rats administered 1.25 ml of ruzu herbal bitter showed no significant difference when compared to positive and negative controls. This showed that Ruzu herbal bitter possess antimicrobial and hyperglycaemic effect.

Keywords: Ruzu herbal bitters; Wistar albino rats; Antimicrobial agents; Diabetes; Hyperglycemia

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INTRODUCTION

Herbal medicines tend to look ancient and unscientific when compared to man-made drugs which are thought to be better than those made from plants. They are still the backbone of about 75-80% of the world population, mainly in the developing countries for primary health care [1].

The Ruzu Herbal Bitter consists of 3 key elements; The Uvaria Chamae (also known as bush banana), Curgulico Pilosa (also known as squirrel groundnut) and Colocythis citrullus (also referred to as bitter apple, desert gourd or egusi) [2].

Uvaria chamae is a Nigerian medicinal plant that belongs to the family, Annonaceae. It is commonly called by the Igala people of Kogi State as Ayiloko, Kaskaifi by the Hausas, Oko oja by the Yorubas in Nigeria as well as Akotompo by the Fula-fainte of Ghana. It is a medicinal plant used in the treatment of fever and injuries [3,4]. They are other oral claims that the plant can cure such as abdominal pain, used as treatment for piles, wounds, sore throat diarrhea etc.

The genus *Curculigo* belongs to the family Hypoxidaceae and consists of approximately 20 species of exclusively tropical origin [5].The members of the family are small to medium herbs, with grass-like leaves and an invisible

stem, modified into a corm or a rhizome. The rhizomes of *Curculigo pilosa* (CP) Schum and Thom, was the first African species to be described of the *Curculigo* genus [6]. It has medicinal usages in Northern Nigeria as a purgative and as a remedy for hernia.

Citrullus colocynthis Schrad, belongs to the family of Cucurbitaceae, popularly named bitter apple or bitter cucumber in English and called Hendevaneh Abujahl (Abujahl watermelon) or Kadu Hanzal (bitter ground) in Persian, is a well-known medical plant used alone or in compounds for many medical purposes [7]. Different parts of the plant including seeds, fruit, root, stem, and leaves, used as either aqueous or oil extracts, dried or fresh, are believed to have antidiabetic [7,8] antihyperlipidemic [9,10], laxative [7,11,12] , anti-inflammatory [12], analgesic [13], vermifuge [14], hair-growth-promoting [15], antibacterial [13], antifungal [13], and antioxidant properties [16].

Diabetes is a serious metabolic disorder with micro and macro vascular complications that results in significant morbidity and mortality [17]. Chronic hyperglycaemia during diabetes causes glycation of body protein that in turn leads to secondary complications affecting eyes, kidney, nerves and artery [18]. These may be delayed, lessened or prevented by maintaining blood glucose values close to normal.

MATERIALS AND METHODS

Experimental Procedures

Collection of materials

Ruzu herbal bitter (an already made extract) was purchased at Ruzu World Company, BCA Road Umuahia, Abia State, Nigeria and Alloxan was purchased from Grace and Mercy Pharmacy, Umuahia, Abia state.

Experimental Animals

Eighteen Wistar albino rats of both male and female sex were used for this study. The animals were obtained from the Animal Breeding Unit of the College of Veterinary medicine, University of Nigeria, Nsukka. They were kept in well ventilated plastic stainless steel cages and left under laboratory conditions for two weeks for acclimatization. The animals were divided into six groups of 3 rats each. They were fed with commercial rat feed and clean tap water ad libitum throughout the period of experiment.

Induction of Diabetes by Alloxan Administration

Diabetes was induced by the use of alloxan. Eighteen animals of both sexes were selected and weighed. The alloxan was dissolved in 10 ml of normal saline which gives l00mg/ml and administered intraperitoneally according to body weight within few minutes of preparation after base line test has been determined. Diabetic state was confirmed after 3 days by means of a glucometer, the rats were fasted for 6 hours and blood was taken from tail artery of the rats. Rats with blood glucose of 185.8-250 mg/dL were taken for the experiment, diabetic condition was produced having fasting blood sugar level of above 185mg/dl and the diabetic state was treated by oral administration of ruzu herbal bitters throughout the experiment.

Experimental Design

The animals were divided into six groups of three animals per group. The blood glucose level of the animals were taken daily for before feeding and treatment. They were fed and treated for ten days as follows:

Group A: received 5 ml of ruzu herbal bitters

Group B: received 2.5 ml of ruzu herbal bitters.

Group C: received 1.25 ml of ruzu herbal bitters

Group D: received 0.6.25 ml of ruzu herbal bitters

Group E: (positive control) received 1ml of diabenese (Standard Diabetic drug).

Group F: (normal control) received 1ml of water.

METHODOLOGY FOR ANTIBACTERIAL AND ANTIFUNGAL STUDIES

In-vitro antifungal and antibacterial activities of plant extracts and standard antibiotic drugs were determined by disc diffusion method [19]. For susceptibility testing, crude extract was made into a suspension using Dimethyl Sulphoxide (DMSO). The concentration of the material was made to 200 mg and further concentration were prepared by doubling dilution sterile disc having diameter of 6mm were impregnated with 25 µL of each concentration of extract, standard antibiotic drugs and dried in an incubator to remove the solvent. On the other hand bacterial strains were spread on the surface of agar plate (nutrient agar for bacteria and sabroud agar for fungal) aseptically by sterile cotton swab. The sterile disc loaded with extracts and standard antibiotic drugs were placed on inoculated surface agar plate with the help of sterile forceps. These plates were incubated for 18-24 hrs at 37°C and 24-72 h at 25°C. The diameter at the zone of inhibition around each of the disc was taken as measure of antibacterial and antifungal activities. Each experiment was carried out in triplicate and the diameter at inhibition was measured in millimetre with the aid of meter ruler.

Method for Determination of Blood Sugar (Glucose) Level

The blood sugar (glucose) level of the rats were determined by the used of glucometer and glucometer text strip. A small amount of blood was obtained by slightly cutting the tail of the rats and was placed on a disposable test strip. The meter reads and the blood sugar (glucose) level was obtained and recorded in units of mg/dl.

RESULT AND DISCUSSION

The result of Table 1 showed that Ruzu herbal bitters inhibited the growth of *Nesseria gonnorhea* with the zone of inhibition of 11mm when compared with Ciprofloxacin with the zone of inhibition of 6.0mm. On the other hand, Ciprofloxacin inhibited the growth of *Pretous mirabilis* and *Klebsiella pnemoniae* with the zone of inhibition ranging 10-12mm when compared with Ruzu bitters with zone inhibition ranging from 5-8 mm.

Table 1. Antimicrobial Activities of Ruzu herbal bitters

Organism	**Ruzu Bitters 5 mL** **Zone of Inhibition in (mm)**	**Ciprofloxacin 50 mg** **Zone of Inhibition in (mm)**
Staph. aureus	9.0 ± 1.0	11.0 ± 1.0
E. coli	11.0 ± 1.0	12.3 ± 2.1
Nesseria gonorrhea	11.0 ± 1.0*	6.0 ± 1.0*
Pretous mirabilis	5.0 ± 1.0*	10.0 ± 1.0*
Klebsiella pneumoniae	8.0 ± 1.0*	12.0 ± 1.0*

Streptococus pneumoniae	10.7 ± 2.1	10.3 ± 1.5
Salmonella typhi	14.0 ± 1.0	11.0 ± 1.0

Values are expressed as means ± SEM. Values with asterisks (*) in the same column are significantly different when comparing groups treated with ruzu herbal bitters and ciprofloxancin.

The result of Table 2 showed that the minimum inhibitory concentration (MIC) of *Staph aureus, Nesseria gonorrhea, Klebsiella pneumonia* is 0.625ml, the MIC of *E.coli* and *Streptococus pneumoni* is 0.3125 ml, the MIC of *Pretous mirabilis* is 1.25 ml and MIC of *Salmonella typhi* is 0.16129 ml.

Table 2. Minimum Inhibitory concentration

Organisms	**2.5 ml**	**1.2 5 ml**	**0.625 ml**	**0.3125 ml**	**0.16129 ml**	**MIC (ml)**
Staph. aureus	5.3 ± 0.5	2.0 ± 3.0	0.4 ± 3.0	0.0 ± 0.1	0.0 ± 0.0	0.625
E. coli	6.0 ± 1.0	2.0 ± 4.0	2.0 ± 4.0	0.3 ± 0.1	0.0 ± 0.0	0.3125
Nesseria gonorrhea	5.6 ± 0.5	2.0 ± 3.0	2.0 ± 3.0	0.0 ± 0.0	0.0 ± 0.0	0.625
Pretous mirabilis	2.3 ± 0.5	0.5 ± 0.9	0.5 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	1.25
Klebsiella pneumoniae	4.0 ± 1.0	1.6 ± 0.5	1.6 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.625
Streptococus pneumonia	5.0 ± 1.0	2.6 ± 0.5	2.6 ± 0.5	0.1 ± 0.5	0.0 ± 0.0	0.3125
Salmonella typhi	7.6 ± 0.5	0.5 ± 1.0	5.0 ± 1.0	0.9 ± 0.5	0.1 ± 0.1	0.16129

Values are expressed as means ± SEM

The result showed that there was a significant ($P<0.05$) increase in Blood glucose level in group treated with 5ml of Ruzu bitters on day 4 and 5 when compared with the controls. Similarly there was a significant ($P<0.05$) increase in blood glucose level groups treated with 2.5ml and 0.625ml of Ruzu bitters on day 5 when compared with the controls. However, group treated with 1.25ml of Ruzu bitters showed no significant ($P>0.05$) difference in blood glucose level when compared with the controls but group treated with standard drug decreased significantly ($P<0.05$) when compared with the normal (Table 3).

Table 3. Blood sugar level of wistar albino rats

Days	**5 ml**	**2.5 ml**	**1.25 ml**	**0.625 ml**	**Diabenese**	**Normal saline**
1	576.5 ± 17.6	413.0 ± 230.5	248.0 ± 35.3	240.0 ± 147.0	254.5 ± 31.8	207.5 ± 3.51
2	549.5 ± 37.4	369.5 ± 245.3	148.5 ± 54.4	201.5 ± 156.2	169.0 ± 26.8	184.0 ± 11.0

3	448.0 ± 31.1	330.0 ± 267.2	75.5 ± 31.8	364.5 ± 31.8	107.0 ± 11.3	190.0 ± 12.7
4	443.0 ± 32.5*	299.0 ± 158.3	91.5 ± 0.7	335.5 ± 34.6	126.0 ± 38.1*	152.5 ± 7.7
5	392.0 ± 57.9*	263.5 ± 58.6*	127.5 ± 9.1	267.0 ± 19.7*	121.0 ± 2.8	173.5 ± 6.3
6	395.5 ± 36.0	259.0 ± 172.6	106.5 ± 4.9	197.0 ± 7.0	166.5 ± 51.6	159.0 ± 5.6
7	247.5 ± 85.5	288.0 ± 104.6	138.5 ± 9.1	212.5 ± 24.7	140.0 ± 43.8	133.0 ± 65.9
8	576.5 ± 17.6	494.5 ± 40.3	426.5 ± 9.1	278.5 ± 129.4	115.5 ± 16.2	354.0 ± 313.9
9	130.0 ± 36.7	175.0 ± 19.7	153.0 ± 164.0	351.5 ± 56.5	150.0 ± 56.5	112.5 ± 6.3
10	175.0 ± 19.7	139.5 ± 3.5	103.0 ± 84.8	261.5 ± 21.9	97.0 ± 62.9	175.5 ± 62.9

Values are expressed as means ± SEM. Values with asterisks (*) in the same column are significantly different when comparing the treatment groups with the controls (diabeness and normal saline groups).

DISCUSSION

Antimicrobial agent is an agent that kills or inhibits the growth of microorganism. In this study, Ruzu herbal bitters inhibited the growth of *Nesseria gonorrhea* compared with Ciprofloxacin (standard drug), this indicates that the herb may contain active principle that can could be as a result of uvaria chamae contained by the Ruzu bitters which have been listed amongst the traditional medicinal plants commonly used in West Africa [20-22]. Addae-Kyereme et al. [23] reported that alkaloid, plelocorpin, kopsmine, eburnamine and pleiomutinine are present in *Uvaria chamae* and these may be responsible for the high antibacterial activity recorded in the study. However, Ciprofloxacin (standard drug) inhibited the growth of *Pretous mirabilis* and *Klebsiella pneumonia* when compared to Ruzu herbal bitters; this could be as a result of its expanded spectrum of action.

Diabetes mellitus has been described as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [24-26] and the major goals in the treatment of diabetes has been to keep both short term and long term glucose levels within acceptable limits, thereby reducing the risk of long term complications [27]. In the present study, there was a significant ($P<0.05$) increase in blood glucose level in groups treated with 5ml, 2.5ml and 0.625 ml of Ruzu herbal bitters on day 4 and 5 when compared with the controls. This could be attributed to glycogenolysis process where glycogen is broken down to glucose molecules or building up of glucose from non-carbohydrates substrate like pyruvate through gluconeogenesis. This is also comparable to mechanism of glucagon injection which raises the concentration of glucose in the blood stream thus the pancrease releases glucagon when the concentration of glucose in the blood stream falls too low and glucagon causes the liver to

convert stored glycogen into glucose which is released in the blood stream. This suggests that Ruzu herbal bitter possesses antimicrobial and hyperglycemic effect.

CONCLUSION

From this study, it can be deduced that Ruzu herbal bitter possesses antimicrobial and hyperglycaemic effect and as such may not be used in the management of diabetes in alloxan-induced diabetic rats but may be used in inhibition of growth of some microorganism.

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