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**Research Article** 

# THE INFLUENCE OF AQUEOUS LEAF EXTRACT OF GOAT WEED (*AGERATUM CONNYZOIDES*) ON ETHANOL-TREATED ADULT WISTAR RATS

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## ABSTRACT

Alcohol has been reported as one of the major predisposing agents of gastric ulcer, liver diseases and renal failures. This study was thus carried out to investigate the influence of the aqueous leaf extract of *Agerantum conyzoides* on ethanol-treated in adult wistar rats by carrying out biochemical and histological on gastric mucosa, hepatocytes and renal tissue. A total of twenty-four (24) rats weighing between 150-320 g were divided into four groups (A, B, C and D) of 6 rats each. Group A served as control (2 ml of distilled water), Group B, C and D were administered ethanol + 10 mg of Omeprazole (standard anti-ulcer drug), ethanol + 500 mg/kg and ethanol + 250 mg/kg body weight of extract, respectively for twenty-eight (28) day. All the groups were fed rat-chow, *ad libitum*. At the end of administration, the rats were fasted over-night, sacrificed and the organs harvested and whole blood collected into heparinized bottles, centrifuged and plasma collected into plain sample bottles for onward biochemical assays. However, the tissues were immediately fixed in 10 % formal-saline, processed histological and stained with H and E stains. The slides were examined under microscope and Photomicrographs taken. The result showed that the high dose of the aqueous plant extract was more potent in ameliorating the ulcer than the low dose.

Keyword: Ageratum conyzoides; Gastric ulcer; Ethanol; Omeprazole

# INTRODUCTION

The use of Traditional medicine is as old as the origin of man. Several lines of evidence indicate that medicinal plants represent the oldest and the most widespread form of medication, especially in developing areas of the world like Nigeria Halberstien, 2005<sup>1</sup>. Traditionally, herbs and herbal products have been considered to be non-toxic and have been used by the general public and tradomedical practitioners to treat a range of ailments. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. The use of traditional and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed UNESSCO, 1996<sup>2</sup>. Ageratum convzoides is a tropical plant that is common in West Africa and some parts of Asia as well as Brazil, Shirwalkar et al., 2003<sup>3</sup>. Ageratum is derived from the Greek words 'a geras', meaning non-aging, referring to the longevity of the whole plant. Though Sani and Bahri, 1994<sup>4</sup> as well as, Fu et al., 2002<sup>5</sup> had reported that ingesting this plant could cause liver lesions and tumor, researches had demonstrated that this plant has other important health benefits. In Central Africa it is used to treat pneumonia, but the most common use is to cure wounds and burns, Durodola, 1977<sup>6</sup>. It's bactericidal, anti dysenteric, and anti-lithic properties have been reported, Borthakur and Baruah, 1987<sup>7</sup>; Almagboul, 1985<sup>8</sup>; Ekundayo et al., 1988<sup>9</sup>; Vera, 1993<sup>10</sup>. It has been used in Cameroon and Congo, to treat fever, rheumatism, headache, and colic, Menut et al., 1993<sup>11</sup>; Bioka et al., 1993<sup>12</sup>. Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhea, rheumatism, spasms, or as a tonic, Negrelle *et al.*, 1988<sup>13</sup>; Oliveira *et al.*, 1993<sup>14</sup>. Ming, 1999<sup>15</sup> and Brasil, 1989<sup>16</sup> have reported that this plant contains vast phytochemical constituents and possess quick and effective action in burn wounds.

According to Ming, 1999<sup>15</sup>, several pharmacological investigations have been conducted to determine efficacy. Duradola, 1977<sup>6</sup> verified inhibitory activities of ether and chloroform extracts against in vitro development of Staphylococus aureus. Almagboul et al., 1985<sup>8</sup> using methanolic extract of the whole plant, verified inhibitory action in the development of Staphylococus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa. Bioka et al., 1993<sup>12</sup> reported effective analgesic action in rats using aqueous extract of A. convzoides leaves (100 to 400 mg/kg). Assays realized in Kenya, with aqueous extract of the whole plant, demonstrated muscle relaxing activities, confirming its popular use as an antispasmotic, Achola et al., 1994<sup>17</sup>. Thus, the dose-dependent ameliorative effects of this plant on ethanol-induced gastric ulceration, as against, a standard anti-ulcer agent like Omeprazole have been investigated in this research by studying histological photomicrographs of the various groups. Also, the ethanol-effect and ameliorative influence of the extract on liver and renal functions have been investigated.

# MATERIALS AND METHOD

# **Preparation of plant extract**

Ageratum conyzoides leaf was carried out by dissolving 402.70 g of the leaf in 4 L of distilled water in a ratio of 2:3 for 72 h after which filtration was carried out using sintered filter and then Whatman no.1 filter paper giving a dark green filtrate. The extract was then concentrated in vaccuo using a rotary evaporator to remove the ethanol at a temperature of 99°C. The syrup that was obtained was dried to powdered form using a freeze-dryer and the weight determined as 16.3 g, while percentage yield was 14.68 %. The evaporated extract was then reconstituted in distilled water at 5 g % (w/v). The reconstituted extract was then stored in a capped container and kept in the refrigerator at about + 4°C until usage period.

#### Animals

Twenty-four adult Wistar rats were used as experimental animals in this study. Their weights were between 150-320 g. The animals were purchased and maintained at the Animal House of the Department of Anatomy, University of Benin and were transferred into their cages. The research ethics committee guidelines of the College of Medicine, University of Benin (CMS/REC/2014/57) was strictly adhered to. The Mature plants of *A. conyzoides* were harvested in whole from bush farms around Benin City in June, 2012. It was identified and authenticated by Mr JO Erhabor, at the Herbarium (UBHa0301) in the Department of Pharmacognosy Faculty of Pharmacy, University of Benin.

## **Drug Administration**

The animals were divided into four groups: A, B, C and D with each group having six Wistar rats which were all weighed prior to administration; all the groups given rat chow and clean water *ad libitum*. Group A which served as the control group received 2 ml of distilled water. Groups B, C and D rats were given 1 ml of 80 % ethanol for one week to induce events that look like gastric ulceration after which a rat each from these groups were sacrificed, gastric sections taken and studied to ascertain the development of signs of ulcerations. Following this, group B were administered 10 mg of Omeprazole, and groups C and D, 500 mg and 250 mg, respectively, of the extract calculated based on weekly weights of the rats, for the next three (3) weeks. The administration of ethanol, the plant extract and omeprazole were given using an oro-gastric tube, Akah *et al.*,  $2010^{18}$ .



Figure 1: Control rat stomach showing normal gastric mucosal epithelial lining A, pits B and glands C (H and E; × 10)



Figure 3: Rat stomach treated with Omeprazole after alcohol administration showing mucosa lining A (H and E; × 10)

#### Blood sample and tissue collection

After period of treatment, the rats were sacrificed using a decapitator, whole blood samples collected using heparinized bottles, centrifuged and the plasma collected into plain sample bottles for onward biochemical analyses of liver function parameters, viz, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB), conjugated bilirubin (CB), total protein (TP) and plasma albumin (ALB); renal function parameters, viz, plasma electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, bicarbonate ion), creatinine and urea. The stomach and duodenum of each rat were quickly harvested and placed in 10 % formal-saline for routine histological studies.

#### **Biochemical assays**

Biochemical analyses were done using ready to use  $\mbox{Randox}\ensuremath{\mathbb{R}}$  assay kits.

#### Statistical analysis

Values were represented as mean  $\pm$  S.E.M. (n = 6), analyzed with one-tail students't-test using the SPSS version 16.0.

## RESULT

The representative section of the stomach, liver and kidney with control and the various treated organs, treated with Omeprazole, *A. conzyiodes* at a higher and low dose respectively



Figure 2: Rat stomach treated with alcohol and sacrificed after one week showing irregularly shaped ulceration extending down to the sub mucosa A (H and E; × 10)



Figure 4: Rat stomach treated with high dose *A. conyzoides* extract showing mucosa lining A and mild lamina propria congestion B (H and E; × 100)

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Figure 5: Rat stomach treated with low dose *A. conyzoides* extract showing patchy mucosa lining erosion A (H and E; × 100)



Figure 7: Rat liver treated with Omeprazole showing unremarkable liver architecture (H and E;  $\times$  400)



Figure 9: Rat liver treated with low dose *A. conyzoides* extract showing mild vascular congestion A, mild kuppfer cell activation B and mild periportal infiltrates of inflammatory cells C (H and E; × 400)



Figure 11: Rat spleen treated with Omeprazole after alcohol administration showing mild follicular activation A and mild histiocyte activation B (H and E; × 40)



Figure 6: Control rat liver showing portal triad A, hepatocytes B and sinusoids C (H and E; × 400)



Figure 8: Rat liver treated with high dose *A. conyzoides* extract showing unremarkable liver architecture (H and E; × 400)



Figure 10: Rat spleen showing red pulp A and white pulp B (H and E; × 40)



Figure 12: Rat spleen treated with high dose *A. conyzoides* extract after alcohol administration showing mild follicular activation A and moderate histiocyte activation B (H and E; × 40)

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Figure 13: Rat spleen treated low dose *A. conyzoides* extract after alcohol administration showing moderate follicular activation A (H and E; × 40)



Figure 15: Rat kidney treated with Omeprazole showing unremarkable cortical architecture (H and E; × 40)



Figure 14: Control rat kidney showing cortical glomerulus A, tubules B and interstitial space C (H and E; × 40)



Figure 16: Rat kidney treated with high dose *A. conyzoides* showing unremarkable cortical architecture (H and E;  $\times$  40)



Figure 17: Rat kidney treated with low dose A. conyzoides showing unremarkable cortical architecture (H and E; × 40)

Table 1: Liver Function Parameters of the Treated Groups with Control

Groups	ALP	ALT	AST	ТВ	СВ	TP	ALB
Α	$42.00 \pm 5.10$	$18.40 \pm 1.44$	$95.80 \pm 11.63$	$0.32 \pm 0.06$	$0.16 \pm 0.04$	$7.14 \pm 0.08$	$3.86 \pm 0.35$
В	$38.00 \pm 9.17$	$19.50 \pm 1.85$	$99.00 \pm 12.72$	$0.20 \pm 0.00$	$0.10 \pm 0.00$	$6.75 \pm 0.25$	$3.70 \pm 0.29$
С	$50.40 \pm 4.12$	$19.40 \pm 2.09$	$90.80 \pm 8.26$	$0.20 \pm 0.00$	$0.10 \pm 0.00$	$7.26 \pm 0.45$	$3.52 \pm 0.26$
D	$63.00 \pm 15.32$	$20.40 \pm 2.32$	$98.60 \pm 5.49$	$0.32 \pm 0.04$	$0.16 \pm 0.56$	$6.46 \pm 0.25$	$3.92 \pm 0.32$

Values are represented as mean  $\pm$  S.E.M. for six (6) determinations, Means with "are significantly different (p < 0.05) compared to the control group

Table 2: Renal Function Parameters of the Treated Groups with Control

Groups	Urea	Sodium	Potassium	HCO <sub>3</sub> <sup>-</sup>	Chloride	Creatinine
Α	$35.00 \pm 2.35$	$136.60 \pm 1.00$	$4.44 \pm 0.20$	$20.20 \pm 1.00$	$104.40 \pm 1.00$	$0.66 \pm 0.05$
В	$41.50 \pm 2.72$	$139.25 \pm 2.00$	$5.25 \pm 0.20$	$22.50 \pm 0.90$	$102.50 \pm 1.00$	$0.95 \pm 0.05$
С	$42.80 \pm 4.73$	$141.80 \pm 1.00$	$4.42 \pm 0.20$	$18.40 \pm 1.00$	$104.20 \pm 0.90$	$0.84 \pm 0.10$
D	$49.40 \pm 3.91$	$143.80 \pm 0.50$	$4.04 \pm 0.20$	$22.00 \pm 2.00$	$105.00 \pm 0.60$	$0.80 \pm 0.03$

Values are represented as mean  $\pm$  S.E.M. for six (6) determinations, Means with \* are significantly different (p < 0.05) compared to the control group

# DISCUSSION

From the result, ALP and AST increased significantly at 250 mg of administered aqueous leaf extract of A. convzoides, but decreased as dose was decreased, compared to the control (Table 1). However, AST reduced significantly. Total and conjugated bilirubin, as well as, plasma total protein and albumin, did not show any remarkable change in levels with respect to administered ethanol and extract. Plasma urea and creatinine levels increased with ethanol administration, but decreased with administered extract. Thus, there was no noticeable influence of both ethanol and plant extract on liver parameters, but ethanol had a marked negative effect on renal function of the rats, though this could not be said to have been ameliorated by the extract. Li et al., 2013<sup>19</sup> reported that excessive alcohol consumption can lead to gastric ulcer. In this study, we tried to see how aqueous leaf extract of A. convzoides could ameliorate morphological changes mimicking gastric ulceration of ethanoltreated rats. The work showed that the aqueous leaf extract of A. conyzoides extract can ameliorate ethanol induced gastric ulcer, Okolie et al., 2013<sup>20</sup>. The mechanism for our findings may be connected with reduction in gastric acid secretion and gradual deposition of mucous on the gastrointestinal wall thus protecting the epithelial layer of the stomach from gastric acid digestion as observed in plate four (4).

## REFERENCES

- Halberstien RA. Medicinal plants: Histological and crosscultural usage patterns. Animals of Epidemiology 2005; 15: 686-699. http://dx.doi.org/10.1016/j.annepidem.2005.02.004
- UNESCO. Culture and health, orientation texts-world decade for cultural development 1988-1997, document CLT/DEC/PRO-1996, Paris France; 1996. p. 129.
- Shirwaikar A, Bhilegaonkar PM, Malini S, Kumar JS. The gastro-protective activity of the ethanol extracts of *Agerantum conyzoides*, Jethno Pharmacol 2003; 86(1): 117-121. http://dx. doi.org/10.1016/S0378-8741(03)00050-3
- Sani Y, Bahri S. Pathological changes in liver due to the toxicity of *Ageratum conyzoides*. Penyakit Hewan (Indonesia) 1994; 26(48): 64–70.
- Fu PP, Yang YC, Xia Q, Chou MC, Cui YY, Lin G. Pyrrolizidine alkaloids-tumorigenic components in Chinese herbal medicines and dietary supplements. Journal of Food and Drug Analysis 2002; 10(4): 198–211.
- Durodola JJ. Antibacterial property of crude extracts from herbal wound healing remedy-*Ageratum conyzoides*. Planta Med 1977; 32: 388–390. http://dx.doi.org/10.1055/s-0028-1097620
- Borthakur N, Baruah AKS. Search for precocenes in *Ageratum conyzoides* Linn. of North-East India. J. Indian Chem. Soc 1987; 64: 580–581.

- Almagboul AZ, Farroq AA and Tyagi BR. Antimicrobial activity of certain Sudanese Plants used in folkloric medicine: Screening for antibacterial activity, Part II. J. Fitoterapia 1985; 56: 103-109.
- Ekundayo O, Sharma S, Rao EV. Essential oil of Ageratum conyzoides. Planta Med 1988; 54: 55–57. http://dx.doi.org/ 10.1055/s-2006-962336
- Vera R. Chemical composition of the essential oil of *Ageratum conyzoides* L. (Asteraceae) from Reunion. Flavour Fragrance J 1993; 8: 256–260. http://dx.doi.org/10.1002/ffj.2730080504
- Menut C, Sharma S, Luthra C. Aromatic plants of tropical central Africa, Part X-Chemical composition of essential oils of *Ageratum houstonianum* Mill. and *Ageratum conyzoides* L. from Cameroon. Flavour Fragrance J 1993; 8(1): 1–4. http://dx. doi.org/10.1002/ffj.2730080102
- Bioka D, Banyikwa FF, Choudhuri MA. Analgesic effects of a crude extract of *Ageratum conyzoides* in the rat. Acta Hort 1993; 332: 171–176.
- Negrelle RRB, Sbalchiero D, Cervi AC. Espécies vegetais utilizadas na terapêutica popular no município de Curitiba, Paraná, Brasil. Univ. Federal do Paraná; 1988.
- 14. Oliveira F, Akisue MK, Garcia LO. Caracterização farmacognóstica da droga e do extrato fluído de mentrasto, *Ageratum conyzoides* L. Lecta 1993; 11(1): 63–100.
- Ming LC. Ageratum conyzoides: A tropical source of medicinal and agricultural products. In Janick, J. Perspectives on new crops and new uses. Alexandria VA: ASHS Press; 1999. p. 469– 473.
- Brasil, Ministério da Saúde, Central de Medicamentos. *Ageratum conyzoides*. In: Programa de pesquisas de plantas medicinais: Primeiros resultados. Brasília; 1989.
- 17. Achola KJ, Munenge RJ, Mwaura AM. Pharmacological properties of root and aerial parts extracts of *Ageratum conyzoides* on isolated ileum and heart. Fitoterapia 1994; 65: 322–325.
- Akah PA, Nnamani CE, Nnamani PO. Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. J. Med. Med. Sci 2010; 1(10): 447-452.
- Li W, Huang H, Niu X, Fan T, Mu Q, Li H. Protective effect of tetrahydrocoptisine against ethanol-induced gastric ulcer in mice. Toxicol. Appl. Pharm 2013; 272(1): 21-29. http://dx. doi.org/10.1016/j.taap.2013.05.035
- Okolie NP, Agu K, Eze GI. Protective effect of ethanolic extract of *Annona muricata* Linn on some early events in cycas-induced colorectal carcinogenesis in rats. J Pharm Sci Innov 2013; 2(4): 14-21. http://dx.doi.org/10.7897/2277-4572.02444

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