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(RESEARCH ARTICLE)

Gastroprotective effects of Methanol leaf extract of *Desmodium velutinum* (Fabaceae) and honey on ethanol-induced gastric ulcer in albino rat: The concept of combination therapy

Ifeanyi Peter Onyeka [1, *], Felix Ahamefule Onyegbule [2], Christopher Obodike Ezugwu [3], Emeka John Dingwoke [4], Chibueze Jeremiah Ike [5], Cyril Onyeka Ogbue [1], Chinelo Kene Ezejiegu [6] and Chiamaka Henrietta Jibuaku [6]

[1] *Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.*
[2] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka*
[3] *Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsuka.*
[4] *Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.*
[5] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.*
[6] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka*

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Abstract

Phytochemicals are known for their therapeutic effects through diverse mechanisms such as therapeutic modulators, substrate ligands, receptor ligands, enzyme cofactors, enzymes precursors, toxins scavengers, microbial growth inhibitors, therapeutic agents, and therapeutic enhancers in the treatment of various diseases and ailments. Folklorically, the indigenous people of Nigeria have used *Desmodium velutinum* (Fabaceae) for treatment of disease conditions such as headache, fever, diarrhea toothaches, ulcers, anti-oxidants, and pains. This study investigated the toxicity, and anti-ulcer effects of a combination of methanol leaf extract of *Desmodium velutinum* and honey in rats. Anti-ulcer studies were carried out using absolute ethanol rat models of ulceration. The rats were distributed into ten groups of five rats each. Group 1 served as a negative control, group 2 served as a positive control, group 3 served as untreated control, the test groups: groups 4 to 6 received crude extract of *Desmodium velutinum* at doses of 100, 250, and 500 mg/kg respectively, groups 7 to 9 received a combination of 1 ml honey and crude leaf extract of *Desmodium velutinum* at doses of 100, 250 and 500 mg/kg while group 10 received 1 ml of honey only. The acute toxicity showed that the extract caused no death in the mice at 5000mg/kg and therefore the LD50 is above 5000mg/kg. The result also showed that 500 mg/kg of the crude extract and 1 ml honey had 42.9% and 71.76 % inhibition of ulceration respectively, while their combination had 100% inhibition of ulceration among the rats treated with the combinations. The combination of methanol leaves extract of *Desmodium velutinum* and honey exhibited gastrotherapeutic effect in rats and suggest its continued usage as an antiulcer agent for the treatment of ulcer.

Keywords: Ulcer; *Desmodium velutinum*; Honey; Combination therapy; Gastro-protective; Anti-ulcer

* Corresponding author: Ifeanyi Peter Onyeka
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.

1. Introduction

Stomach ulcer is one of the most common digestive diseases. It can be chronic in nature and the lesion occurs in any part of the digestive tracts in the stomach, small intestine, and esophagus. Previous studies found that the incidence of uncomplicated peptic ulcer disease in the general population was roughly one case per 1000 persons per year whereas that of the complicated ulcer was roughly 0.7 cases per 1000 persons per year [1]. The prevalence study of seven developed countries based on doctors' diagnoses showed a prevalent rate that ranged from 0.1 to 1.5 percent whereas the prevalent rate based on hospitalization was estimated to be 0.1 to 0.19 percent [2]. It is estimated that Peptic ulcer disease (PUD) claimed more than 300,000 lives worldwide in 2013 and its incidence in Africa is increasingly high [3]. The disease condition is as a result of an imbalance between the aggressive factors (acid and pepsin) and mucosal defense factors (blood flow and endogenous prostaglandins) [4] that maintain an equilibrium of the stomach. The main causes of peptic ulcers are said to be either by the bacteria Helicobacter pylori or heavy use of non-steroidal anti-inflammatory drugs (NSAIDs) [5] among other causes. The bacteria or NSAIDs play a role in the pathogenesis of ulcers by damaging the mucus of the stomach lining, thereby exposing the interior of the stomach to acid which irritates the tissue and causes peptic ulcers, which is essentially a lesion in the stomach with a burning sensation. NSAIDs cause ulceration by preventing the synthesis of prostaglandins due to the inhibition COX-1 enzyme thereby leading to a reduction in the production of gastrointestinal mucus, bicarbonate, and mucosal blood flow which are the protective factors of the stomach [6]. Although using antibiotics, proton pump inhibitors such as omeprazole, analogs of prostaglandin, and H2 receptor blockers such as cimetidine, ranitidine, and famotidine reduce the mortality associated with peptic ulcers. These drugs have various gastrointestinal toxicities associated with their use [7] and therefore remain an impediment to their application in clinical practice. For example, misoprostol (prostaglandin E-analogue) which is used as a cytoprotective agent in ulcer disease cannot be used to treat ulcers in pregnant subjects because of its tendency to cause abortion [8], also cimetidine can cause reversible impotence and retard the metabolism of other drugs thereby enhancing their toxic effect because of its ability to inhibit cytochrome p450 [9, 10]. It is, therefore, suitable to explore the natural flora in the search for an effective, affordable, acceptable and readily available anti-ulcer agent that is devoid of side effects and toxicity.

Utilizing plant-based medications may provide an alternative source of medication for the treatment of ulcer disease that is relatively devoid of side effects, cheap readily available, and relatively safe if prepared properly [11]. Medicinal plants have been used from time immemorial for the treatment of different disease conditions because they are rich in phytochemicals that exhibit various therapeutic effects. *Desmodium velutinum* (Fabaceae) is used in traditional medicine in the Eastern part of Nigeria where it is called'Ikeagwuani' to treat inflammatory disorders. It is the specie of shrub that belongs to the family Fabaceae. Its branches are often dark red, yellowish-brown when young, and have short hooked-hairy. Pharmacological studies of *Desmodium velutinum* have been conducted and reported for antipyretic activity [12], antioxidant activity [13] Hypolipidaemic effect [14]; analgesic activity [15]. Traditional healers in Anambra State use *Desmodium velutinum* for the treatment of ulcers though this has not been scientifically documented.

Honey is a natural by-product of flower nectar and the digestive tract of the honey bee with a wide range of applications including its use as food supplements and as a therapeutic substance [11, 16]. It is dark amber or black viscous liquid that is useful in modern medicine for the management of gastrointestinal, cardiovascular, inflammatory, and neoplastic states [16]. Major components of honey include fructose, glucose, amino acids, vitamins, minerals, and enzymes [16-18], which work together to provide varying therapeutic effects such as antioxidants [19]; Cardioactive and vasoactive effect [20], and antimicrobial [21-23]. It has been reported that oral administration of honey and glucose or fructose showed protection against gastrointestinal diseases such as gastric ulcers [24].

Although it has been reported that dose-dependent crude leaves extract of *Desmodium velutinum* showed gastro protection against experimentally induced ulcers in Wistar rats using ethanol [25], this is the first report on the gastro-protective effects of a combination of crude methanol leaf extract of *Desmodium velutinum* and honey on ethanol-induced gastric ulcer in albino rat models. This study evaluated the acute toxicity, gastrotherapeutic and gastro-protective effect of crude methanol leaf extract of *Desmodium velutinum* combined with honey in rats.

2. Material and methods

2.1. Chemicals and Drugs

Omeprazole (Prilosec®) was purchased from Bewell Pharmaceuticals, Awka, and Anambra State, Nigeria. Other reagents and chemical were of analytical grades and purchased from Sigma Aldrich USA.

2.2. Collection and Identification of plant material

Fresh leaves of *Desmodium velutinum* were collected from their natural habitat in, Awka South Local Government Area, Anambra State. The plant was properly identified by a plant taxonomist Mr. Felix Nwafor at the Department of

Pharmacognosy and Environmental Medicine, University of Nigeria Nsuka. Voucher number PCG/UNN/0336/Fabaceae was obtained.

2.3. Preparation of plant Extract

The freshly collected leaves of *Desmodium velutinum* was thoroughly washed with tap water and air-dried under a shade for 14 days. The dried leaves were pulverized using electric blender (model MS-223; Blender/Miller III, Taiwan, China) and stored in a sterile container. Exactly 1 gram of the grounded sample was weighed and extracted with 1.5 L of absolute methanol by cold maceration. This mixture was covered in order to prevent solvent evaporation and was allowed to stand for 74 hours. The solution obtained was filtered using Whatman filter paper No. 1. The filtrate was concentrated to dryness using bench top freeze dryer (Labconco™) at 4°C for approximately 4 hours. The concentrated extract was scrapped into a sterile bottle and the percentage yield was calculated. It was covered properly with foil paper and kept in a refrigerator as the crude extract. Upon required, it was reconstituted in distilled water to give the varying doses mg/kg body weight of the extract used in this study.

2.4. Phytochemical Screening

The Phytochemical analyses were conducted using standard laboratory procedure to identify secondary metabolites as described by [26-30] to determine the presence of secondary metabolites in the leave extract of *Desmodium velutinum* and in the honey.

2.5. Acute toxicity test of *Desmodium velutinum*

The acute toxicity test to determine the LD_{50} as an index of safety of the extract was carried out using the method employed by [31] as modified by [32].

Experimental Animals

Following institutional ethical approval, adult albino rats of either sexes weighing between 150 to 170 g were purchased from Pharmacology and Toxicology Department, University of Nigeria Nsukka. The animals were acclimatized for 7 days, under 12 hours dark and light cycles. They were fed with water and standard pellet *ad libitum*. Feeding of the animals was withdrawn 24 hours prior to the experiment, but they were allowed access to water *ad libitum* and kept under standardized conditions of temperature of 21 ^{0}C.

2.6. Collection of Honey

The honey used in this study was a multifloral honey and was obtained from natural honey hives at Opi-Nsukka, in Nsukka Local Government Area, Enugu State, Nigeria.

2.7. Anti-ulcer studies

The anti-ulcer studies were done using standard laboratory procedure of ethanol-induced ulcer model described by [33] using Omeprazole as the standard drug.

2.8. Induction of Acute Gastric Lesion by Ethanol and Gastroprotective studies

In ethanol induced animals were fasted for 36 hours but allowed free access to water ad libitum [33]. They were randomly selected and allocated into ten groups of five rats each. Group 1 served as negative control that received only water; group 2 served as positive control that received Omeprazole; group 3 served as untreated control group 4 to 6 received crude extract of *Synedrella nodiflora* at doses of 100, 250 and 500 mg/kg body weight respectively; group 7 to 9 received combination of 1 ml honey and crude extract of *Synedrella nodiflora* at doses of 100, 250 and 500 mg/kg while group 10 received only 1 ml of honey. Thirty minutes after administration of the extract, honey, water and standard drugs, ulceration was induced by gastric instillation of 1 ml of 96% ethanol (5 mL/kg, po), except the normal group that served as normal control. One hour after administration of ethanol, the animals were anesthetized with intraperitoneal administration of ketamine (50 mg/kg) and were sacrificed by cervical displacement and the stomach was removed, opened to examine any ulcerative lesions. The number, length and severity of ulceration were scored as follows: 0= normal colored stomach, 0.5= red colored, 1= spot ulcers, 1.5= hemorrhagic streak, 2= ulcers, 3= perforation.

Ulcer index was expressed as: Ulcer index= (UN+ US+UP) x 10^{-1}. Where UN is the average of number of ulcers per animal, US is the average of severity of ulcer and UP is the percentage of animals with ulcer. Also, the Percentage inhibition of ulceration (PIU) was calculated using the formula:

$$\text{PIU [24]} = \frac{\text{ulcer control} - \text{ulcer treatment} \times 100}{\text{Ulcer control}}$$

2.9. Analysis of result

All result was analyzed using statistical package for social science version 20.0. One-way ANOVA was used to analyze for the variation between means of each group while Duncan multiple range test was used to separate the means. The data were expressed as mean ± SEM while ulcer inhibition was expressed as index number and percentage. P values less than 0.01 were considered significance.

3. Results

3.1. Phytochemical evaluation

The result of the qualitative and quantitative phytochemical evaluation are presented in Table 1 and Figure 1. It revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids carbohydrates and reducing sugar in both samples. There is significant different ($P<0.05$) in the concentration of alkaloids, carbohydrates, flavonoids, reducing sugar, tannins and steroids contents of honey and *Desmodium velutinum* (Appendix 1). Quantitatively (Figure 1 and appendix 1), alkaloids in *Desmodium velutinum* (13.74) are higher than alkaloids content in honey (0.24). The result also showed that both *Desmodium velutinum* (16.0) and honey (17.01) have high concentration of flavonoids. Tannins content is significantly higher in *Desmodium velutinum* (17.85) than honey (0.669). Saponins content is significantly higher in honey (1.76) than in *Desmodium velutinum* (0.23). Steroids are higher in honey (0.08) than *Desmodium velutinum* (0.05). Carbohydrates and reducing sugar are significantly higher in honey (approximately 89 and 12.1) than in *Desmodium velutinum* (37 and 7) respectively. Terpenoids is significantly higher in honey (21.41) than *Desmodium velutinum* (0.5).

Table 1 The phytochemical constituents of *Desmodium velutinum* and honey

Bioactive compound	**Qualitative**	
	Desmodium velutinum	**Honey**
Alkaloids	Present	Present
Flavonoids	Present	Present
Tannins	Present	Present
Saponins	Present	Present
Steroids	Present	Present
Terpenoids	Present	Present
Carbohydrates	Present	Present
Reducing Sugar	Present	Present

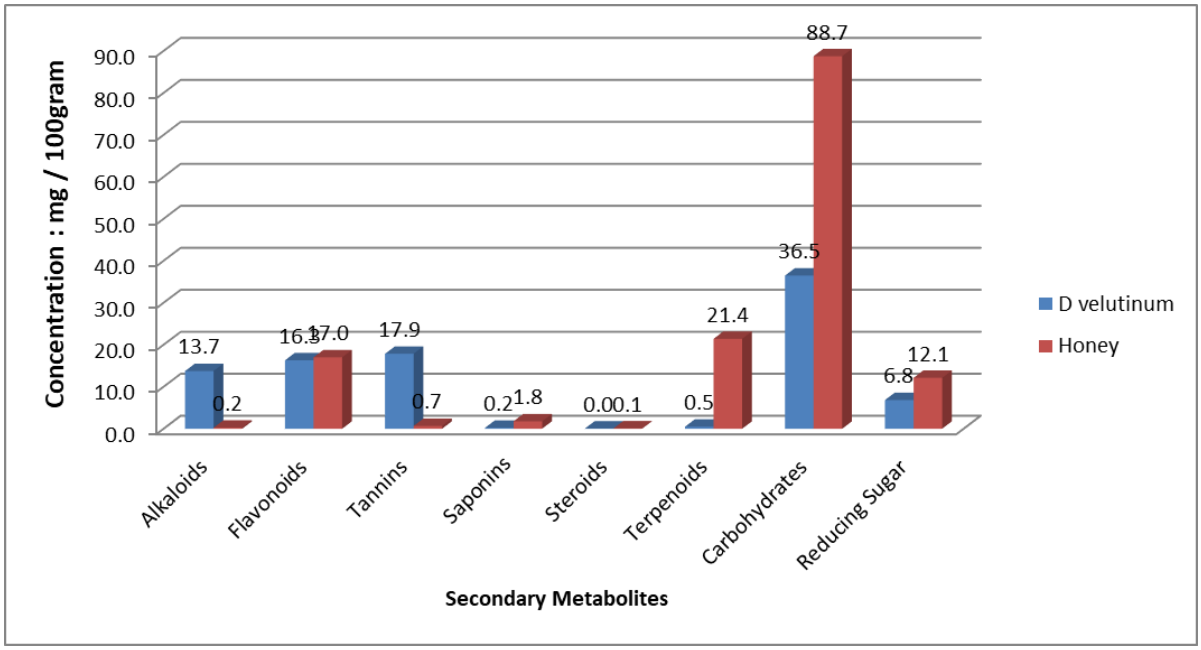

Figure 1 Quantitative phytochemistry result of *Desmodium velutinum* and honey

3.2. Acute toxicity test of *Desmodium velutinum*

Result of the acute toxicity studies of the methanol leaves extract of *Desmodium velutinum* is presented in Table 2. It showed that the LD_{50} is above 5000 mg/kg because no death was recorded above 5000 mg/kg in rats when administered orally and this implies that there is no toxicity associated with its consumption at normal doses. More so, there is no toxicity associated with honey.

Table 2 Acute toxicity test of *Desmodium velutinum*

Phase	Dose (Mg/kg)	No of animals	Death ratio
Phase 1	10	3	0/3
	100	3	0/3
	1000	3	0/3
Phase 2	1200	1	0/1
	1600	1	0/1
	2900	1	0/1
	5000	1	0/1

3.3. Anti-ulcer studies

The results of the antiulcer studies are presented in Figures 2 to 5 and appendix 2.

Ulceration was confirmed by necrosis and depletion of the stomach lining. Omeprazole was administered as anti-ulcer agent that acts by protecting the stomach from depletion of the layer.

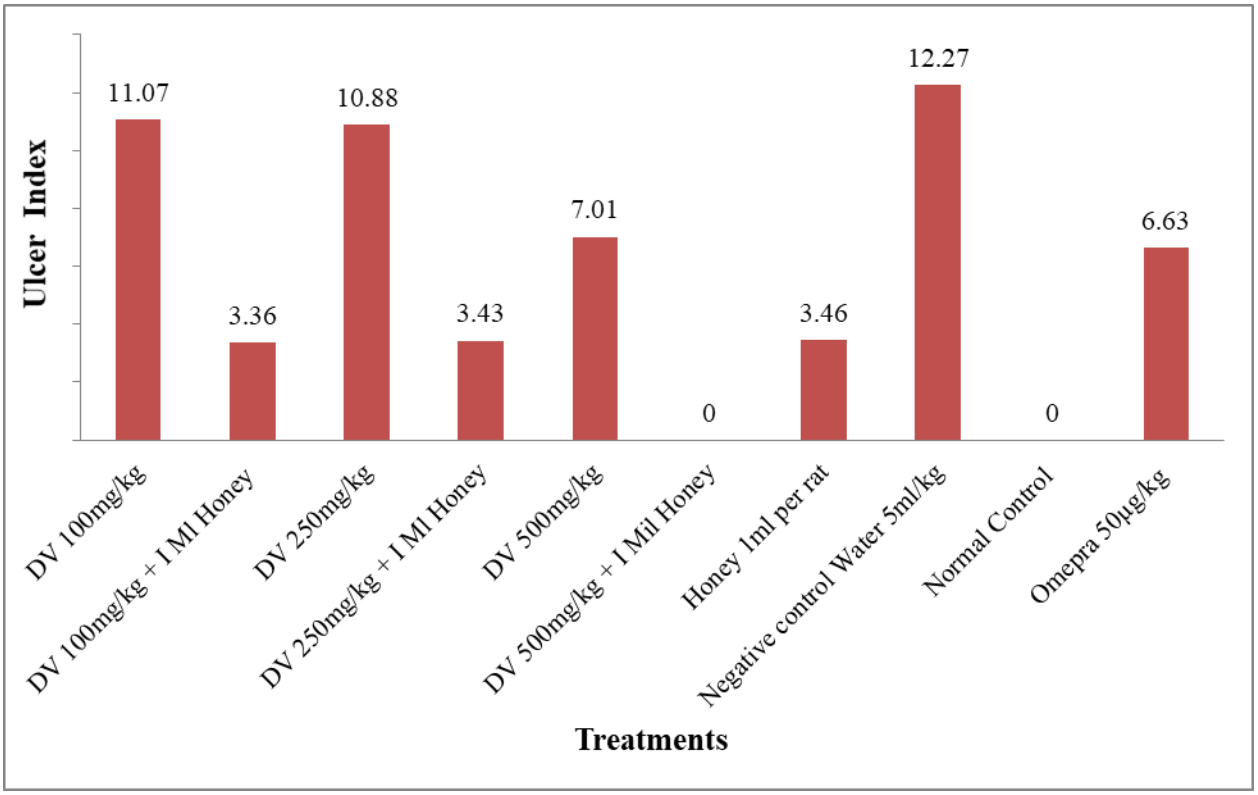

Figure 2 Ulcer Index of *Desmodium velutinum* combined with honey in ethanol induced ulceration in rats. (DV= *Desmodium velutinum* extract)

The result of the ulcer index is presented in Figure 2 and appendix 2. It was observed that the rats treated with water (negative control) had severe ulceration as shown by the ulcer index (12.3) and evident on plate B1 and B2 which are characterized by perforations. This is followed by the group that was pre-treated with 100 mg/kg and had ulcer index of 11.1 and supported by plate D1 and D2. The group that received 250 mg/kg had ulcer index of 10.9 with lesion shown on plate E1 and E2. The 500 mg/kg group had moderate ulceration with an ulcer index of 7.0. Both the standard control with ulcer index of 6.6 and the group that received honey alone with an ulcer index of 3.5 had the least ulceration among the treatment group which was characterized by mild ulceration, mild spots and colorations. A combination of the extract and honey showed reduced ulceration as follows: at 500 mg/kg plus 1 ml of honey the treatment showed no ulceration among the treatment group while at 100 mg/kg and at 250 mg/kg (with the same ulcer index of 3.4 for both doses) combined with honey showed slight ulceration. This implies that the extract showed greater protection against ethanol-induced ulceration in rats at 500 mg/kg and had synergistic effect with honey against the ulceration.

3.4. Percentage inhibition of ulceration by the extract, honey and the combination

The result of the percentage inhibition of ulceration is presented in Figure 3 and appendix 2. The result showed that there is significant different ($P < 0.05$) in level of ulceration between the treated group and negative control (Appendix 2). It showed that there was a synergistic effect when the crude extract of *Desmodium velutinum* was administered in combination with honey (1 ml) across all the doses of 100, 250, and 500 mg/kg with corresponding inhibition of 72.6%, 72.1%, and 100% respectively. These effects are shown on plate G to I respectively. This indicated that the extract at different doses 100, 250 and 500 mg /kg in combination with honey had improved anti-ulcer activity. The improvement in inhibition of ulceration showed the following: At 100 mg/kg plus 1 mill of honey the extract showed percentage improvement in inhibition of ulceration from 9.9% (100 mg/kg alone) to 72.6% (100 mg/kg plus 1 ml honey), while 250 mg/kg of the extract improved from 11.3% (250 mg/kg alone) inhibition of ulceration to 72.1% inhibition of ulceration (250 mg/kg plus 1 ml of honey) while 500 mg/kg improved from 42.9% to 100 % inhibition of ulceration. The result showed great synergy in action against ulcer by *Desmodium velutinum* crude extract when combined with honey.

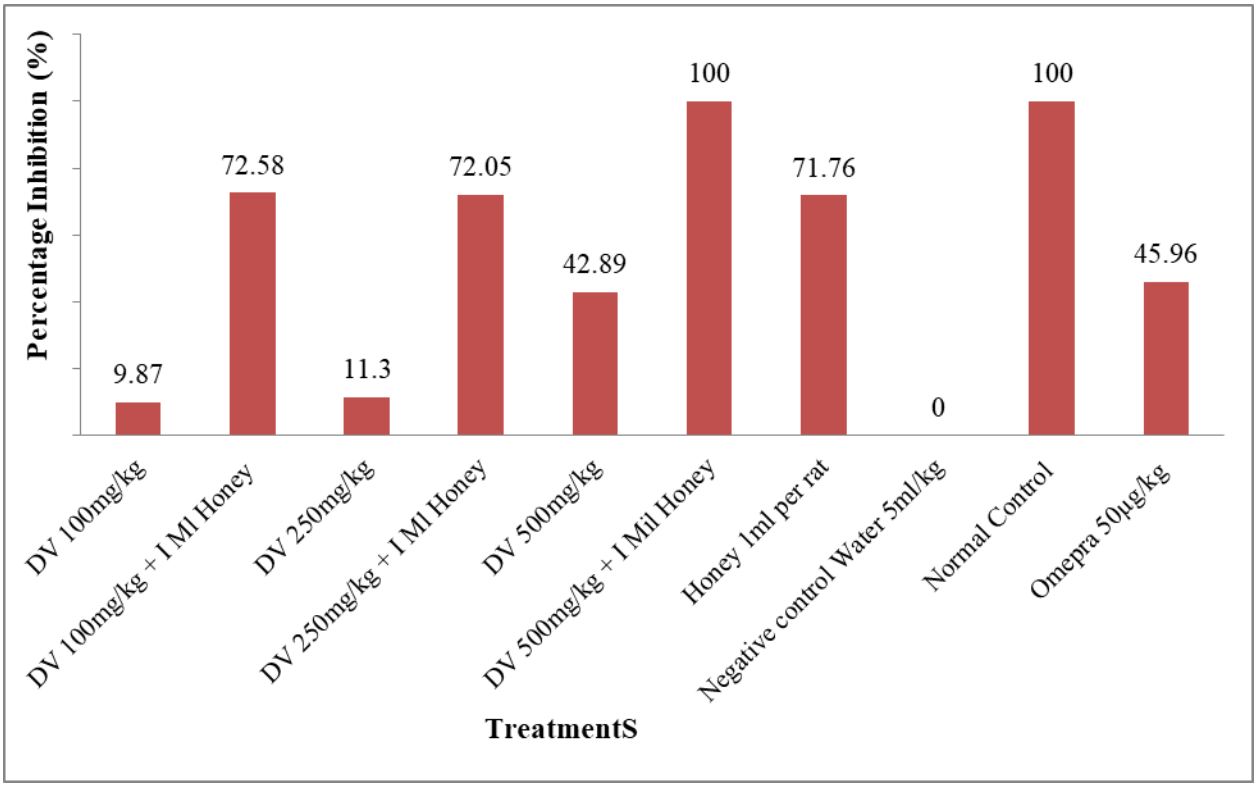

Figure 3 Effect of *Desmodium velutinum* combined with honey in ethanol induced ulceration in rats. (DV= *Desmodium velutinum* extract)

3.5. Macroscopic evaluation of stomach of the rats pretreated with *Desmodium velutinum* in the ethanol-induced ulcer models

The result of the macroscopic evaluation of the stomach of the rats pretreated with the methanol leaves extract of *Desmodium velutinum* is presented in Figure 4A to 4I and it showed gross reduction in ulceration for all the treatment doses; single doses and in combination with honey at 100, 250 and 500 mg/kg (Figure 4D to I), compared to the negative control (Figure 4B) that received only distilled water. Also, rats pretreated with Omeprazole (Figure 4C) showed reduction in ulceration, compared to the negative control (Figure 4B). The macroscopic result supported the result of the ulcer index and percentage inhibition of ulceration respectively and this implied that the methanol leaves extract of *Desmodium velutinum* when given orally either in combination or as single dose of 500 mg/kg had anti-ulcer activity. It further showed that honey when combined with the extract of *Desmodium velutinum* had enhanced antiulcer effect.

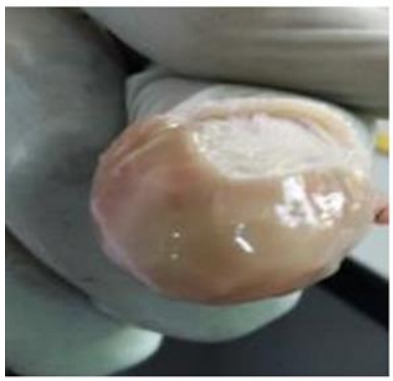	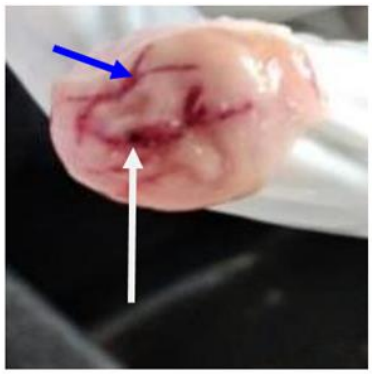	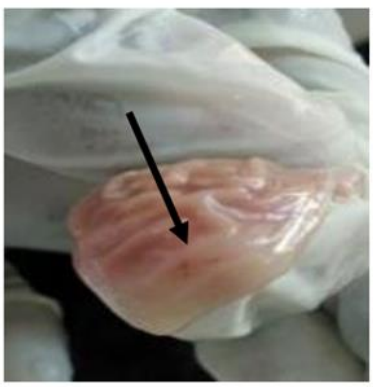
Figure 4A Normal control characterized by normal stomach without ulceration	**Figure 4B** Negative Control (5 ml/kg of H2O) showing severe ulceration characterized by perforation (white arrow) and streak blue arrow.	**Figure 4C** Positive control (Misoprostol 50 µg/kg) characterized by spot ulceration (black arrow)

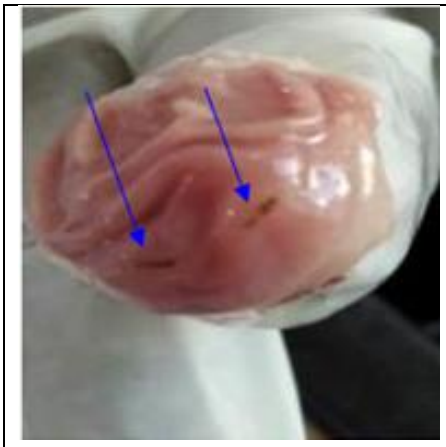	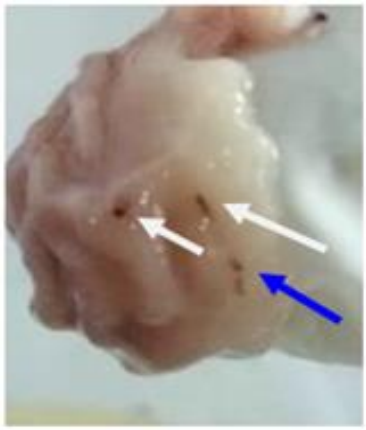	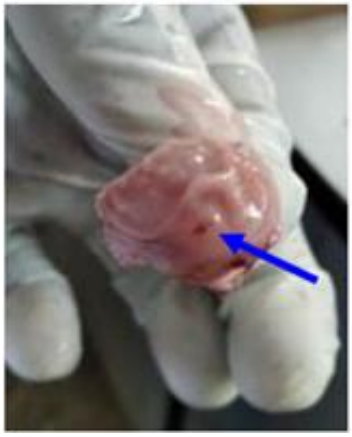
Figure 4D Extract 100 mg/kg (blue arrow) characterized by 2 streak of ulceration	**Figure 4E** Extract 250 mg/kg characterized by 1 streak (blue arrow) and 2 coloration (white arrows) of ulceration.	**Figure 4F** Extract 500 mg/kg characterized by one coloration (blue arrow).
		
Figure 4G Extract 100 mg/kg plus 1 ml honey (blue arrow) characterized by 1 spot ulceration.	**Figure 4H** Extract 250 mg/kg plus 1 ml honey (blue arrow) characterized by 1 spot ulceration.	**Figure 4I** Extract 500 mg/kg plus 1 ml honey characterized by normal stomach without ulceration.

3.6. Histopathology evaluation of stomach of the rats pretreated with the methanol leaves extract of *Desmodium velutinum* in the ethanol-induced ulcer models

The result of the histopathology is presented in Figure 5 A to 5I and the result showed significant reduction in ulceration for all the treatment doses; single doses and in combination with honey at 100, 250 and 500 mg/kg (Figure 5D to 5I), compared to the negative control (Figure 5B) that received only distilled water. Also, rats pretreated with Omeprazole (Figure 5C) showed reduction in ulceration, compared with the negative control (Figure B2). The histopathology results supported the result of the ulcer index and percentage inhibition of ulceration and this implied that leave extract of *Desmodium velutinum* when given orally either in combination or as single dose of 500 mg/kg possessed anti-ulcer activity.

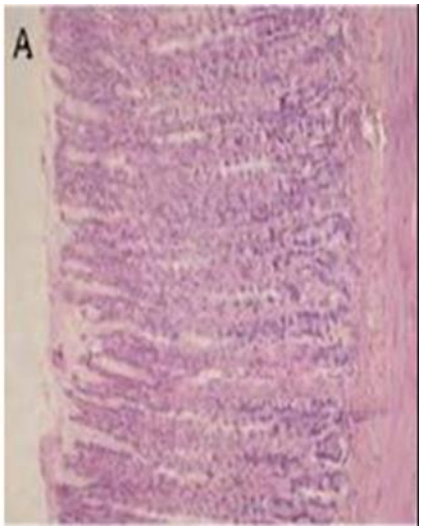	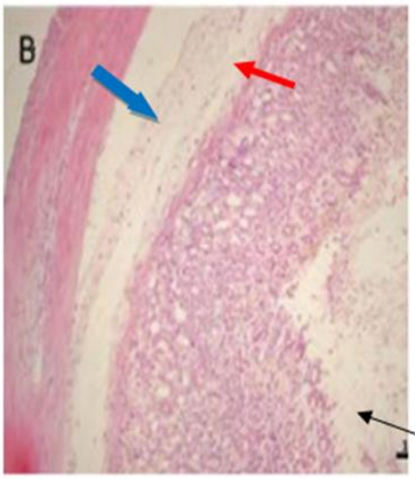	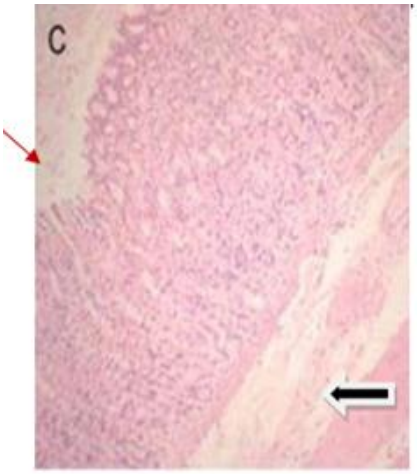
Figure 5A Normal control which showed typical gastric histoarchitecture with intact epithelium and glands.	**Figure 5B** Negative control water 5 ml/kg. Plate B displayed several changes in the of gastric mucosa, such as severe desquamation (black arrow) and loss of surface epithelial cell (red arrow), necrosis, vacuolization, edema and dilated gastric glands along with infiltration of inflammatory cells (blue arrow)	**Figure 5C** Positive control Omeprazole 50 µg/kg. Showed decreased gastric lesions compared to plate B. The gastric mucosa exhibited focal loss of superficial gastric epithelium (black arrow). The gastric glands were almost normal in appearance(red arrow)
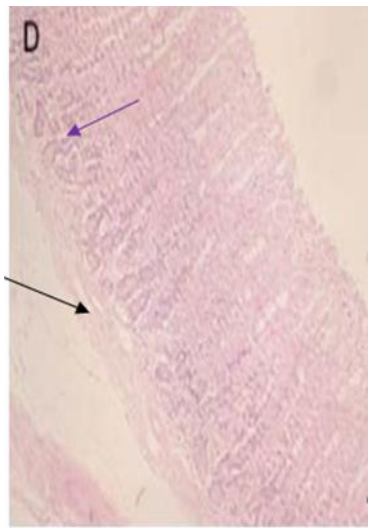	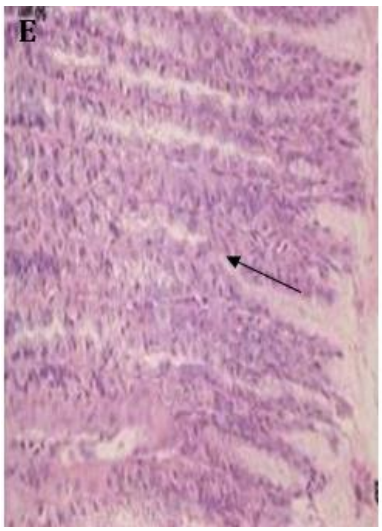	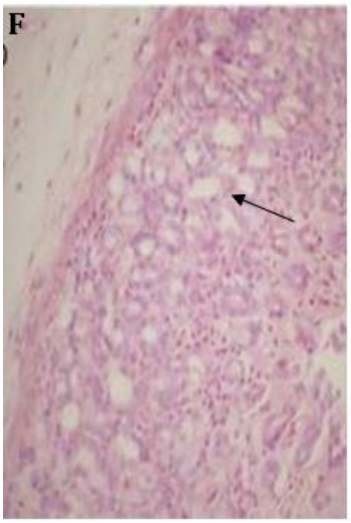
Figure 5D Extract 100 mg/kg, resulted in gastric lesions, characterized by focal areas of disruption, mucosa showed almost normal gastric glands (purple arrow), with mild edema and limited esinophilic infiltration (black arrow)	**Figure 5E** Extract 250 mg/kg, displayed very mild erosion of superficial epithelial cells loss and mild hemorrhages (arrow)	**Figure 5F** Extract 500 mg/kg, the mucosa was infiltrated by inflammatory cells (arrows) that almost displayed extensive edema

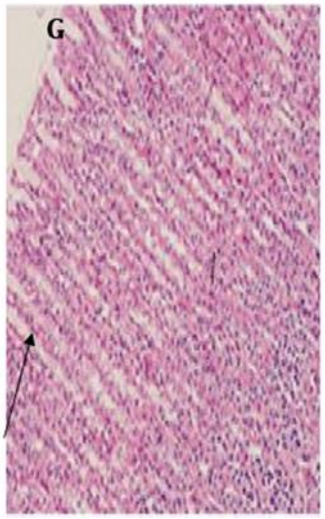	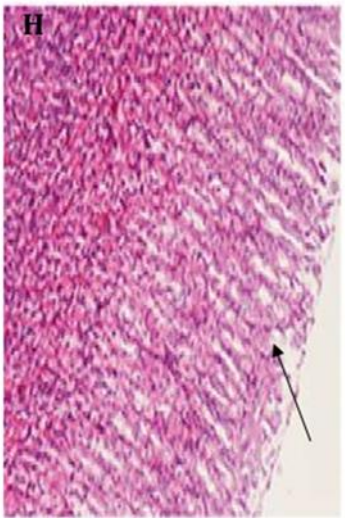	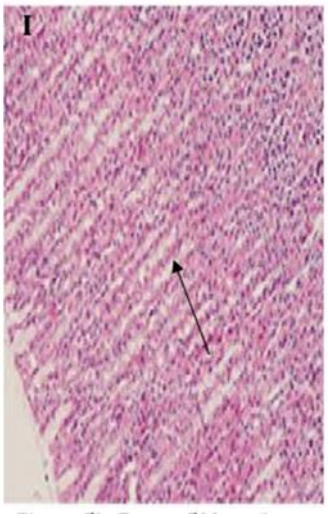
Figure 5G Extract 100 mg/kg + Honey. revealed few superficial surface of mucosa, gastric glands appeared normal without inflammatory cells infiltration (arrow)	**Figure 5H** Extract 250 mg/kg + Honey. revealed superficial surface of mucosa gastric glands appearing normal and necrosis cannot be seen (arrow)	**Figure 5I** Extract 500 mg/kg + Honey. It is characterized by mucosal lesions (arrow) with mild haemorrhage, but edema almost disappearing.

4. Discussion

Phytochemicals formed the basis of folk medicine which has existed from time immemorial to the present times. It has shown, preclinical evidence as antiulcerogenic agents as reported by the following studies [34-39] and hence can play a vital role in the emergence of new alternative drugs with potent antiulcer effect. In the light of this development, the antiulcer activity of a combination of methanol leaves extract of *Desmodium velutinum* and honey was investigated in a rat model using absolute ethanol as ulcerogenic. The result of the phytochemicals analysis of *Desmodium velutinum* showed a high concentration of tannins (17.9) flavonoids (16.3) and alkaloids (13.2) which are known phenolics while honey also contained a reasonable concentration of phenolics. Phenolics are well established and known for wound healing and anti-ulcer property. Phenolics such as flavonoids, tannins, and terpenoids have been reported to possess antiulcer properties [40-43]. Tannins are scientifically established to precipitate protein and 'tar' the outermost layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritants [44]. Flavonoids on the other hand are known for antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion [45] and protection of the stomach lining through a cascade of endogenous and exogenous mechanisms. The progressive wound retraction and re-epithelialization observed in the treatment groups, notably the group 9 animals that received a combination therapy of 1 ml of honey and extract 500 mg/kg body weight strongly indicated that methanol leaves extract of *Desmodium velutinum* , and honey possess gastrotherapeutic effects, which gave an excellent ulcer healing activity synergistically as shown in plate H, with the corresponding 100% inhibition (Figure 3). This suggests that the combination of honey and the extract of *Desmodium velutinum* has a gastroprotective effect which could be by the protection of the epithelium and release of pre-formed mucus following response to toxicological injury.

Gastric ulceration was confirmed by the observed acute lesion (plate B1) in the negative control animals (group 1) following ethanol instillation. Administration of ethanol causes gastric necrotic damage and subsequent inflammatory cell infiltration and reduces the secretion of bicarbonate, gastric mucus, and nitric oxide [46]. In this study, the significant increase in ulcer index in the negative control (Figure 2) as shown in Figures 4B and 5B may be a result of oxidative stress, formulation of reactive oxygen species, and inhibition of prostaglandin synthesis caused by exposure to ethanol. It has been reported that ethanol reduces the gastric blood flow and induces oxidative stress by increasing the production of malondialdehyde and reducing glutathione production [46, 47] and may also attenuate other endogenous anti-inflammatory and anti-oxidant moieties such as binding proteins (MBPs), Uric acid (UA), Melatonin (MEL), Bilirubin (BIL) and Polyamines (Pas) in the rat stomach endogenous system to fight the free radicals thereby inhibiting ulceration. The experiment described in this study showed that methanol leaves extract of *Desmodium velutinum* , and honey at different doses of 100, 250, or 500 mg/kg reduced and repaired the histopathologic damages

and the number (inhibition) and size of gastric ulcers caused by ethanol instillation Wistar rats. The cytoprotective and gastrotherapeutic activities of the leave extract and honey are most probably due to the medicinal properties of their secondary metabolites. These phytometabolites exert potency as drugs through diverse mechanisms as drugs such as modulators of endogenous defensive factors, and or scavengers of free radicals released by the ethanol, inhibitors and could be a potent anti-ulcer drug. The antiulcer activity of the D. velutinum could be attributed to the high content of flavonoids and tannins which are established to have antiulcer activity due to their antioxidant properties [48].

The Fabaceae family are among the most studied shrubs and have been shown to possess promising wound healing, antioxidant, anti-inflammatory, cytoprotective, gastric secretion inhibition, and mucus production and this supports its folkloric usage for the treatment of wounds. The antiulcer activities of the Fabaceae family are evidently supported by the studies [49-50]. The leaf extract and honey used in this study may have exhibited the observed anti-ulcer activity could be through an antioxidative mechanism or by blocking the hydrogen/potassium adenosine triphosphatase enzyme system (by inhibition of gastric proton pump) primarily responsible for the acidification of the stomach contents and the activation of the digestive enzyme pepsin.

It was found that administration of the methanol leaves extract of *Desmodium velutinum* alone resulted in approximately 72% inhibition of ulceration and this finding is in agreement with a similar study that reported the gastroprotective effect of leaves to extract of *Desmodium velutinum* against an ethanol-induced ulcer in experimental rats [25]. However, in this study, the concept of combination therapy of natural flora is introduced for the treatment of peptic ulcers which is evidently reported by [11, 33] to be an effective treatment strategy. As evident in the group of animals that received a combination of 500 mg/kg of the extract and 1ml of honey, there was an excellent repair of the stomach lesion as shown in plate H with a corresponding zero ulceration indicating 100% inhibition of ulceration (Figure 2). In a side-by-side comparison to the group that received 500 mg/kg of the extract alone, although suggestive of gastroprotective attributes by the observed significant ulceration healing (plate F1) with a corresponding 72 % inhibition of ulceration, the inability of the extract to completely inhibit the negative effect of the ethanol is evident that combination therapy is more effective and potent, hence the choice of the treatment strategy. The gastroprotective effect of this phytomedicine (*Desmodium velutinum* combined with honey) could be by the release of gastric mucus formed in response to the mechanical injury caused by ethanol. Therefore, the released mucus offers protection against stomach lesions by providing buffering capacity for the neutralization of luminal acid that aids the formation of acute gastric mucosal lesions, thereby enhancing the repair of stomach lesions caused by pharmacological irritants such as ethanol. Based on the pathological features of the ulceration in these rats and the healing processes, it can be said that these rat models are a prototype of human ulceration and the combination therapy used in this would have a good safety profile using appropriate dosage therefore this suggests its continued usage for the treatment of stomach ulcer.

5. Conclusion

This study has demonstrated that methanol leaves extract of *Desmodium velutinum* and honey exerts gastroprotective gastrotherapeutic effects. The combination therapy gave the desired therapeutic effect, as evident in the excellent ulceration protection & healing on the ethanol-induced ulcer in albino rat models. Consideration the rising inherent toxic side effects of synthetic drugs, natural flora of plants such as the leaves of *Desmodium velutinum* in combination with honey is potential source of anti-ulcer agents, believed to be non-toxic, safe, effective, readily available and affordable. This therefore indicates that the crude extract when combined with honey could possibly possess combined or additive anti-ulcer potential by inhibition of malondialdehyde, inhibition of free radicals & oxidation and stimulation of binding proteins (MBPs), glutathione production (GHS), Uric acid (UA), Melatonin (MEL), Bilirubin (BIL) and Polyamines (Pas) in the rat stomach system to fight the free radicals thereby inhibiting ulceration.

Abbreviation

- DV: *Desmodium velutinum*

Appendix

Appendix 1 The Phytochemical constituents of *D. velutinum*

SN	**Bioactive compound**	**Qualitative**		**Quantitative**	
		D velutinum	**Honey**	***D velutinum***	**Honey**
1	Alkaloids	Present	Present	13.740 ± 0.052 [b]	0.244 ± 0.006 [a]
	Flavonoids	Present	Present	16.290 ± 0.017 [a]	17.01± 0.023[b]
4	Tannins	Present	Present	17.850 ± 0.017 [b]	0.669 ± 0.006 [a]
6	Saponins	Present	Present	0.228 ± 0.002 [a]	1.761 ± 0.006 [b]
7	Steroids	Present	Present	0.049 ±0.002 [a]	0.081 ±0.002 [b]
9	Terpenoids	Present	Present	0.483 ±0.001 [a]	21.410 ± 0.066 [b]
10	Carbohydrates	Present	Present	36.486 ± 0.029 [a]	88.733 ± 0.333 [b]
13	Reducing sugar	Present	Present	6.837 ± 0.029 [a]	12.120 ± 0.000[b]

Appendix 2 Anti-ulcer potential of methanol extract of *D. velutinum* combined with honey in rats

Groups /Treatment Mg/Kg	**Ulcer Index**	**Percentage inhibition of Ulceration %**
Negative control Water 5ml/kg	12.27 ± 0.27 [e]	-
Omeprazole 50µg/kg	6.63 ± 0.32 [c]	45.96
Normal Control	0.00 ± 0.00 [a]	100.00
Extract 100mg/kg	11.07 ± 0.47 [d]	9.87
Extract 250mg/kg	10.88 ± 0.18 [d]	11.30
Extract 500mg/kg	7.01 ± 0.29 [c]	42.89
Extract 100mg/kg + I Ml Honey	3.36 ± 0.03[b]	72.58
Extract 250mg/kg + I Ml Honey	3.43 ± 0.10 [b]	72.05
Extract 500mg/kg + I Mil Honey	0.00± 0.00 [a]	100.00
Honey 1ml per rat	3.46 ± 0.03 [b]	71.76

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest among the authors.

Author contributions

All authors contributed to the conceptualization, investigation, methodology, supervision, formal analysis and writing of the manuscript.

Statement of ethical approval Statement of ethical approval

All experimental procedures were approved by the Faculty of Pharmaceutical Sciences research and ethical committee. Also, one of the authors Mr. Ike CJ is licensed to handle laboratory animals.

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