

Interaction between aqueous extract of *Moringa oleifera* leaves and rabeprazole in treatment of ethanol induced gastric ulcer in Wistar rats

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Abstract

Background: The effect of concomitant use of herbs and conventional drugs is not widely known because it hasn't been a common practice especially among the elite and those resident at the urban centers but very common among the rural dwellers for co-administered herbs/supplements and conventional drugs. This study was carried out to investigate the interaction between *moringa oleifera* and rabeprazole in gastric acid secretion.

Methods: Twenty (20) adult Wistar albino rats of both sexes weighing 300-450 g were randomly allocated into four (4) groups of five (5) animals per group. Group 1 served as the control and was given normal saline without administration of acid alcohol. Animals in group 2 were given acid alcohol (0.25 ml of 36% HCl + 75% ethanol 0.25 ml/100 g body weight). Animals in group 3 were given 50 mg/100 g bw of *Moringa oleifera* 1 hour before acid alcohol was administered. Group 4 were given: Rabeprazole 20 mg /kg body weight + acid Alcohol (36% HCL 0.25 mls+ 75% Ethanol (0.25 mls). A 30% isoflurane (inhalational anesthesia) by API Manufacturer with FDA, UK, marketed by Macfes medical store, high level Markurdi, Benue State, Nigeria. And 3.5% isoflurane (of the mixture of isoflurane 30%) at 100% oxygen was soaked in a cotton wool and dropped in clean and covered plastic container, and was used to anesthetized the rats, for the purpose of the aqueous extracts administration through a gastric fistula created by a surgical procedure. 10 minutes after aqueous extracts administration, aliquot samples were collected over 4 hours. Gastric acid secretion was measured by titrating the aliquots to a phenolphthalein endpoint.

Results: showed that *Moringa Oleifera* significantly delayed the time of onset of action of rabeprazole when co-administered. *Moringa* alone was a better inhibitor of gastric acid secretion than *moringa* co-administered with rabeprazole.

Conclusion: *Moringa* delays the time of onset of action of Rabeprazole when co-administered.

Keywords: *Moringa*; Rabeprazole; Gastric acid secretion; Proton pump; Gastric mucosa

1. Introduction

Moringa Oleifera is a commonly used herb in Nigeria [1]. It has been shown to have an inhibitory effect on gastric acid secretion [2]. Concomitant use of herbs/supplements containing *Moringa Oleifera* with conventional drugs is common among patients as it is believed that *moringa* is a multi-therapeutic plant [3]. This may lead to herb-drug interactions in the same way that two or more co-administered drugs may interact [4]. Herbal constituents that are substrates for the same enzymes or transporters of conventional drugs may induce or inhibit the enzymes and/or transporter activity.

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Pharmacokinetic endpoints such as area under the curve (AUC), time to maximum plasma concentration (T_{max}), peak plasma concentration (C_{max}), trough concentration (C_{min}), clearance (CL), volume of distribution (V_d/F) and half-life (T_{1/2}) may be altered significantly resulting in toxicity, more severe adverse effects, sub-therapeutic drug concentrations and treatment failure [4]. However, herbal medication has been effective in several health conditions, especially now that study had proven that, treatment of gastric ulcer using conventional therapy faces a major setback [16]. *Moringa oleifera* leaves possess many documented actions including, gastro-protection which means protection against gastric mucosal injury [15]. Synergistic effects of *Moringa oleifera* on gastro-protective action and gastric acid secretory effect of proton pump inhibitor (rabeprazole) has limited studies in Nigeria. This study seeks to identify the effects of *Moringa oleifera* and rabeprazole when co-administered. It has been established that both *Moringa oleifera* and rabeprazole, separately have protective effects on gastric mucosal injury [2].

Rabeprazole is a proton pump inhibitor used in the management of GERD (gastroesophageal reflux disease), co-administered with antibiotics (Amoxicillin and Clarithromycin) for the eradication of *H. Pylori* Peptic Ulcer Disease (PUD) and also used for other hyper gastric acid secretory conditions.

2. Material and Method

A total of 20 Wistar rats of both sexes, 14-16 weeks old (weighing 350-400 g) were obtained from the Animal House, College of Health Sciences Benue State University Makurdi. Animals were kept in plastic cages wood saw dust for beddings and were allowed to acclimatize for 2 weeks before commencement of treatment. The rats were maintained in a standard condition at room temperature (27 ± 2 °C) and relative humidity ($50 \pm 5\%$), with 12 hours Light / dark cycle. They were randomized into four groups of five animals each in a cage. They were handled in line with international protocol.

2.1. Preparation of aqueous Extract

Fresh *Moringa oleifera* leaves were collected from the natural habitat in Makurdi, Benue state, Nigeria. A sample of leaves collected was submitted to the Department of Botany in the Faculty of Science, Benue State University and certified by the Botanist. Consequently, the sample was allocated a voucher number (HBI - 002- BSU23) and deposited in the herbarium.



Figure 1 Leaves of *Moringa Oleifera*

The leaves were sorted out to obtain only the fresh leaves and washed with distilled water without squeezing to remove debris and dust particles. They were shade-dried for ten days and dried leaves were pulverized with an electric blender. A portion (300 g) of the powdered leaves was soaked in 1500 ml of distilled water for 72 hours with the solution thoroughly stirred twice daily according to the method of [5]. The extract was filtered with WHATMAN no1 filter paper. The filtrate was dried using an evaporator and then reconstituted before use.

The treatment protocol is as follows:

- Control group: Administered normal saline orally 5 -10 mls in 6 hours. [6].
- Acid Alcohol group: (36% HCL 0.25 ml) + 75% Ethanol 0.25 ml/100 g body weight [7].
- *Moringa oleifera* group: 50 mg /100 g body weights given 1hr before acid Alcohol (36% HCL (0.25 ml) + 75%Ethanol (0.25 ml) / 100 g body weights [8].
- Rabeprazole + *Moringa oleifera* (50 mg /100 g) Group: Rabeprazole 20 mg/kg body weight + acid Alcohol (36% HCL 0.25 mls+ 75% Ethanol (0.25 mls).

Surgical Procedure and Induction of Ulcer After a 12-hour fast, each animal was anesthetized with 3.5% isoflurane (from the mixture of isoflurane 30%) at 100% oxygen was soaked in a cotton wool and dropped in clean and covered plastic container, which was effective almost immediately. A tracheostomy was performed. A nasogastric tube was passed. A duodenostomy was performed and normal saline was used as gastric lavage to wash out the debris from the stomach until clear effluent was obtained. A duodeno-gastric cannula was passed and ligated in situ for subsequent collection of gastric acid secretion.

Measurement of gastric acid secretion was done according to the method of [6], and as modified by [7]. Basal acid secretions were collected for the first 2 hours and titrated every 10 minutes for each hour. *Moringa oleifera* leaf aqueous extract was administered one hour before acid alcohol was given. The aliquots were collected every 10 minutes for 120 mins (2 hrs). Each aliquot was titrated to a phenolphthalein endpoint using 0.01 M NaOH and the acid output or concentration was calculated as described by Ibu [7]. As follows:

Where Normality = Molarity

$$MA \times VA = MB \times VB \dots\dots\dots (1)$$

$$MA = (MB \times VB) \div VA \dots\dots\dots (2)$$

Where,

MB = Molarity of base known (0.01 N) = 10 mMol

VB = Volume of the base known (titrate of NaOH) used

VA = Volume of acid (effluent volume) = 10 ml

Substituting for MB and VA

$$MA = 10 \times VB \div 10 \dots\dots\dots (3)$$

$$\text{Therefore } MA=VB \dots\dots\dots (4)$$

$$\text{Acid output / 10 minutes} = VB \text{ mMol / L / 10 mins} \dots\dots\dots (5)$$

$$\text{Acid output per hour} = VB \times 6 \text{ mMol/ L / hour as stated by [7].} \dots\dots\dots (6)$$

2.2. Data Analysis

Data obtained from the study was expressed as mean ± SEM. The differences between the groups were analyzed by one-way analysis of variance (ANOVA), followed by Turkey’s post hoc test for multiple comparisons using SPSS statistical tool version 22.

2.3. Ethical clearance

Ethical clearance for the use of animals for experiment was obtained from the ethical committee in the College of Health Sciences, Benue State University, Makurdi (THS REC No: CREC/THS/0002).

2.4. Chemicals

A 30% isoflurane (inhalational anesthesia) by API Manufacturer with FDA, UK, marketed by Macfes medical store, high level Markurdi, Benue State, Nigeria. And 3.5% isoflurane (mixture of isoflurane 30%) at 100% oxygen was soaked in a cotton wool and dropped in clean and covered plastic container, and was used to anesthetized the rats, for the purpose of the experiment. These were purchased from EMOLE chemical shop, old Otukpo Road High-Level Makurdi, Benue State Nigeria.

2.5. Animals

Adult Wistar albino rats weighing 300-450 g of either sex were purchased from the disease-free stock of the animal house of the College of Health Sciences, Benue State University, Makurdi, and used for the study. They were maintained in normal laboratory conditions of temperature 28 °C relative humidity (with a 12 hour light-dark cycle) and adequate ventilation. The animals were fed with a commercial diet (Vital Feed Nig. Ltd.) and water ad libitum. Food was withheld 12 hours before the experiments, but they had free access to water. Permission for the use of animals and animal house were obtained from the Animal Ethics Committee of Benue State University Makurdi, before experimentation.

3. Result

There was a significant ($p < 0.01$) increased in gastric acid secretion with Moringa co-administered with rabeprazole compared to Moringa alone after 1 hours, suggesting inhibitory effects of Moringa on rabeprazole after 1 hours, as seen in figure 2 below.

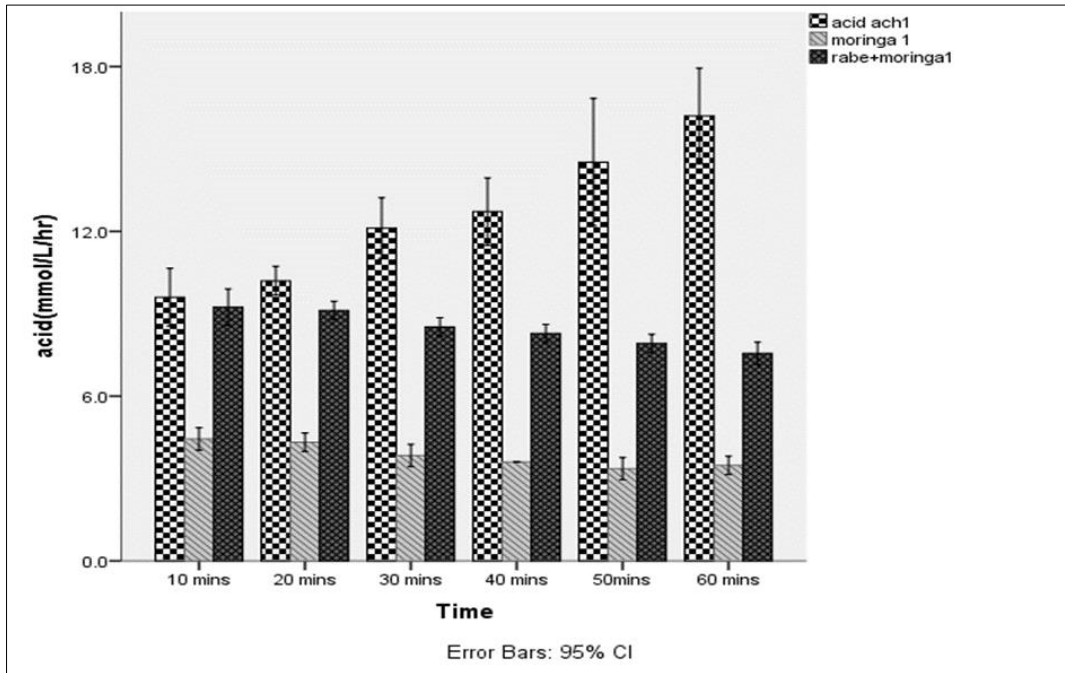


Figure 2 Gastric acid secretion in control, Moringa, and in rabeprazole+Moringa group every 10 minutes after 1 hour

There was a significant ($p < 0.01$) increased in gastric acid secretion with Moringa co-administered with rabeprazole compared to Moringa alone after 2 hours, suggesting inhibitory effects of Moringa on rabeprazole after 2 hours, as seen in figure 3 below.

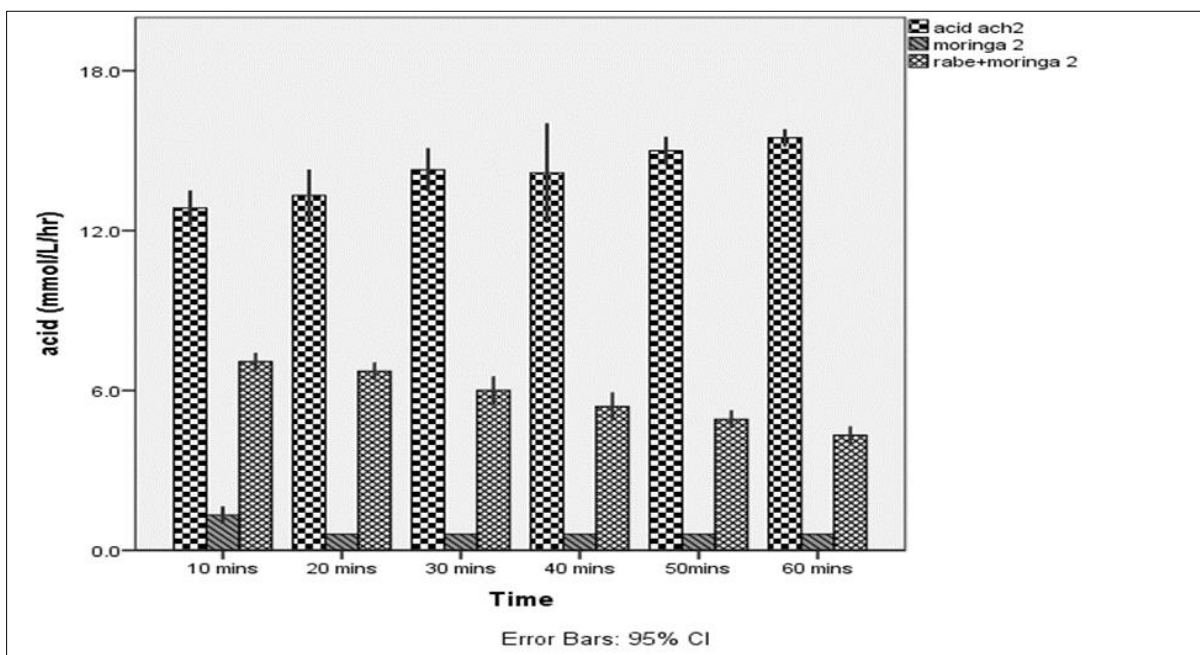


Figure 3 Gastric acid secretion in control, Moringa, and in rabeprazole + Moringa group every 10 minutes after 2 hours

There was an increased in gastric acid secretion with Moringa co-administered with rabeprazole compared to Moringa alone after 3 hours, suggesting inhibitory effects of Moringa on rabeprazole after 3 hours, however not significant. Results here, suggested a gradual loss of inhibitory effects of Moringa on rabeprazole after 3 hours. As seen in figure 4 bellow.

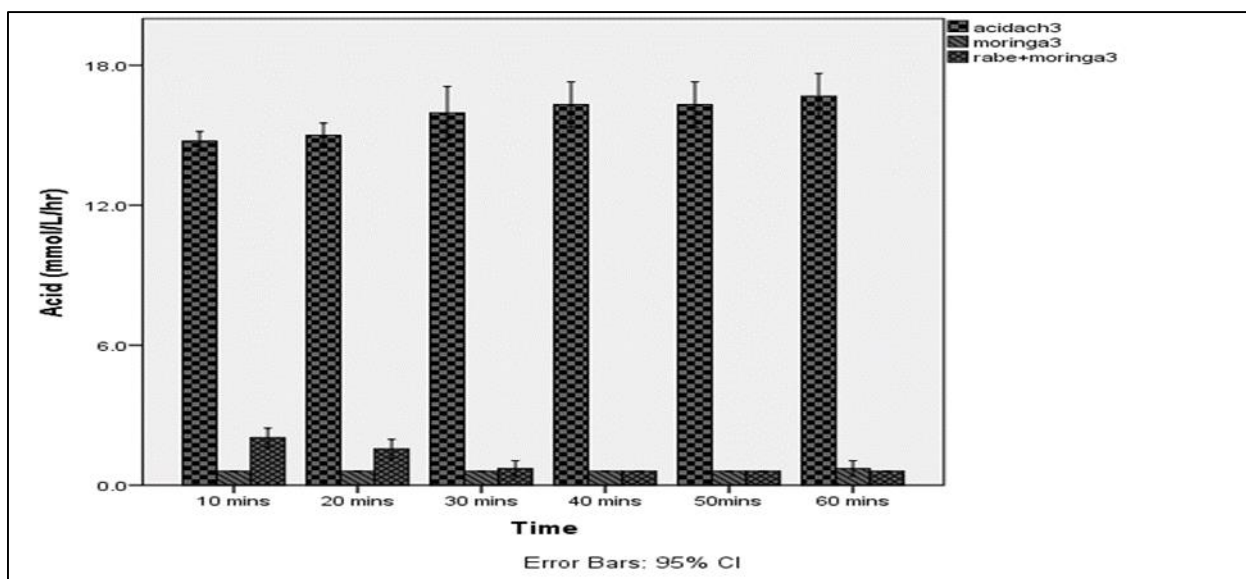


Figure 4 Gastric acid secretion in control, Moringa, and in rabeprazole+Moringa group every 10 minutes after 3 hours

Results showing an initial inhibitory interaction between Moringa and rabeprazole but however loss after 4 hours in moringa co-administered with rabeprazole compared to Moringa alone after 4 hours, suggesting loss of inhibitory effects of Moringa on rabeprazole after 4 hours. As seen in figure 5 below

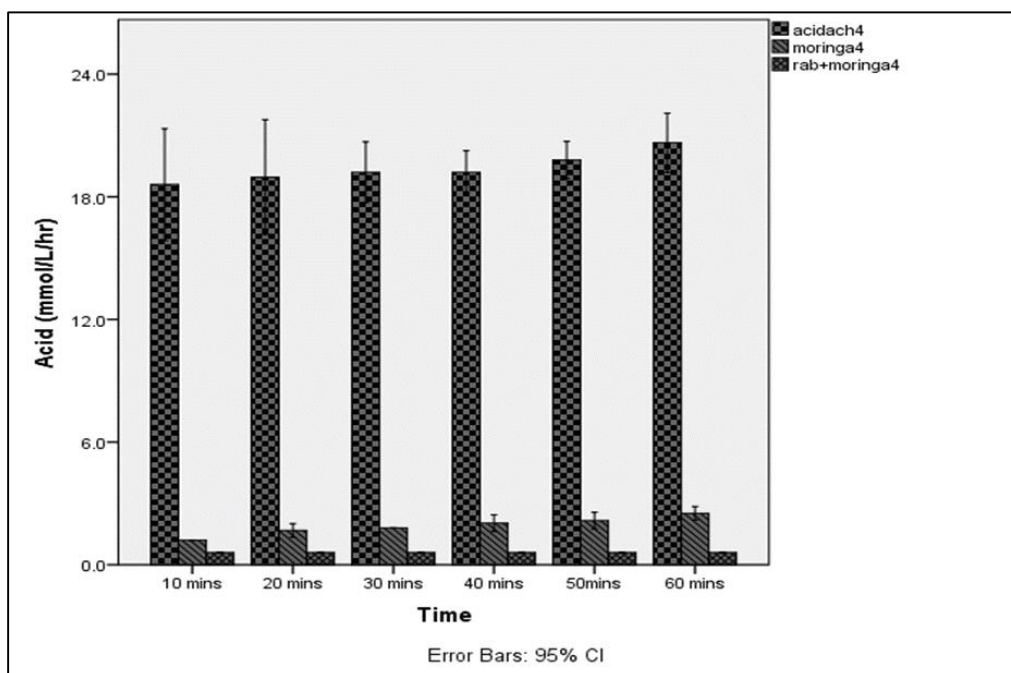


Figure 5 Gastric acid secretion in control, Moringa and in rabeprazole + Moringa group every 10mins after 4 hours

4. Discussion

Rabeprazole is the choice of drug for physicians in managing GERD because of its faster onset of action compared to other proton pump inhibitors [10]. . However, results from this study as shown in Figure 2 at 10 minutes after 1 hour

that is, 70 minutes after administration of rabeprazole and *Moringa Olefera*, there was no significant difference between the acid alcohol group and Moringa + Rabeprazole group ($p > 0.05$). This was also observed by [11], that *Moringa Oleferaco* administered with Amodiaquine significantly increases the T_{max} of Amodiaquine and its active metabolite Desethyl amodiaquine thus increasing its time of onset of action. Similarly, other authors [12]. in their work (interaction of Enalapril with Moringa by affecting angiotensin Converting enzyme invitro and ex vitro) concluded that co-administration of Moringa with Enalapril reduced Enalapril ACE inhibitor's effect.

Results from this study also showed that the time of onset of action of Moringa alone was faster to reach than rabeprazole co-administered with moringa. This shows that Moringa possibly formed an insoluble complex with rabeprazole that is slowly absorbed. Moringa has been shown to have constituents that can form insoluble complexes [11]. These constituents include ascorbic acid, β - sitosterol, iron, calcium, phosphorus, copper, α - tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β - carotene, protein, and in particular essential amino acids such as methionine, cysteine, tryptophan and lysine [13]. These constituents modify gastric pH or form insoluble compounds in the gastrointestinal tract, thereby affecting the time of onset of action of rabeprazole. Moringa is also known to have antispasmodic properties due to its constituent Glucomorinhin [14]. This may delay gastric emptying time and eventually delay absorption of rabeprazole as shown in figures 1 and 2.

Results from this study in Figure 3 showed that Rabeprazole co-administered with moringa achieved the same therapeutic effect with moringa alone at 40 minutes after 3 hours of administration. This shows that the maximum effect of moringa as an inhibitor of gastric acid secretion is 270-280 minutes after administration. However, the effect of rabeprazole + moringa persisted till the end of the experiment that is 6 hours after administration.

5. Conclusion

This study established interaction between *Moringa Olefera* and Rabeprazole which affects the time of onset of action of rabeprazole. This delay was detrimental to the animal used as Moringa co-administered with Rabeprazole did not offer to prevent mucosal ulceration caused by acid alcohol.

Recommendation

Moringa co-administered with rabeprazole did not offer to prevent mucosal ulceration caused by acid alcohol and should be avoided in animals and by implication, co-administration of herbal and orthodox medications be avoided in human. There will be need for gene expression studies and possible clinical studies for knowledge on this interaction in future research.

Limitation of the study

A financial resource was lacked to carry out the gene expression (CGRP genes) and to study on the phytochemical components of sunflower seeds, available in our environment.

Compliance with ethical standards

Acknowledgment

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study involved use of Wistar rats. The handling of the animals was done in line with the guideline laid down by the institutional ethical committee. Ethical approval for the study was conveyed in certificate number CREC/THS/0002.

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