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Effect of Aqueous Garlic (Ag) Extract on Aspirin Induced Gastric Mucosal Lesion in Albino Wistar Rats.

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Abstract: The aim of this study was to evaluate the effect of aqueous garlic (AG) extract on aspirin induced gastric mucosal lesion. It was carried out on 72 albino Wistar rats. Animals were divided into two groups. Gastric ulcer group (n=36) and gastric secretion group (n=36). Each group was subdivided into control (n=16) and study groups (n=18). Control group was further subdivided into 3 subgroups including (1) Oral distilled water subgroup (n=6); (2) Oral aspirin (150mg/kg) subgroup (n=6) and (3) Oral AG extract (150mg/kg) subgroup (n=6).The study group was also subdivided into (1) Oral aspirin (150mg/Kg) + AG extract (150mg/kg) subgroup(n=6); (2) Oral aspirin (150mg/Kg) + AG extract(300mg/kg) subgroup(n=6) and (3)Oral aspirin(150 mg/kg) + Cemitidine(50mg/kg) I.P subgroup((n=6).All groups were fasted for 36 hours before pyloric ligation. Gastric secretions were collected, centrifuged and analysed.Gastric ulcer parameters were also studied for gastric ulcer group. There was significant decrease($p<0.05$) of titratable acidity and total acid output in aspirin(150mg/Kg) + AG (150mg/Kg) and aspirin(150mg/Kg) + AG (300mg/kg) when compared with aspirin(150mg/Kg) alone group. Also there was significant decrease ($p<0.05$) in ulcer index and ulcer score in both groups than aspirin alone group. In comparison between garlic groups and cemitidine group there was more decrease in these parameters in cemitidine groups than garlic group. It seems likely that AG extract offered protective effects on aspirin induced gastric mucosal lesion.

Key words: antioxidant, garlic extract, gastric secretion, gastric ulcer

INTRODUCTION

Aqueous garlic (AG) extract could be the most promising garlic preparation for the prevention of diseases (Sumiyoshi, 1997). AG extract exerts antioxidant action by scavenging reactive oxygen species, enhancing cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase (Borek, 2001). AG extract inhibit lipid peroxidation and inhibit activation of oxidant induced transcription factors (Sener *et al*., 2005). Garlic and its derivatives possess antioxidant properties to scavenge toxic radicals (Kalayarasan *et al*., 2008). According to the recent pharmacological findings garlic has a preventive rather than therapeutic in peptic ulcer (Sumiyoshi, 1997). Peptic ulcers are open sores in mucous lining of the stomach and duodenum. The annual mortality rate due to peptic ulcer is n low and deaths are largely due its complications such as hemorrhage and perforation (Isemberg and Soll, 1996). The risk of developing an ulcer depends upon the specific type of NSAID, dose, duration of use and individual patient factors (Andrew et al., 1991). The pathogenesis of peptic ulcer is far from clear and so also the mechanism of anti ulcer drugs (Akimoto *et al*, 1998). NSAID stimulate HCl secretion and cause weakness of mucous gel layer which act as barrier by decreasing mucin production and increasing the secretion of bicarbonate from gastric and duodenal mucosa (Huang *et a.l*, 2002). Non steroidal anti inflammatory drugs block COXI activity and reduce gastric mucosal prostaglandin results in decrease mucosal blood flow, decrease secretion of mucus and bicarbonate (Burke *et al*., 2006).

The aim of the present study to evaluate the effect of aqueous garlic extract on aspirin induced gastric mucosal lesion and secretion.

MATERIALS AND METHODS

Plant Material: The garlic was identified and authenticated in Herbarium section of the Department of biological sciences, Ahmadu Bello University Zaria with a voucher number 211.

Animals used: A total number of 72 albino Wistar rats of both sexes weighing between 120g-150g were used. The animals were randomly divided into gastric secretion group (n=36) and gastric ulcer group (n=36).

Drugs and chemicals used: Cemitidine 200mg/2ml (SK&F®), Ketamine (Ketalar-Parkes and Davis®) and all other chemicals used were of analytical grade.

Method of extraction: About 250grams of the plant material was peeled, washed, crushed and then put into a conical flask. About 500mls of distilled water was added and shaken by mechanical shaker for 4 hours and left

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overnight. It was then filtered with a Whatman filter paper (size 1) and filtrate was evaporated to dryness in an oven at 40˚C.The residue obtained was then used as the aqueous extract.

Experimental design:

A: Gastric ulcer groups (n=36)

I– Control (n=18) classified as follows:

a Plain control (n=6) given orally distilled water (5mls/Kg) body weight.
b Aspirin control (n=6) given orally aspirin (150mg/kg) body weight.
c Aqueous garlic (AG) extract control (n=6) given orally at a dose of 150mg/kg body weight.

II– Study group (n=18) classified as follows:

a Aspirin (150mg/kg) + AG extract (150mg/Kg) body weight (n=6) orally.
b Aspirin (150mg/kg) + AG extract (300mg/kg) body weight (n=6) orally.
c Aspirin (150mg/kg) orally + Cemitidine (50mg/kg) body weight I.P. (Abdelaziz *et al.*, 2006). (n=6).

B: Gastric secretion groups (n=36)

I– Control (n=18) classified as follows:

a Plain control (n=6) given orally distilled water (5mls/Kg) body weight.
b Aspirin control (n=6) given orally aspirin (150mg/kg) body weight.

d Aqueous garlic (AG) extract control (n=6) given orally at a dose of 150mg/kg body weight.

II– Study group (n=18) classified as follows:

d Aspirin (150mg/kg)+AG extract (150mg/Kg) body weight (n=6) orally.
e Aspirin (150mg/kg)+AG extract (300mg/kg) body weight (n=6) orally.
f Aspirin (150mg/kg) orally+Cemitidine (50mg/kg) body weight I.P.(Abdelaziz *et al.*, 2006). (n=6).

Induction of gastric ulcer: The animals were fasted for 36 hours in separate cages with raised wide wire mesh to avoid coprophagia (Basso *et* al., 1983), but with water given *ad libitum*. After 4 hours under light anesthesia by katamine, animals were killed, stomach removed and opened on greater curvature and examined for ulceration. Evaluation of degree of ulceration was expressed in terms of ulcer score which is calculated by dividing the total number of ulcers in each group by number of rats in that group (Robert et al., 1968). The degree of ulceration was also expressed as ulcer index and calculated by multiplying ulcer score x 100 (Radwan *et al*., 2003). Preventive index was calculated according to the method of Hano *et al*., 1976.

$$\text{Preventive index} = \frac{\text{U.I control} - \text{U.I. treated}}{\text{U.I. treated group}} \times 100$$

$$\%\ \text{of ulceration} = \frac{\text{number of ulcerated rat} - \text{number of non ulcerated rat}}{\text{Number of rat in group}} \times 100$$

Collection of gastric secretion by pyloric ligation (Shay *et al*., 1945): The animals were fasted for 36 hours in separate cages with raised wide wire mesh to avoid coprophagia (Basso *et* al., 1983), but with water given ad libitum as in gastric ulcer group. After administration of drugs to animals one hour later (Abdelaziz et al., 2006) and under anesthesia by Katamine hydrochloride 50mg/kg I.P. abdominal wall was opened, the pylorus identified, ligated and abdomen was then closed. After 4 hours, the animals euthanized by chloroform anesthesia, abdomen opened again, stomachs ligated from esophageal opening and removed, opened at greater curvature, gastric juice collected and centrifuged for studying of gastric secretion parameters including volume in (ml), titritable acidity, Meq/L, titritable acid output MEq/h and proteolytic activity by pepsin concentration (mg 1ml)

Determination of titratable acidity (Deverport, 1972): 0.2ml of centrifuged gastric juice was titrated using phenol red as an indicator with end point at 7.0pH against 0.01 NaOH .Titratable acidity was calculated in Meq/L.

Total titratable acid output Meq/L amount of NaOH that neutralize 100mg of gastric juice.

Determination of Proteolytic activity (Hawk *et al.*, 1960): 0.2ml of centrifuged gastric juice + 3ml of casein 3% for each rat test and blank. Then 10ml of 6% trichloracetic acid added to blank to stop enzyme activity. Both blank and test tubes incubated in water bath with temperature 37˚C for 30 minutes .Then 10ml of trichloracetic acid added to test tubes, shaken well and filtered. Proteolytic activity determined spectromotometrically by optical density measured at 280 W.L.

Statistical analysis: All data were presented as mean ± SEM.The data were analyzed by one way analysis of variance (ANOVA).The Dunnett's post-hoc test was used to determine differences between the groups. Values of $p<0.05$ was regarded as statistically significant.

RESULTS

As regard to gastric secretion parameters there was significant increase ($p<0.05$) of volume, titratable acidity and total acid output in control group that received aspirin than the group that received distilled water. In AG extract group there was significant increase ($p<0.05$) in the volume of gastric secretion when compared with distilled water group as shown in Table (1).There was no significant change in the pepsin concentration.

Table 1: Gastric secretion parameters in control (distilled water), aspirin (150mg/kg) and garlic extract (150mg/kg) orally.

	Group		
Parameter	Distilled water group.n=6	Aspirin group (150mg/kg). n=6	AG extracts group (150mg/kg). n=6
	Mean ± SE	Mean ± SE	Mean ± SE
Volume (ml)	2.24 ± 0.2	5.05 ± 0.96[a]	3.95 ± 0.15[a]
Titritable acidity (Meq/L.)	43.3 ± 0.08	85±1.69[a]	40.1±1.3[ns]
Total acid output (MEq/lh)	117±2.94	190 ±0.76[a]	114±2.32[ns]
Pepsin concentration (mg/ml)	8.8 ±2.1	10.7 ± 2.4[ns]	8.2 ± 1.8[ns]

a=significant (P<0.5)
ns=Not significant (P>0.5)

Table 2: Gastric ulcer parameters in control (distilled water), aspirin (150mg/kg) and garlic extract (150mg/kg) orally

	Group		
Parameter	Distilled water group.n=6	Aspirin group (150mg/kg). n=6	AG extracts group (150mg/kg). n=6
	Mean ± SE	Mean ± SE	Mean ± SE
Ulcer score	3.0±0.15	5.0±0.95[a]	3.0±0.1[ns]
Ulcer index	300	500[a]	300[ns]
Preventive index	83.3	16.7	66.7
% ulceration	16.6%	83.3%	33.3%

a = significant (P<0.5)
ns= Not significant (P>0.5)

Table 3: Gastric secretion parameters in aspirin(150mg/Kg), aspirin(150mg/Kg)+ AG extract 150mg/kg, aspirin(150mg/Kg)+ 300mg/kg orally and aspirin(150mg/Kg)+ Cemitidine (50mg/kg) i.p.

	Group			
Parameter	Aspirin (150 mg/kg) Group n=6	Aspirin (150 mg/kg) +AG extract (150mg/kg) n=6	Aspirin (150 mg/kg) +AG extract (300mg/kg) n=6	Aspirin (150 mg/kg) Cemitidine (50mg/kg) n=6
Volume (ml)	5.05±0.96	3.85±0.13[ns]	3.8±1.3[ns]	1.3±0.14[a]
Titritable acidity (Meq/L.)	85±1.69	72±1.2[a]	81±2.1[a]	56.25±6.3[a]
Total acid output (Meq/lh)	190±0.76	133±1.92[a]	198±2.8[a]	89.92±2.8[a]
Pepsin concentration (mg/ml)	10.7±2.4	6.7±1.6[a]	3.16±1.4[a,]	7.9±1.9[ns]

a = significant (P<0.5)
ns= Non significant (P>0.5)

Table 4: Gastric ulcer parameters in aspirin(150mg/Kg), aspirin(150mg/Kg)+ AG extract (150mg/kg), aspirin(150mg/Kg)+ AG extract (300mg/kg) and aspirin+ cemitidine 50mg/kg. (n=6) each.

	Group			
Parameter	Aspirin (150 mg/kg) Oral	Aspirin (150 mg/kg) +AG extract (150mg/kg)	Aspirin (150 mg/kg) +AG extract (300mg/kg)	Aspirin (150 mg/kg) Cemitidine 50mg I.P
Ulcer score	5.0±0.95	1.2±0.29[a]	2.3±0.58[a]	3.2±1.0[a]
Ulcer index	500	120[a]	230	230[a]
Preventive index	16.97	66.7[a]	89.25[a]	83.3[a]
%ulceration	83.3%	33.3%	16.7%	16.7%

a = significant (P<0.5)

As regard to ulcer parameters there was significant increase (p<0.05) in ulcer score and ulcer index in aspirin (150mg/kg) group than both distilled water and AG extract (150mg/kg) group. There was an associated low preventive index and high percentage of ulceration as shown in Table (2).

In the study groups, as regard to gastric secretion parameters, there was a significant decrease (p<0.05) in titratable acidity and total acid output in the two extract groups {(aspirin 150mg + garlic 150mg/Kg) and (aspirin 150mg/Kg +garlic 300mg/kg)} than the aspirin (150mg/kg) group. When compared with aspirin (150mg/Kg) + Cemitidine (50mg/kg) there was also a significant decrease (p<0.05) in gastric volume, titritable acidity and total acid output than aspirin (150mg/kg) group alone as shown in Table (3).

There was a significant decrease (p<0.05) in the pepsin concentration in the extract treated groups only.

As regard to gastric ulcer parameters there was a significant decrease (p<0.05) in ulcer score, ulcer index and ulcer score in (aspirin + AGE 150mg/Kg) and (aspirin + AGE300mg/kg) groups. This was accompanied by increased preventive index and decrease percentage of ulceration. In the aspirin + cemitidine 50mg/kg i.p. group, there was also a significant decrease (p<0.05) in ulcer index accompanied by increase preventive index and decrease percentage of ulceration as shown in Table (4).

DISCUSSION

In this study of gastric secretion parameters, aspirin150mg/kg + AG extract 150 and 300mg/kg groups show significant decrease (p<0.05) in titratable acidity and total acid output than aspirin 150mg/kg alone. In gastric ulcer parameters, there was significant decrease(p<0.05) in ulcer index and ulcer score in aspirin + AG extract 150 and 300mg/kg groups than aspirin alone without significant differences between AG extract 150 and 300mg/kg groups. In comparison, aspirin 150mg/kg + cemitidine 50mg/kg i.p. group produced more significant decrease (p<0.05) in gastric secretion parameters. These results could be explained by that prostaglandins normally protect the gastrointestinal mucosa from damage by maintaining blood flow and increasing mucosal secretion of mucous and bicarbonate (Voultilainen et al., 2001). Synthetic non-steroidal anti-inflammatory (NSAIDS) like aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and block diffusion of H^+ (Roa et al., 1999). Aspirin blockade of cyclooxygenase-1 (Cox-1) and (Cox-II) results in reduction of prostaglandin synthesis. The interruption of prostaglandin synthesis results in impairment of mucosal damage repair, thus facilitating mucosal injury (Burke *et al.*, 2006). Aspirin and related non-steroidal anti-inflammatory drugs and alcohol can aggravate or interfere with the healing of peptic ulcers. Smoking is also known to slow ulcer healing (Rydning *et al.*, 1982). In parietal cells, H^+ ion secretion is an oxidant process. H^+ ion dissociates from H_20 or H_2CO_3 also in an oxidant process. H^+ is pumped out in exchange with K^+ as an active transport process and Cl^- ion in exchange with HCO_3^- in basolateral membrane of parietal cells (Ganong, 2005). Cl^- ion is then pumped and transported into gastric lumen. These increase oxidant process and increase hydrogen peroxide production. In presence of Cl^- and H_2O_2, hydrochloric acid will be formed and this is a very toxic oxidant. This will results

in mucosal membrane lipid peroxidation and mucosal soreness and disruption (Moncada and Higgs, 1993). Lipid peroxidation and lipid derived products have been implicated in pathogenesis of a variety of diseases (Moriel *et al*., 2000). Extracts of fresh garlic contain anti-oxidant phytochemicals that prevent oxidants damage (Kalayarasan *et al*., 2008). These include unique water soluble organosulphur compounds, lipid soluble organosulphur compounds and flavonoids (Borek, 2001).The antioxidant enzymes activities superoxide dismutase, reduced glutathione,catalase and glutathione peroxidase in lipopolysaccharide and D-galactosamine hepatic injury has been reversed to normal with garlic extract (Elbasbishy, 2008). Garlic extract was found to be the most effective in the prevention of aflatoxin-induced toxicity and free radical generation in rats (Abdelwahhab and Ali, 2003). Burn injury caused significant increases in malondialdehyde and protein oxidation and decrease glutathione, garlic extract reversed these oxidant responses. It seems likely that garlic extract protects tissues against oxidative damage (Sener *et al*., 2003). It seems that aqueous garlic extract protect gastric mucosa from aspirin injury through neutralization of released free radicals and inhibition of Hcl secretion. These results concurs with Borek (2001) who reported that aqueous garlic extract inhibits lipid peroxidation and inhibits the activation of oxidant induced transcriptation factor and nuclear factor Kappa B, thus protecting endothelial cells from injury by the oxidizing molecules. Sener *et al.* (2005) who reported that it seems likely that garlic aqueous extract with its antioxidant and oxidant scavenging properties may be of potential therapeutic value in protecting against oxidative injury due to hepatic ischemia reperfusion. Gedik *et al*. (2005) also suggested that AG extract with it antioxidant and anti-fibrotic properties may be of potential therapeutic value in protecting liver fibrosis and oxidative injury. However, these results contradicted the work of Oboh (2005) who reported that garlic induced oxidative stress and produced hepatotoxicity.

CONCLUSION

Aqueous garlic extract offered some protection against aspirin induced gastric mucosal damage. The antioxidant compounds present in AG extract play protective role against the production of reactive oxygen species and lipid peroxidation. The present study revealed that AG extract has promising phytochemicals for the development of alternative treatment against gastric ulcer.

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