Effect of Aqueous Extract of *Vernonia amygdalina* (Bitter Leaf) on GASTRIC $_{p}$H and Serum Bicarbonate of Wistar Rats

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DOI: 10.56201/ijmepr.v7.no2.2023.pg1.12

ABSTRACT
Vernonia amygdalina is a shrub commonly consumed in Nigeria as a vegetable in soup. The aim of the present study was to determine the effect of aqueous extract of Vernonia amygdalina on gastric pH and post-prandial serum bicarbonate in wistar rats. A total of 20 male wistar rats weighing 180-250g were divided into four groups of 5 rats each; Group I served as control, Group II received 400mg/kg Aqueous bitter leaf extract, Group III received Omeprazole (20mg/kg) and Group IV received a combination of Omeprazole and Aqueous bitter leaf extract. 45 minutes after administration of the extract, the pH of the gastric effluent decreased significantly whereas the post-prandial serum bicarbonate concentration increased significantly compared to control ($p < 0.05$). Addition of the extract with omeprazole caused significant reduction in gastric pH but increased serum bicarbonate compared to the omeprazole-only group. The results suggest that the extract would increase gastric acid while preserving the serum bicarbonate. The Aqueous bitter leaf prevented excessive reduction in gastric pH that occurred with the drug alone. Hence, the study concluded that aqueous bitter leaf alone increased the level of gastric acid secreted while in combination with Omeprazole, it acted as a buffer to stabilize the gastric pH
Keyword: Vernonia amygdalina, Serum Bicarbonate, Omeprazole

Background to Study

Vernonia amygdalina

Vernonia amygdalina is a shrub or small tree of 2.5cm tall with many branches. *Vernonia amygdalina* belongs to the family of plants known as Asteraceae and genus Vernonia and is fully classified as follows; Kingdom- plantae, Class - Dicotyledone, Order - Asterales, Division Angiospermae, Subclass - Gamopetalae, Family - Asteraceae, Genus - Vernonia, Species -Vernonia amygdalina and Botanical name – Vernonia amygdalina (Dutta ., 2005)

In Nigeria, *Vernonia Amygdalina* is commonly found in the south east ecological zones of Cross river, Akwa-ibom, Rivers, Bayelsa, Anambra, Abia, Imo and Enugu states. It is also found in some countries in Africa, namely; Togo, Kenya, Tanzanian, Cameroon, Ghana, etc. (Egedigwe.; 2010)

Vernonia amygdalina, commonly known as 'bitter leaf, is called different names in different locations in Nigeria. It is known in igbo language as 'onugbu', in yoruba as 'ewuro', in efik/Ibibio as 'etitot', in edo as 'oriwo', in hausa as 'chusardoki' while in ugep, cross-river state, it is referred to as 'kedemiti sekedali' in their native language (Egedigwe.; 2010).
Its trunk measure up to 40cm in diameter sometimes with barks that are grey to brown in color. The trunk is initially smooth, gradually becoming fissured with young branches that are densely pubescent. Its leaves appear alternate, are simple with absence of stiple and has petiole about 6mm in diameter and elliptic in shape. The leaves are green with a characteristic odor and a bitter taste. The bitterness is caused by sesquiterpene lactones known as vernodalin, vernolepin and vernomygdin and steroid glycosides known as vernoniosides (Challand et al.; 2009). It is propagated by seed but most farmers use stem cuttings for their cultivation. Cuttings used for propagation of mature stems are selected on the basis of their attributes which include their degree of bitterness, leaf size and growth characteristics. Cuttings maybe planted erect or slanted at an angle of 45 degrees to obtain more side shoots and cutting have been observed to grow faster than seedlings. (Kalanda and Ligowski.; 1995).

Economic Importance of Vernonia Amygdalina
Vernonia amygdalina is commonly used in traditional medicine for various purposes; extracts from the leaves are used to treat fever, malaria, diarrhea, dysentery, hepatitis and cough. It is also used as a laxative and as fertility inducer. Some other people use it for the treatment of scabies, headaches and stomach-ache. Its root extracts are also used as treatment for malaria and gastrointestinal disorders (Abosi and Raseroka; 2003). In Nigeria, its leaves are placed on a wound as a substitute for iodine. One of the most common medicinal use of *vernonia amygdalina* is as a treatment against intestinal worms including nematodes. Not only humans but also chimpanzees ingest the pith of *vernonia amygdalina* for the control of intestinal nematode infections (Attangwho et al., 2007). Bark infusions are also used to treat fever and diarrhea while the dry flowers serve as a source of cure against stomach disorders. *Vernonia amygdalina* is also used as a control agent against disease in plant.

Gastric Acid
Gastric acid, gastric juice, or stomach acid, is a digestive fluid formed within the stomach lining. With a pH of between 1 and 3, gastric acid plays a key role in digestion of proteins by activating digestive enzymes, which together break down the long chains of amino acids of proteins. Gastric acid is regulated in feedback systems to increase production when needed, such as after a meal. Other cells in the stomach produce bicarbonate, a base, to buffer the fluid, ensuring a regulated pH. These cells also produce mucus - a viscous barrier to prevent gastric acid from damaging the stomach linings. The pancreas further produces large amounts of bicarbonate and secretes bicarbonate through the pancreatic duct to the duodenum to neutralize gastric acid passing into the digestive tract.

The active components of gastric acid are protons and chloride. Often simplistically described as hydrochloric acid, these species are produced by parietal cells in the gastric glands in the stomach. The secretion is a complex and relatively energetically expensive process. Parietal cells contain an extensive secretory network (called canaliculi) from which the "hydrochloric acid" is secreted into the lumen of the stomach. The pH of gastric acid is 1.5 to 3.5 in the human stomach lumen, a level maintained by the proton pump H+/K+

ATPase. (Elaine et al 2018) while the parietal cell releases bicarbonate into the bloodstream in the process, which causes a temporary rise of pH in the blood, known as an alkaline tide.

The highly acidic environment in the stomach lumen degrades proteins. Peptide bonds, which comprise proteins, are labilized. The gastric chief cells of the stomach secrete enzymes for protein breakdown (inactive pepsinogen, and in infancy, rennin). The low pH activates pepsinogen into the enzyme pepsin, which then aids digestion by breaking the amino acid bonds in a process known as proteolysis. In addition, many microorganisms are inhibited or destroyed in an acidic environment, preventing infection or sickness.

There are three phases in the secretion of gastric acid which increases the secretion rate in order to digest a meal (Harvey 2016).
I. The cephalic phase: Thirty percent of the total gastric acid secretions to be produced is stimulated by anticipation of eating and the smell or taste of food. This signaling occurs from higher centers in the brain through the vagus nerve (Cranial Nerve X). It activates parietal cells to release acid and ECL cells to release histamine. The vagus nerve (CN X) also releases gastrin releasing peptide onto G cells. Finally, it also inhibits somatostatin release from D cells.

II. The gastric phase: About sixty percent of the total acid for a meal is secreted in this phase. Acid secretion is stimulated by distension of the stomach and by amino acids present in the food.

III. The intestinal phase: The remaining 10% of acid is secreted when chyme enters the small intestine, and is stimulated by small intestine distension and by amino acids. The duodenal cells release entero-oxyntin which acts on parietal cells without affecting gastrin.

Regulation of Secretion
Gastric acid production is regulated by both the autonomic nervous system and several hormones. The parasympathetic nervous system, via the vagus nerve, and the hormone gastrin stimulate the parietal cell to produce gastric acid, both directly acting on parietal cells and indirectly, through the stimulation of the secretion of the hormone histamine from enterochromaffine-like cells (ECL). Vasoactive intestinal peptide, cholecystokinin, and secretin all inhibit production.

Justification of Study
The potency of *vernonia amygdalina* as an herbal medicine is undebatable, but there is little or no information on the assumptions on which the drug is being administered by traditional medicine practitioners in Africa. In south eastern Nigeria, this informed the basis for this study, to find out the effects aqueous extracts of the plant has on gastric acid and serum bicarbonate secretions in the intestinal lumen and to determine if the extract can be used for the management of intestinal disorders such as peptic ulcer disease.

Aim and Objectives of Study
Aim
The aim of this study is to evaluate the effect of aqueous extract of bitter leaf on the gastric acid and serum bicarbonate secretions in wistar rats.

The various objectives included:
1. To determine the effect of aqueous extract of bitter leaf on gastric pH.
2. To determine the effect of administration of aqueous extract of bitter leaf on serum postprandial bicarbonate concentration.
3. To evaluate the effect of co-administration of aqueous extract of bitter leaf and omeprazole on gastric pH and serum postprandial bicarbonate concentration.

Significance of Study
This study will help to inform the public on the role of the extract on gastric acid secretion. It will add to existence knowledge and also help to add laws that will help to regulate the consumption of *vernonia amygdalina* (bitter leaf)

Materials and Methods
The following are the materials used during the study:
Animal cage and wire gauze, top feed (finisher) and water, trough, broom and parker, disinfectants, dry saw dust, laboratory coat, masking tape, weighing balance, rubber hand gloves, 2ml syringe, lithium heparin bottle, universal bottle, dissecting board, dissecting kit, drip set, normal saline, 100ml beaker, 50ml beaker, dropper, ethyl carbamate (urethane), burette, 0.01 NaOH solution, phenolphthalein, distilled water, blending machine, maceration jar, whatman filter paper (model no: 10001- 125), evaporating dish, water bath, weighing balance (model no: AUW-220D).

Collection of Plant
Fresh leaves of bitter leaves were bought from a local market in Omoukiri Aluu in rivers state municipality, Rivers state. It was identified as *V. amygdalina* by a taxonomist from botanical unit of the department of biological science, University of Port Harcourt, Nigeria.

Preparation of Extract
The aqueous method was prepared, according to the methods describe by Sofoworo in 1984. Fresh leaves of *V. amygdalina* (bitter leaves) were cut off their stalk and stripped down the midrib. The stripped leaves where thoroughly rinsed in clean water and left overnight to be properly drained of the wash water. The leaves where then air dried under sun light. The dried leaves were then grinded to powder using an electrical blender. 25g of the powder form were immersed in 100ml of ionized water in various macerated jars for 24 hours using a mechanical aggregation at room temperature. The suspension was filtered using what man filter paper

Animal Grouping
Twenty (20) animals were randomly assigned to 4 groups (1, 2, 3, 4) Group 1 served as control group and received distilled water, Group 2, 3 and 4 received Extract only, Omeprazole only, then Omeprazole and extract respectively.

DETERMINATION OF GASTRIC pH

The rats were fasted overnight with free access to clear water. The study was carried out using the continuous stomach perfusion technique described by Ghosh and Schild (1958) and modified by Amure and Ginsburg (1964). The animal was anaesthetized with 25% (w/v) urethane (Ethylcarbamate) at a dose of 0.6ml/100g body weight. A tracheal cannula was

inserted via an incision on the neck to ensure normal breathing throughout the cause of experiment. An abdominal incision throughout the linea alba was made to expose the stomach and a semi transection made at the junction of the pylorus with the duodenum. A pyloric cannula was inserted to collect gastric content. An orogastric infusion tube was carefully passed from the mouth through the oesophagus to the stomach and ligated just behind the trachea cannula to prevent influx. Care was taken not to damage the vagus nerve. Then a perfusion of pre- warned 0.9% normal saline was inserted at a rate of 1ml per minute. The animal was kept warmed with 100watts electric bulb. Also cotton wool soaked in normal saline was placed in the open abdominal cavity to avoid dehydration. Gastric effluents were collected via the pyloric cannula at intervals of 15mins (i.e. 15, 30, 45) for each rat.

After which the pH of the gastric effluent was determined using a pH meter (model: 2211, Hanna: Italy). Then the results of the gastric effluent were graphically represented according

After collecting the last gastric effluent at 45mins, blood sample was collected from each rat into a lithium heparin bottle for analyzing the serum post prandial bicarbonate concentration. This was done using colorimetric method using appropriate kits. The test is based on the principle of that bicarbonate in the samples react with the phosphoenolpyruvate in the presence of the phosphoenolpyruvate carboxylase (PEPC), to produce oxaloacetate and phosphate (Erchinger et al, 2016). One hydrogen ion from a NADH analogue is transfer to the oxaloacetate using porcine malatdehydrogenase (MDH) to produce malate and NAD+Analogue. The consumption NADH result in a decreased absorbance at 390nm that is directly proportional to concentration in the sample.

RESULTS

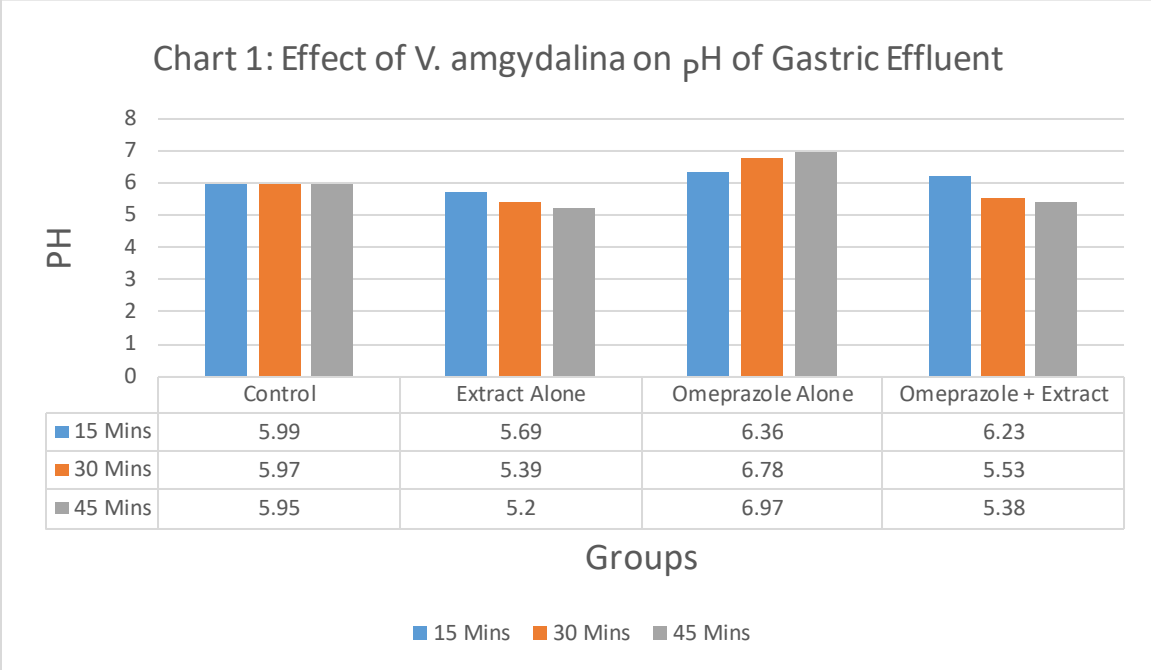
All data were analyzed using statistical package (SPPSS version 20.0). The data was handled both manually and electronically. The analyzed data were presented in tables. Mean comparison (Descriptive analysis) were done with one way (ANOVA). A confidence limit of 95% group differences were considered significant at $P<0.05$. Results are shown as mean ± SEM.

Table 4.1: The effect of *Vernonia amgydalina* (bitter leaf) on the pH of gastric effluent

	TIME INTERVALS		
GROUPS	15MINS	30MINS	45MINS
GROUP 1 (CONTROL)	5.99 ± 0.03	5.97 ± 0.01	5.95 ± 0.00
GROUP 2 (EXTRACT ALONE)	5.69 ± 0.20	5.39 ± 0.37	5.20 ± 0.06
GROUP 3 (OMEPRAZOLE ALONE)	6.36 ± 0.05	6.78 ± 0.03^{ad}	6.97 ± 0.01^{abd}
GROUP 4 (OMEPRAZOLE + EXTRACT)	6.23 ± 0.31	5.53 ± 0.15	5.38 ± 0.27

Values represent Mean ±, Standard error of mean (SEM), n=5

Where a=statically significant i.e. (P<0.05) when compared to group 1
b=statically significant i.e. (P<0.05) when compared to group 2
c= statically significant i.e. (P<0.05) when compared to group 3
d= statistically significant i.e. (P<0.05) when compared to group 4



Analysis of Result of V. Amygdalina on the pH of Gastric Effluent

Table 4.1 above shows the effect of *V. amygdalina* (bitter leaf) on the pH of gastric effluence at different time intervals in wistar rats. The result for 15 minutes interval showed no significant (P>0.005) change when compared to control and test groups. However, for the 30 minutes interval, only group 3 (treated with Omeprazole) showed significantly (P<0.005) elevated pH of gastric effluence when compared to control and group 4.

For the results of the 45 minutes interval, only group 3 (treated with omeprazole) showed significantly (P>0.005) elevated pH of gastric effluence when compared to control group 1, group 2 and group 4.

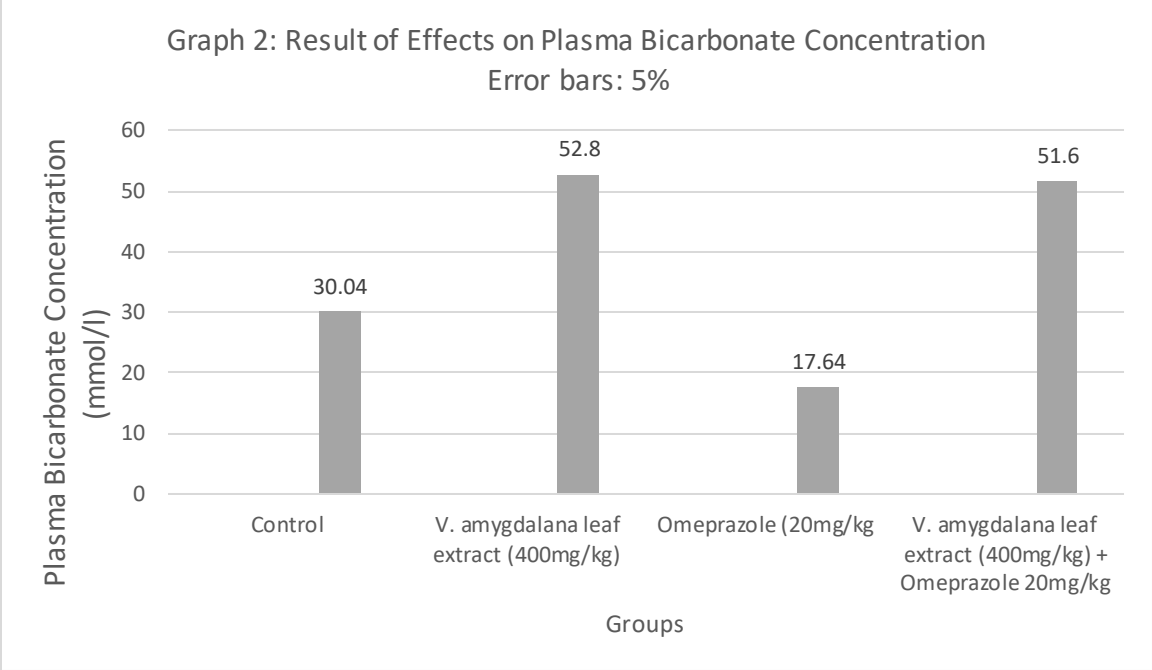
Table 4.2 showed the effect of V*ernonia amgydalina* on plasma bicarbonate

GROUPS	PLASMA BICARBONATE CONCENTRATION (Mmol/L)
GROUP 1 (CONTROL)	30.04 ± 0.09^{b}
GROUP 2 (EXTRACT ALONE)	52.80 ± 0.86
GROUP 3 (OMEPRAZOLE)	17.64 ± 0.12^{ab}

GROUP 4 (OMEPRAZOLE + EXTRACT)	51.60 ± 0.60^{ac}

Values represent mean ±, standard error of mean (SEM), n=5

Where a=statically significant i.e. (P<0.05) when compared to group 1
b=statically significant i.e. (P<0.05) when compared to group 2
c= statically significant i.e. (P<0.05) when compared to group 3
d= statically significant i.e. (P<0.05) when compared to group 4



Analysis of Result on the Plasma Bicarbonate Concentration

Table 4.2 above shows the effect *vernonia amgydalina* (bitter leaf) on the plasma bicarbonate concentration in wistar rats. The result for group 1 showed no significant (p>0.005) change when compared to group 3 and 4, but when compared to group 2 (treated with extract alone) there was a statically significant (P<0.05) decrease change in the plasma bicarbonate concentration. While the result for group 2 (treated with extract alone) showed statistically significant (P>0.05) elevated change in plasma bicarbonate concentration when compared to group 1.

However, the result for group 3 (treated with omeprazole alone) showed a statistically significant (P >0.005) decreased change in plasma bicarbonate concentration when compared to group 1 and group 2, but no significance when compared to group 4.
Lastly, the result for group 4 (treated with omeprazole + extract) showed a statistically significant (P<0.005) elevated change in plasma bicarbonate concentration when compared to group 1 and group 3.

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussions

Effect on Gastric pH

In this study, it was shown that intra-gastric administration of 400mg/kg crude aqueous bitter Leaf (Vernonia amygdaline) markedly decreased the pH of the gastric effluent gradually reaching a maximum of 5.20 after 45 mins. Owu et al. (2006) reports that Vernonia amygdalina crude aqueous extract caused a dose dependent increase in gastric acid output in albino-wistar rats. The exact mechanism by which the extract decreases the gastric pH is unknown. Phytochemical analysis of the leaves of V. amygdalina revealed the presence of saponins (Hack, 2000). Saponins have been reported as strong hemolytic agents, that irritates the gastrointestinal tract and the mucous membrane. It has been suggested that saponins upon irritating the gastrointestinal tract stimulates the oxyntic cells of the stomach and thereby increase gastric secretion. The excoriating effect of the 400mg/kg bitter leaf extract could also be suggestive of the activity of saponins in the extract. Trease and Evans, on the other hand found tannins to have astringent property on both gastrointestinal smooth muscles and blood vessels thus decreasing blood flow to the stomach.

A decrease in blood supply to the stomach reduces stomach secretion. This therefore suggests that the decreased gastric pH produced by *Vernonia amygdalina* may be a net resultant balance of both the inhibitory and stimulatory effect of the extract. Also, when 400mg/kg bitter leaf was used alone, the initial pH of gastric effluent (at 15mins) was slightly lower than the control but gradually reduced till the 45 minutes mark, meaning that bitter leaf may have a rapid onset of action in increasing gastric acid secretion. Omeprazole which is a standard drug (proton pump inhibitor) used for gastric acid inhibition on the other hand, greatly decreased the gastric acid secretion when compared to results from both the negative control group and the 400mg/kg bitter leaf group (Table 4.1). This result is backed by the earlier findings stated in the study conducted by Andersen, Andrade and Wang (2003) on the effects of Omeprazole on inhibition of gastric acid secretion during digestion in the toad *Bufo marinu* which also showed that the administration of Omeprazole during digestion in toads successfully inhibits gastric acid secretion. Co-administering Omeprazole and 400mg/kg bitter leaf also showed a decrease in gastric pH at 45 minutes mark. However, the result obtained from co-administration of 400mg/kg bitter leaf and Omeprazole show that bitter leaf could have a synergistic effect when combined with Omeprazole to bring about a decline in the gastric pH and as well, helps stabilize the effects of Omeprazole on gastric acid pump inhibition upon prolonged administration to bring about a pH level that is favorable for gastric functions.

Effects on Post-Prandial Bicarbonate Concentration

The serum post-prandial bicarbonate concentration was analyzed to get clarification on the particular mechanism of action employed by 400mg/kg bitter leaf in decreasing the gastric pH as seen in the result obtained. This is because measurement of the post-prandial bicarbonate concentration gives one another route (indirect method) of determining gastric acid secretion since proton pump actively transports one molecule of bicarbonate into the plasma for every molecule of Hydrogen ion pumped into the gastric effluent. Hence, Flemstron and Garner (1982) stated that the level of plasma bicarbonate is directly proportional to the level of gastric acidity after a meal. The results from the analysis of the

various groups to that of the negative control as seen in Table 4.2 showed that administration of 400mg/kg bitter leaf, Omeprazole wherefore, 400mg/kg bitter leaf could have elevated the acid secretion due to the high serum post-prandial bicarbonate concentration, while Omeprazole eliminated the secretion of acid in gastric lumen by blocking the proton pump, thereby leading to an increase in the bicarbonate secretion.

Also from the work of Andersen, Andrade and Wang (2003) which illustrated the effect of inhibition gastric acid secretion on arterial acid-base status during digestion in the toad Bufo marinus, it was observed that digestion affects acid-base status because of the net transfer of HCI from the blood to the stomach lumen which leads to an increase in serum HCO3 levels in both extra-and intracellular compartments, hence the ability of Omeprazole to inhibit gastric acid secretion which in turn inhibits secretion of serum bicarbonate. This shows that administration of Omeprazole alone can cause severe reduction in plasma bicarbonate leading to metabolic acidosis and its consequences but its co administration with 400mg/kg bitter leaf showed a marked increase in the level of post-prandial serum bicarbonate concentration when compared to that observed in administration of omeprazole alone (Figure 4.2). Hence 400mg/kg bitter leaf acts as a buffer in omeprazole stimulated gastric acid inhibition to helps avert the problems associated with low levels of serum bicarbonate concentration such as; fatigue, confusion, tachypnea and so on.

Conclusion

This study hence evaluated the effects of aqueous bitter leaf extract on eliciting or inhibiting gastric acid secretion as well its effects on co-administration with Omeprazole (a known proton pump inhibitor). The result showed that upon administration, aqueous bitter leaf extract decreased acid output in the gastric lumen and increased the postprandial serum bicarbonate concentration, thereby suggesting a stimulatory effect of bitter leaf on the activity of the proton pump. By showing a rapid onset in decreasing gastric pH, the study suggests that this was an enhancement of Omeprazole on gastric acid pump inhibition.

Recommendation

From the result of the study, it is strongly recommended that peptic ulcer diagnosed patients should stay away from bitter leaf rich substances as these would increase the level of gastric acid secreted.

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