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ACUTE AND CHRONIC *ALOE BARBADENSIS* MILLER GEL MEDIATES ANTI-ULCEROGENIC AND GASTRIC ANTI-SECRETORY EFFECTS VIA HISTAMINERGIC H_2 RECEPTORS

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ABSTRACT
Aloe is an ethno-pharmacological plant with huge medical potentials in alleviating myriad ailments including gastrointestinal disorders. This study aimed to investigated the effect of chronic and acute administration of *Aloe barbadensis* Miller gel on gastric acid secretion and gastric ulcers in rats. 30 albino Wistar rats were placed on either normal rat chow + drinking water, and/or *Aloe barbadensis* Miller gel for 21 days. Another group (acute group) of normal rats was only given *Aloe* gel on the day of sacrifice. Results revealed that gastric acid output (GAO) reduced significantly ($p<0.001$) from a basal value of 2.36 ±0.76mmol/L/hr to 1.62 ± 0.30mmol/L/hr following acute administration of *Aloe* gel. Chronic administration of *Aloe* gel significantly ($p<0.01$) reduced the mean basal GAO from 4.10 ±0.14mmol/L/hr, to 2.56 ±0.29mmol/L/hr. Administration of histamine thereafter, increased the GAO to 4.26 ± 0.45mmol/L/hr and 5.02 ±0.43mmol/L/hr, in acute and chronic groups respectively, while cimetidine reduced it to 4.20 ± 0.44mmol/L/hr and 4.9 ±0.63mmol/L/hr respectively. The mean ulcer scores reduced significantly in the groups fed with acute (16.80 ± 1.20, $p<0.05$) and chronic (10.4 ±1.75, $p<0.01$) *Aloe* gel compared with control (24. 20 ± 3.11). In conclusion, *Aloe barbadensis* Miller gel appears to be a potent inhibitor of gastric acid secretion and cyto-protective agent under normal physiological conditions, possibly via blocking the H_2-histaminergic receptor.

KEYWORDS: Aloe *Barbadensis* Miller gel, gastric secretion, ulcer, rats.

INTRODUCTION
Aloe is a word derived from the Arabic word "Alloeh" meaning shining and bitter substance. Aloe vera is a succulent perennial medicinal plant belongs to the lily family (Boudreau and Beland, 2006; Reynolds, 2004). Aloe vera plant is believed to have originated in Northern Africa. It is now widely grown in Africa, especially in Madagascar, Nigeria, Ghana, Ethiopia etc. Other countries where this plant is found include United States of America (USA), Japan, the Caribbean and the Mediterranean countries (Akinyele and Udiyi, 2007; Sharma *et al*., 2013).

Aloe vera exists in about 360 different species. Of the genus only five family members are considered to have therapeutic properties, namely, Aloe vera, Aloe saponaria, Aloe perryl, Aloe arborensis and Aloe ferro (Atherton, 2002; Anonymous, 2008a; Anonymous, 2008b). Aloe vera is known by different names like Chinese Aloe, Indian Aloe, True Aloe, Barbados Aloe, Burn Aloe, First Aid Plant, Wand of Heaven and Miracle Plant (Liao *et al*., 2004; Ombrello, 2008).

Aloe vera leaf contains more than 200 different chemical constituents which include acemannan, emodin, aloins, enzymes, mucopolysaccharides, minerals, galactomannans, phenols, saponins, salicylic acid, alkaloids, flavonoids, terpenoids and lectins (Eshun and He, 2004; Boudreau and Beland, 2006; Joseph and Justin, 2010; Adesuyi *et al*., 2012).

Aloe vera plant has huge medicinal values, it cures stomach and intestinal diseases especially ulcers, it is effective against frostbite, radiation burns and other external pains as well as aiding in digestion and combating constipation, inflammation, kidney stones and tissue damage from X-ray exposure (Balch and James, 2000; Leigh, 2005; Wynn, 2005; Reddy *et al*., 2011). Aloe also has bactericidal, virucidal and fungicidal actions as well as the ability to reduce bleeding, fever, inflammation and vascular spasm (Atherton, 2002; Jasso de *et al*., 2005; Ferro *et al*., 2003; Jyotsana *et al*., 2008). Topical application of aloe is useful in the management of insect bites, bee stings, boils, haemorrhoids, gum sores, gout, arthritis, acne and rheumatism. Aloe vera also acts as blood glucose normalizer and as antioxidant

(Gordon and David, 2001; Raamachandra, 2001; Botes *et al*., 2008; Snezana, 2007; Joseph and Justin, 2010).

The stomach is a bean-shape, dilated portion of the gastrointestinal tract which is situated in the epigastric, umbilical and at the left hypochondriac regions of the abdominal cavity. The gastric glands are responsible for the secretion of digestive juices while the pyloric gland secretes mucous for pyloric mucosa protection, while parietal cell secretes hydrochloric acid (Guyton, 2006). Gastric ulcers ensue when the protective barrier of the stomach is broken.

With the alarming incidence of gastric ulceration and the widespread liberal consumption of Aloe vera plant for its purported healing potentials, it was therefore the aim of this research work to investigate the effect (if any) of acute and chronic administration of *Aloe barbadensis* Miller gel on gastric acid secretion and gastric ulceration in rats.

MATERIALS AND METHODS

Experimental animal

Male albino Wistar rats weighing between 200 and 250g were the animal of choice for this experiment. They were obtained from the animal house of the department of Pharmacology, University of Calabar, Nigeria, and housed under standard environmental conditions according to international guideline for animal care (CCAC, 2009).

Experimental plant

The experimental plant was purchased from a local market in Calabar Metropolis and was identified as *Aloe barbadensis* Miller by a Taxonomist, Mr. Frank, in Botany Unit of the Department of Biological Sciences, University of Calabar, Nigeria, where a specimen voucher was deposited.

Preparation of Aloe vera gel

After collecting some fresh samples of Aloe vera plant, the leaves were plucked and washed thoroughly. The leaves were cut open and the whitish marshy area were scrapped off and put in a clean beaker (Pyrex, 500ml) until it was sufficient enough for blending, the gel was thereafter homogenized using an electric blender. No water was added this was to give a 100% concentration of the stock solution.

Experimental protocol

A total of thirty (30) male albino Wistar rats were used for this study. They were divided into 2 batches of 15 rats each and were used utilized for the gastric acid secretion and cyto-protection study respectively. Each batch was further assigned into 3 groups of five rats each. Group 1 (control group) received only normal rat chow and drinking water. Group 2 (chronic group) had oral administration of Aloe vera gel (0.2ml) once daily for thirty days. Group 3 (acute group) had Aloe vera gel (0.2mL) treatment on the last day of the experiment.

Measurement of gastric acid secretion:

Measurement of gastric acid secretion was done by the continuous perfusion method of Ghosh and Schild (1958) modified by Osim *et al*, (1991). Rats from the control and test groups were fasted for 18-24 hours before the start of the experiment. The rats were anaesthetized with 0.6ml/100g body weight of 25% (wt/v) solution of urethane (Sigma, UK) given intra-peritoneally. The trachea was exposed and cannulated. An infusion tube 75cm length and 3mm diameter connected to 60ml syringe carried by a pump was passed to the stomach through mouth and oesophagus. A ligature to stop back flow was made around the oesophagus in the neck. The abdomen was opened along the *linea alba* to minimize bleeding. The small intestine was reached and a semi-transection of 1-2cm away from the pylorus was made and a fistula 8cm long passed gently into the stomach through the pyloric sphincter and knotted.

Normal saline solution pH 7.00 placed in the pump was perfused through the stomach at 1ml/minute via a perfusor. After an initial wash, the perfusate collected every 10 minutes interval and was titrated with 0.01N NaOH solution in a 25ml burette using phenolphthalein as indicator with pink coloration indicating the end point.

The pH of the saline was maintained by passing the perfusion tube through a water bath maintained at temperature of 37^{o}C. Also a low wattage bulb was placed above the animal to warm it and the body temperature monitored. A rectal thermometer was inserted via the anus to ensure that the body temperature was at 37^{o}C, care had to be taken not to ligate the vagus nerve or other blood vessels. To each 10 minute perfusate was added 2 drops of phenolphthalein indicator before titration against 0.01N NaOH (Analar BOH, England) to determine total acidity.

Analysis of gastric acid.

Gastric acid output was measured by titrimetric analysis. The calculation of acid in millimole per litre per hour (mol/L/hr) follows the principle that states that a gram equivalent of acid balances a gram equivalent of the base at neutralization point. This means that:

Normality (N) of Acid x Volume (V) of Acid = Normality of Base x Volume of Base:

i.e. $N_A \times V_A = N_B \times V_B$

From the above equation since Normality (N) of base is known i.e. 0.01N and the volume of base needed for neutralization is known, the gram equivalent can be calculated thus: $N_B \times V_B$. This at the end points to the gram equivalent of the acid. If the volume is in mls, the acidity end point is in milli-equivalent of acid. For a small animal like the rat milliequivalent will be too small and is always converted to µeq or µmol.

Ulcer studies

Gastric ulceration was induced in rats as described by Tekeuchi *et al*, (2001) by oral instillation of 1ml of 0.1N

HCl + 70% ethanol through intubation after an over night fast. One hour later, the animals were sacrificed using over dose of diethyl ether/chloroform and the stomachs were removed and opened along the greater curvature. Haemorrhagic lesions were examined macroscopically and scored as described by Elegbe (1978).

Ulcer scoring

Score	Description
0	Plan
0.5	0-6mm
1	2-3mm
2	>3mm

Statistical analysis

The results are all expressed as mean ± standard error of mean (SEM). The results were compared with the unpaired Student's t-test; (that is between the control and test groups). The P-value of less than 0.05 was considered to be statistically significant. The analysis was done with the help of computer software (SPSS and Excel for windows XP, Brian series, China).

RESULTS

The Effect of acute administration of *Aloe barbadensis* Miller gel, histamine and cimetidine on gastric acid secretion in rats

As shown in fig.1, the mean basal gastric acid output was 2.36 ± 0.76mmol/L/hr. Upon administration of Aloe vera gel, the gastric acid output reduced significantly ($p<0.001$) to a mean output of 1.62 ± 0.30mmol/L/hr.

When histamine was added thereafter, the mean output increased to 4.26 ± 0.45mmol/L/hr, (with a peak value of 5.04 ± 0.23mmol/L/hr), although this increase was not significant when compared with the basal mean output.

Cimetidine thereafter, decreased gastric acid output was to a mean value of 4.20 ± 0.44mmol/L/hr, producing a significant reduction when compared with the mean basal output.

The Effect of chronic administration of *Aloe barbadensis* Miller gel, histamine and cimetidine on gastric acid secretion in rats

Fig. 2, the mean basal output in control group was 4.10 ±0.14mmol/L/hr, it reduced significantly ($p<0.01$) to 2.56 ±0.29mmol/L/hr following chronic feeding with aloe vera gel for 21 days.

When histamine was administered thereafter in the chronic group, the mean gastric output increased to 5.02 ±0.43mmol/L/hr, producing no significant change in mean gastric acid output compared with control. Cimetidine added thereafter caused a slight reduction in the mean gastric output to 4.9 ±0.63mmol/L/hr, producing no significant change in gastric acid output compared with the mean basal gastric acid output.

Administration of histamine in the control group significantly ($p<0.01$) increased the mean gastric output to 6.06 ±0.37mmol/L/hr, (with a peak of 7.68 ±0.29mmol/L/hr) from a basal level of 4.15 ±0.27mmol/L/hr. When Cimetidine was added thereafter, the mean gastric acid output reduced to 5.80 ±0.56mmol/L/hr, and was significant ($p<0.05$) when compared with the mean basal output.

Comparison of the Effect of Histamine and cimetidine on gastric acid secretion in the in Control and Test Groups

As shown in Fig. 3, the peak gastric outputs following the administration of histamine in the control, chronic and acute treated groups were 7.68 ± 0.29mmol/L/hr, 6.72 ± 0.47mmol/L/hr and 5.04 ± 0.24mmol/L/hr respectively, producing a significant ($p<0.01$) reduction in acute group compared with the control.

Mean gastric acid output following administration of cimetidine in control, chronic and acute treated groups were 5.80 ± 0.56mmol/L/hr, 4.90 ± 0.63mmol/L/hr and 4.20 ± 0.44mmol/L/hr respectively. It was significantly ($p<0.05$) reduced in acute group compared with control group.

Comparison of the ulcer score in control and test groups

As shown in fig 4, the mean ulcer score in the control group was 24. 20 ± 3.11, it was significantly reduced in chronic (10.4 ±1.75, $p<0.01$) and acute (16.80 ± 1.20, $p<0.05$) groups compared with control.

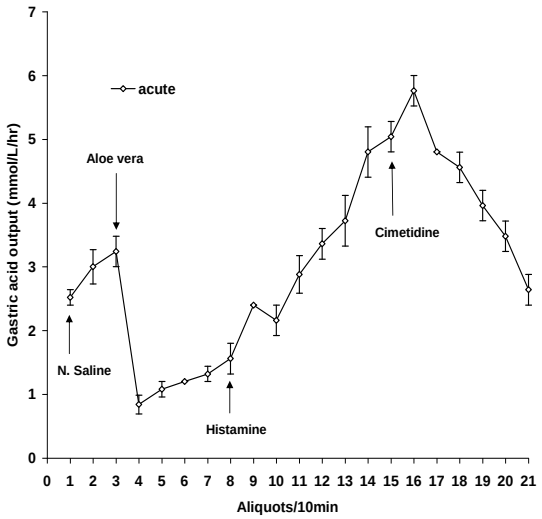

Figure 1: Showing effect of acute administration of Aloe barbadensis Miller gel, histamine and cimetidine on gastric acid output in rats.

Values are expressed as mean ±SEM, n = 5.

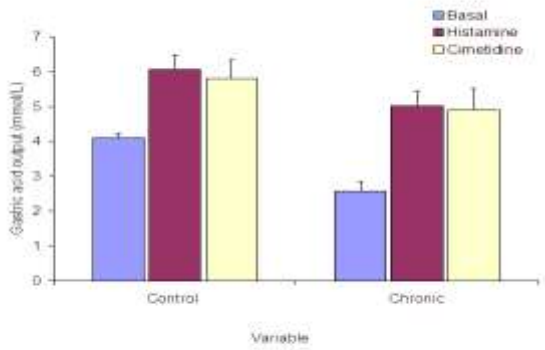

Figure 2: Comparison of basal gastric acid output, effect of histamine and cimetidine in control and chronic groups

Values are expressed as mean ±SEM, n = 5

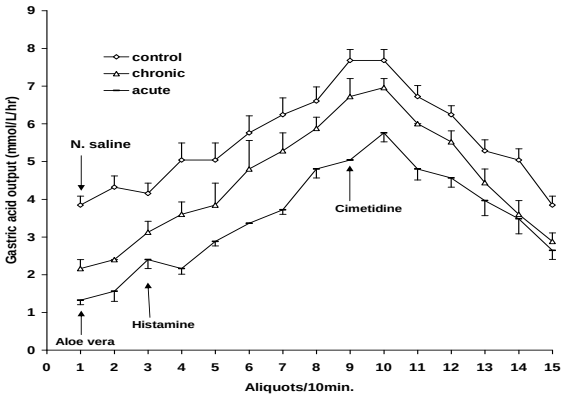

Figure 3: Showing the effect of cimetidine on activity of histamine on gastric acid secretion in control, acute, chronic aloe vera gel treated rats.
Values are expressed as mean ±SEM, n = 5.

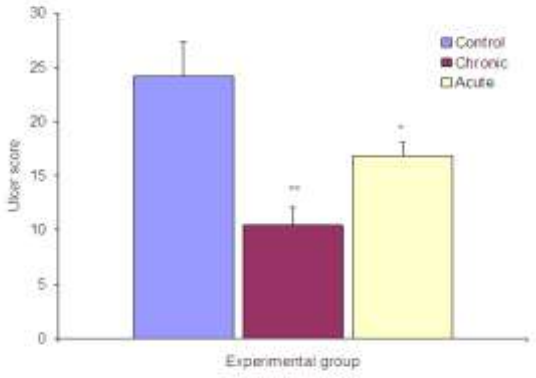

Figure 4: Comparison of mean ulcer scores in control, chronic, acute aloe vera gel treated rats

Values are expressed as mean ±SEM
*p<0.05, **p<0.01 vs control

DISCUSSION

The effect of acute and chronic administration of *Aloe barbadensis* Miller gel on gastric acid secretion and ulceration was studied in Wistar rats. The results obtained showed that acute and chronic administration of *Aloe barbadensis* miller gel significantly reduced gastric acid output and gastric ulcers in rats, with the acute administration being more potent than the chronic. However, both acute and chronic administration of aloe gel inhibited the effect of histamine induced gastric acid secretion.

Histamine was found to significantly increase the gastric acid output of rats in this study but its effect was blocked by administration of cimetidine. This is in agreement with previous reports (Ganong, 1997). Subcutaneous administration of histamine stimulates copious secretion of acid in the stomach of rats through the H_2 receptor. Histamine from enterochromaffin-like cells may well be a primary modulator, but the magnitude of the stimulus appears to result from an intricate augmented interaction of signals of each type (Samuelson and Hinkle, 2003). For example, the low amounts of histamine released constantly from mast cells in the gastric mucosa, only weakly stimulates acid secretion. Also, pharmacologic antagonist of these molecules can block acid secretion (Samuelson and Hinkle, 2003). On the molecular level, the mechanism of action of Histamine on the parietal cell has been described to be through activation of adenylate cyclase, leading to elevation of intracellular cyclic AMP concentrations and activation of protein kinase A (PKA). One effect of PKA activation is phosphorylation of cytoskeleton proteins involved in transport of the H+/K+ ATPase from cytoplasm to plasma membrane (Yao and Forte, 2003).

The inhibition of histamine induced acid secretion by the administration of *Aloe barbadensis* Miller gel; suggests that it interact with the H_2 receptors. It is however possible that other pathway may be involved which were not investigated in this study. It is seen that Cimetidine competitively inhibits the action of histamine at the H_2 receptor antagonist. This is further supported by the fact that the aloe gel augmented the effect of administered Cimetidine on gastric acid output in the rats.

It is obvious that crude aloe vera gel could be administered to peptic ulcer patients or those who are susceptible to gastric ulcer to help alleviate gastric ulceration.

In conclusion, *Aloe abrbadensis* Miller gel appears to be a potent inhibitor of gastric acid secretion and cyto-protective agent under normal physiological conditions, possibly via blocking the H_2-histaminergic receptor.

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