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A Systematic Comparative Study of Morinda Tinctoria and Vitex Negundo for Their Anti-Ulcerogenic Potential

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ABSTRACT

*Since many researchers previously reported that M. tinctoria and V. negundo possess antiulcer activity thus the main objective of our study is to compare the antiulcer activity of both plants. Pharmacogenetic, phytochemical, and pharmacological assessments will be used to identify the antiulcer effect of the M. tinctoria and V. negundo. By using a vacuum rotary evaporator, M. tinctoria and V. negundo alcoholic extracts were prepared. The OECD criteria for acute toxicity and sub-acute oral toxicity were done for 14, 28 days. For antiulcer activity determination, in-vitro and in-vivo methods were used. In the Pylorus ligation-induced gastric ulcer model, 400mg/kg M. tinctoria was significantly different from the standard and revealed a 4.820±00** ulcer index, whereas 400mg/kg V.negundo revealed a 4.980±04*** ulcer index. At the dose of 400 mg/kg of M. tinctoria ulcer index observed 0.46 ± 0.02 and V.negundo was 0.49 ± 0.21 substantially reduced the effect of ulcers and the ulcer index observed in swimming stress-induced ulcers. M. tinctoria and V.negundo observed 4.1±0.37 and 4.5±0.19 ulcer index in NSAIDs-induced stomach ulcers in rats administered by oral route at the dose of 400 mg/kg. With an IC50 of 35 µg/ml, M. tinctoria significantly reduced ATP hydrolysis by goat stomach ATPase. For the positive control group, Omeprazole (10-50µg/ml) was used to lower Hydrogen Potassium ATPase activity, with an IC50 of 29 µg/ml. In comparison to V.negundo, pharmacological studies show that M. tinctoria extract (MTE) successfully protects the stomach mucosa from ulcers generated by different agents.*

Keywords: *M. tinctoria, V.negundo, Peptic ulcer, Pylorus ligation, H+-K+ ATPase*

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INTRODUCTION

Plants are the major source of therapeutic agents to cure human disease. nowadays it's a debatable topic that, natural drugs are healthier in comparison to allopathic drugs. The kingdom Plantae has between 300 and 315 thousand plant species, many of which have been used as remedies for centuries. Numerous researches have looked into the function of medicinal plants and their potential to heal wounds. Because of the growing interest in the use of medical plants around the world, which is expanding at a pace of 7-15 percent per year, there has been a significant increase in medicinal plant-based industries in recent years. Despite significant advances in contemporary medicine, the development of innovative drugs derived from natural sources remains a priority. Traditional treatment based on herbal medical principles has been tried and true for thousands of years and is widely accepted across all cultural and socioeconomic groups (Kaur *et al*., 2012; Vimala & Gricilda Shoba, 2014; Dorjsembe *et al*., 2017; Mostofa *et al*., 2017; Singh *et al*., 2017; Dons & Soosairaj, 2018; Elzayat *et al*., 2018; Pereira *et al*., 2018; Sumantri *et al.,* 2022).

A peptic ulcer is a digestive system condition known as corrosive peptic injury.Ulcers can occur in the oesophagus and the Mackel diverticulum, but they occur in the stomach or the duodenum. Many researchers have reported that ulcer formation can be caused by a variety of factors throughout the previous 30 years. Hospitalization, mortality, and incidence ratios have all decreased dramatically during the last 30 years. The lifetime probability for the occurrence of the disease is near about4-19 percent in the general population with an annual occurrence rate of around 0.1-0.3 percent (Waldum *et al*., 1993; Andersson *et al*., 1999; Gary & Kevin, 2004; Tripathi, 2008; Amandeep *et al*., 2012; Tortora & Tortora, 2014; Vomero & Colpo, 2014; Waugh & Grant, 2014; Dennis *et al*., 2015; Mirski *et al*., 2016; Lanas & Chan, 2017; Kavitt *et al*., 2019; Kuna *et al*., 2019; Keikha *et al*., 2022).

Morinda and Negundo species are also known as traditional tropical plants that have been utilized for many years as herbal medicines. M.Tinctoria thrives as a wild shrub in the agroecosystems, and because all portions of the M. tinctoria are clinically active. it may be utilized to treat a variety of ailments. Researchers discovered that the leaf, root, and fruit extracts of M.Tinctoria had a variety of medicinal qualities, including ulcer-fighting properties (Mathivanan *et al*., 2006; Bagavan *et al*., 2008; Vadivu *et al*., 2008; Sahoo *et al*., 2009; Sivaraman & Muralidharan, 2010; Sivakumar *et al*., 2018; Rex Jeya Rajkumar *et al*., 2018). Nirgundi, Sambhalu, or monk's paper are all names for Vitex negundo L. It grows in wet locations, often along river banks, in India up to a height of 1500 gigameters as well as in the Mediterranean and Central Asian regions. It is thought to

offer several medicinal properties, as well as antiulcer properties (Ullah *et al*., 2012; Vangoori *et al*., 2013; Gill *et al*., 2018; Chandra & Meel, 2020; Júnior *et al*., 2020; Devi, 2021; Jongjai *et al*., 2021; Khan *et al*., 2021; Neha *et al*., 2021; Gouthami *et al*., 2022; Mehta *et al*., 2022; Tasfiyati *et al*., 2022; Vannur *et al*., 2022; Wang *et al*., 2022).

MATERIALS AND METHODS

A completely grown fresh Leaf of V. Negundo was ordered from Kanpur, while the M. tinctoria plant was taken from Tamil Nadu. Plants were authenticated by a renowned botanist. We employed Suresh *et al*. techniques for hydroalcoholic extraction of M. tinctoria leaves and Vitex Negundo, and standard procedures to recognize different constituents of an active component such as lipid, proteins, amino acids, and so on.

For the Acute, Subacute, and screening models, male Wistar rats (150-200gm) were used. The rats were kept, in a room at a temperature of 22-30°C, with 65% relative humidity, and a 12 hour light/dark cycle supported by artificial light.Within 7 days, the bedding was changed 3 times. Standard diet and water were provided for all rats. All experiments followed the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guideline (CPCSEA/AC/09/1273).

Acute toxicity study

The OECD 423 recommendations for extracts used an acute oral toxic class technique to determine safer levels. Four male Wistar rats were given initial doses of MTE and VNE of 500, 1000,2000&4000 mg/kg body weight by oral route, while one was maintained normal. Rats were followed for three days to see if there were any major physiological changes, such as weight gain or other toxicity indicators. The technique was then repeated for a total of 7 days with the same dose level, with the 14th day serving as the evaluation (Oecd, 1994; Radwan *et al.,* 2022).

Sub-acute toxicity study

Following OECD Guideline 407, repeat oral toxicity assessments were performed. The animals were divided into nine groups of four rats in a group. Group firstwas given 10 milliliters per kilogram of distilled water (bd.wt.) and treated normally. MTE and VNE were administered to groups 2nd and 3rd,4th and 5th, 6thand 7th, and 8th and 9th at doses of 500,1000,2000 & 4000 mg/kg body weight by oral route. for 28 days.Medicine was given at precise times each day, andchanges in signs, symptoms, death were recorded at least two timesa day. Animals weight was taken weekly, then euthanized on the last day of the fourth week for estimation of differentbiochemical parameters, and hematological tests.

Anti-Ulcer models

Pylorus ligation (induced) gastric ulcers in rats

The animals fasted for a day before the experiment water was permitted to drink as much as they wanted. Male rats weighing 175-250 g were separated into four groups, each with four rats. Normal saline was given to Group 1st, Omeprazole was given to Group 2nd as a standard (20 mg/kg), MTE was given to Group 3rd and 4th and VNE was given to Group 5th and 6th (p.o.) 1 hour after treatment, all rats were anesthetized and the abdomen was opened. According to Shay *et al*., 1945, the pylorus of the small intestine was elevated and ligatedvertically (Shay, 1945). The stomach along with pylorus was restored with care and abdominal wall repair by suture. Rats were CO2 euthanized after 4 hours of pyloric ligation. Content of the stomach was kept in the centrifugation tube after the abdomen was sliced open. The complete stomach fluid was measured and centrifuged at 3000 revolutions per minute for nearly 8-10 minutes. The pH, acidity, and free acidity were all measured. The ulcer on the stomach's surface had been examined macroscopically and evaluated on millimeter-square paper. The Ulcer Index was used to represent the amount of surface area (mm2) (UI) (Goel *et al*., 1985; Rao *et al*., 2004; Bafana & Balaraman, 2005; Parmar & Prakash, 2006; Zakaria *et al*., 2011). The Ulcer index was calculated using the equation provided (Vogel & Vogel, 1997; Zavalishina *et al.,* 2022).

pH determination

A one-ml aliquot of stomach fluid was dissolved in one ml of water. A pH meter was used to determine the pH of the solution.

Total acidity determination

A one-millilitregastric juice supernatant was diluted with one-milliliter distilled water and transferred to a 50 ml conical flask, and titrated with 0.01N NaOH in presence of phenolphthalein indicator until a persistent pink color developed. The consumed amount of 0.1N NaOH was given. The total acidity of gastric juice is expressed in milliequivalents per liter (mEq/L) using the following equation:

$$Activity = \left(volume\ of\ NaOH \times N \times \frac{100mEq}{L}\right) \div 0.1 \quad (1)$$

Free acidity determination

Before a persistent pink color was discovered, the supernatant of stomach fluid was titrated with 0.01N NaOH in presence of Topfer's reagent indicator. The amount of 0.01N NaOHwas used as specified.For the determination of total acidity same equation was used.

Swimming stress-induced gastric ulcers in rats

Before the experiment, the animals fasted for a day,water was permitted to drink as much water as they desired. Male Wistar rats weighing 175-250 g were separated into six groups each with four rats. Group 1streceived normal saline, whereas Group 2nd received standard drug famotidine (10 mg/kg, bd.wt, p.o.) as a control. MTE was administered to Groups 3rd and 4th at 200, 400 mg/kg, respectively (bd.wt, p.o.), whereas VNE was given to Groups V and VI at 200,400 mg/kg, respectively (p.o). The rats were forced to swim up to 30cm in a circular container of water after 30 minutes of post-drug therapy (height 45cm, diameter 30cm). The water temperature was regulated between 28 and 290 degrees Celsius. The rats were euthanized three hours later by an anaesthetic ether overdose. The stomach was taken, opened, and removed from the body.Onmillimetre-square paper, the

ulcer regions on the stomach's surface were investigated macroscopically. The Ulcer Index was a measure of the amount of area (mm2) (UI). The equation was used to calculate the Ulcer index as described by Suresh *et al*.

Aspirin-induced gastric ulcers in rats

Before the experiment, the animals were deprived of food for a day,water wasdrink as much water as they desired. Male Wistar rats weighing 120-130 grams were placed into seven groups of four animals each. For 7 days, Groups I and II were given normal saline, Group III was given Omeprazole as a standard (20 mg/kgp.o.), Groups IV and V were given 200, 400 mg/kg of MTE, and Group VI and VII was given 200,400 mg/kg of VNE. On the seventh day, 30 minutes after the medication treatment, every rat except the normal control (positive control) group got aspirin (200mg/kg p.o.). The rats were given a high dosage of ether and were euthanized four hours later.The stomach was taken down and opened along the larger curvature. In every stomach, the mucosa was examined for an accurate count and severity of ulcers. The equation was used to calculate the Ulcer index as described by Suresh *et al*.

H^+/K^+ ATPase inhibition activity

Hydrogen potassium ATPase was made from goat mucosal following the process described by Cheon *et al*. (2001) Drinking water was used to clean the stomach of a recently butchered goat. The stomach mucosa membrane was scraped and homogenized within ice-cold phosphate buffer (pH 7.4). at 18,000 rpmfor 15 minutes. The resulting supernatant was centrifuged once more. The particle has been resuspended in the homogenization buffer. Discontinuous density gradient centrifugation of Ficoll sucrose was utilized to prepare preparations for Hydrogen potassiumATPase. Lowry *et al*. techniques were utilized to determine protein concentrations (Bradford, 1976).

Assay of H^+/K^+ ATPase

To make a volume of 1 ml, various concentrations of extract (10 gm/ml to 50 gm/ml) were incubated in the reaction mixture (40 mM Tris-HCl buffer, pH 7.4, including 2 mM MgCl2 and 10 g membrane protein).To begin the reaction, the whole formulation was incubated at 37°C for 15 minutes with 2 mM ATP Tris salt.The reaction was allowed to be eliminatedby the addition of 1 mL of trichloroacetic acid (ice-cold,10% v/v) to the mixture. The behavior was determined by the presence and absence of hydrogen potassium ATPaseby using various dosages of both the extracts and omeprazole. At 400 nm, the amount of inorganic phosphate buffer released by ATP was quantified spectrophotometrically (Reyes-Chilpa *et al*., 2006).

RESULTS AND DISCUSSION

In this study, the antiulcer properties for MTE and VNE plants were assessed in albino rats using in vitro and in vivo ulceration models. In the current study, the antiulcer potential of alcoholic extracts of MTE and VNE was assessed at two dosages (200 and 400 mg/kg). toxicity tests are usually completed in two months.Four male Wistar rats were given an initial dose of MTE & VNE of 500,1000,2000 and 4000 mg/kg body weight by oral route, and one was maintained normal. There was no significant difference in body weight between pre-and post-diagnosis, and there was no sign of toxicity. When the treatment was repeated for another 7 days with the same dose quantity, no alterations in normal behavioral patterns were seen, and no clinical indications of toxicity or deaths were observed after 14 days. The rats were given different dosages of M. tinctoria and V. Negundo hydro-alcoholic extract (500, 1000, 2000, and 4000 mg/kg) did not affect their body weight **(Figure 1)**, and there were no major differences in the organ weights of treated and control rats. The stomach and other organs were not harmed by M. tinctoria and V. Negundo because their gross and net weights were not substantially different from control values.

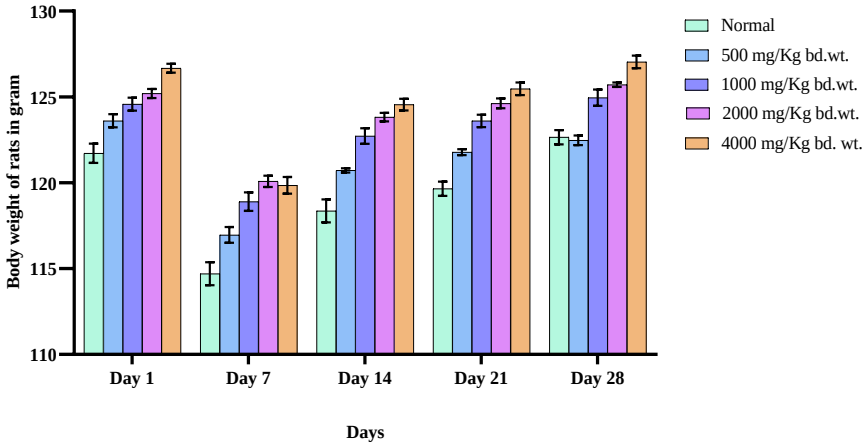

Figure 1. Graphical representation of body weight with different dos

During the required research duration, no behavioral changes, therapy-related odd symptoms, or mortality risks were observed. Cholesterol profile,Glucose (before and post-treatment), or Total Protein. **Table 1** shows the results of the study. In Wistar rats given a greater dose of 4000 mg/kg, no death was observed. In the groups under investigation, all

estimated hematological parameters were reported at healthy values. Hematological measures such as Red blood cells, white blood cells (Total), hemoglobin, and Platelets were not significantly different for MTE and VNE in both control and treated rats. There are no significant differences in hematological parameters in the extract-treated animals compared to controls, suggesting that the active ingredients of the MTE and VNE did not cause blood cell lysis or limitation in blood cell production **(Table 2)**. M. *tinctoria* and V. *Negundo* are non-toxic to rats, according to the findings.

Table 1. Biochemical parameters of *Morinda tinctoria* & Vitex *negundo* extracts in rats (values represented as Mean ± SEM).

Parameter/Dose	TC(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	Glucose (g/l) (BT)	Glucose (g/l) (PT)	T P (g/dl)
Control	123.13±0.04*	79.23 ±0.05*	67.13 ±0.00*	0.81 ±0.04*	0.79 ±0.08*	7.4±1.0*
MTE 500 mg/kg	101.51 ±0.03*	76.13 ±0.05*	56.60 ±0.06*	0.93 ±0.02*	0.63 ±0.03*	7.1±1.2*
VNE 500 mg/kg	100.12 ±0.04*	77.71 ±0.01*	57.23 ±0.02*	0.94 ±0.06*	0.65 ±0.08*	7.1±1.8*
MTE 1000 mg/kg	253.21 ±0.04*	134.71 ±0.03*	42.52± 0.04	0.75±0.02*	0.92 ±0.03*	6.7±1.0*
VNE 1000 mg/kg	252.31 ±0.05*	136.66 ±0.01*	43.51± 0.06	0.74±0.08*	0.93 ±0.02*	6.6±1.0*
MTE 2000 mg/kg	90.12 ±0.01*	71.37±0.04*	21.62 ±0.05*	0.85 ±0.03*	0.80 ±0.01*	7.3 ±1.1*
VTN 2000 mg/kg	91.12 ±0.01*	72.52±0.04*	23.23 ±0.04*	0.87 ±0.04*	0.83 ±0.06*	7.6 ±14*
MTE 4000 mg/kg	66.20 ±0.03*	64.02 ±0.03*	19.21 ±0.00*	0.95±0.03*	0.73 ±0.34*	7.1±1.0*
VNE4000 mg/kg	68.22 ±0.03*	60.31 ±0.02*	20.71 ±0.00*	0.97±0.08*	0.76 ±0.22*	7.2±1.2*

Table 2. Haematological parameters of *Morindatinctoria* & *Vitexnegundo* in rats (values represented as Mean ± SEM).

PARAMETERS	Haemoglobin (g/dl)	WBC (×10³/µl)	RBC (×10⁶/µl)	Platelet(×10³/µl)
CONTROL	11.3 ± 0.31*	7.20 ± 0.40*	9.30 ± 0.21*	990± 0.12*
500mg/kg MTE	10.2 ± 0.81*	7.12± 0.20*	8.40± 0.40*	980 ± 0.12*
500mg/kg VNE	10.0± 0.21*	7.80± 0.22*	8.57± 0.34*	982 ± 0.12*
1000mg/kg MTE	11.10± 0.23*	8.50± 0.36*	9.80 ± 0.12*	972± 0.20*
1000mg/kg VNE	10.20± 0.43*	8.10± 0.12*	9.10 ± 0.30*	975 ± 0.46*
2000mg/kg MTE	11.10 ± 0.80*	8.50 ± 0.30*	9.10± 0.45*	978± 0.20*
2000mg/kg VNE	10.15± 0.20*	8.10 ± 0.62*	8.60± 0.42*	982± 0.22*
4000mg/kg MTE	11.50± 0.24*	7.86± 0.30*	10.20± 0.05*	984± 0.20*
4000mg/kg VNE	10.20± 0.14*	7.20± 0.56*	10.10± 0.32*	981± 0.60*

Rats treated with MTE and VNE with different dose levels exhibited a significant reduction in the ulcers number and the ulcer index in the Pylorus ligation-induced gastric ulcer model (UI). MTE at the dose level 200 mg/kg showed 5.60± 0.02* ulcer index, whereas VNE with the same dose level showed 5.49± 0.02* ulcer index, although MTE and VNE are not statistically significant against omeprazole. The test group that received 400 mg/kg MTE and VNE, on the other hand, saw a reduction in total ulcer count and ulcer index (4.82± 0.00 and 4.98± 0.04***, respectively). In comparison to the control and standard groups, the Ulcer Index at the dosage level of 400mg/Kg was substantially relevant **(Figure 2)**.

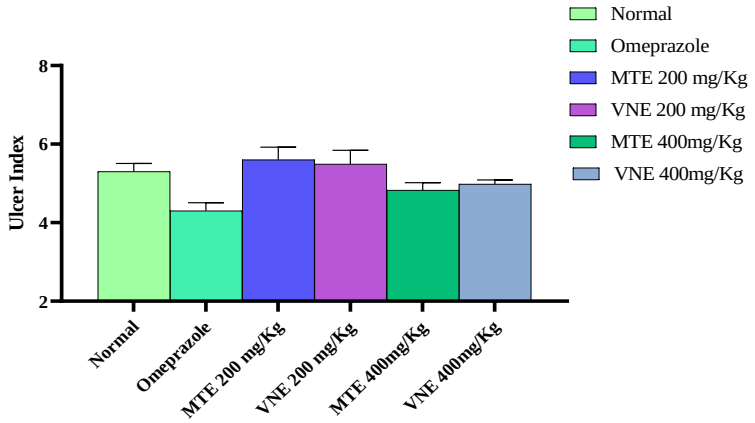

Figure 2. Ulcer Index of *Morinda tinctoria &* Vitex *negundo in* Pylorus ligation induced model

In gastric ulcer induced by swimming Stress, the test group which received 200mg/kg dose of MTE and VNE exhibits prevent from ulcer as the index was 0.65 ± 0.01 and 0.58 ± 0.01 respectively. A decrease in ulcer number and ulcer index was observed. While at the dose level 400mg/Kg was also reduced ulcer number and ulcer index (0.46 ± 0.02 and 0.49 ± 0.21 respectively). In comparison to famotidine (0.45 ± 0.03), at the dose level of 400mg/Kg, MTE and VNE both were significantly reduced ulcer formation in rats **(Figure 3)**.

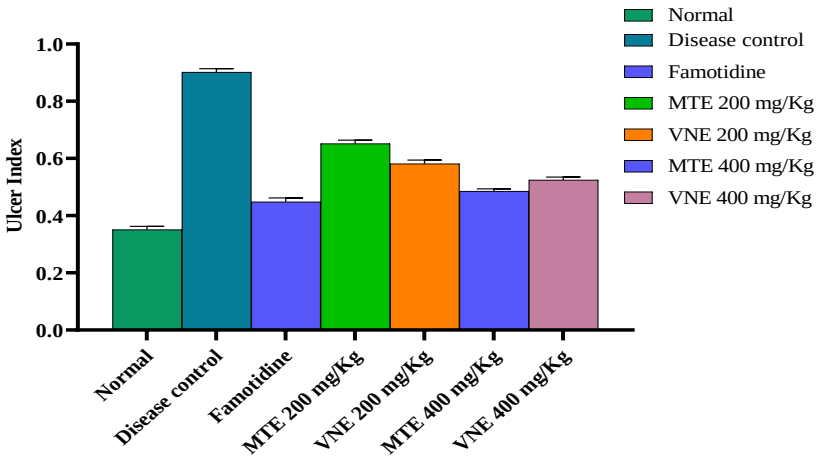

Figure 3. Ulcer Index of *Morinda tinctoria & Vitex negundo*\ on Swimming stress-induced ulcer

Aspirin, a nonsteroidal anti-inflammatory medicine, causes ulcers in the stomach by inhibiting cyclooxygenase enzymes and lowering prostaglandin production. These medicines also elicit an inflammatory response in the stomach mucosa, increasing reactive oxygen species.Leaves off MTE and VNE have been shown to have oxygen scavenging properties in previous research. In the present work, MTE and VNE at the dose level of 200 and 400mg/Kg caused a significant reduction in ulcer index in comparison to standard omeprazole. The positive control group had no ulcers, but the negative control group, which was given aspirin, had an ulcer index of 7.8±0.18 with stomach mucosal hyperplasia and inflammation. There was a decrease in the ulcers number and the ulcer index. At the dose level of 200mg/Kg bd. Wt. MTE and VNE decrease ulcer index 5.6 ± 0.13 and 5.3± 0.084 respectively and At the dose level of 400 mg/Kg bd. wt. 4.1 ± 0.37 and 4.5 ± 0.19 ulcer indexeswere observed. Alcoholic extract of MTE and VNE both reduce ulcer formation in comparison to standard omeprazole (4.0 ± 0.16). MTE possesses good antiulcer activity as compared to VNE in the aspirin-induced gastric model **(Figure 4)**.

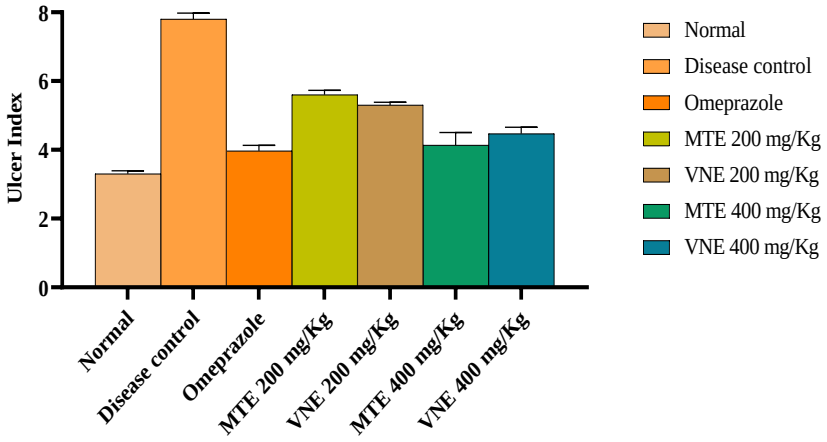

Figure 4. Ulcer Index of *Morinda tinctoria & Vitex negundo* on gastric ulcer induced by Aspirin

Dose-manner enzyme inhibition by standard and extract were examined in hydrogen potassiumATPase Inhibition Activity, revealing that MTE and VNE were significantly decreasing the enzyme hydrogen potassium ATPaseactivity,which is required for acid secretion and equivalent in action to Omeprazole. In comparison to VNE 37 µg/ml, M. tinctoria hydroalcoholic extract significantly reduced Adenosine triphosphate hydrolysis by goat stomach ATPase with an IC50 of 35 µg/ml. Omeprazole (10-50µg/mL) was used as a positive control to lower hydrogen potassiumATPase activity, with an IC50 of 29 µg/ml **(Figure 5)**.

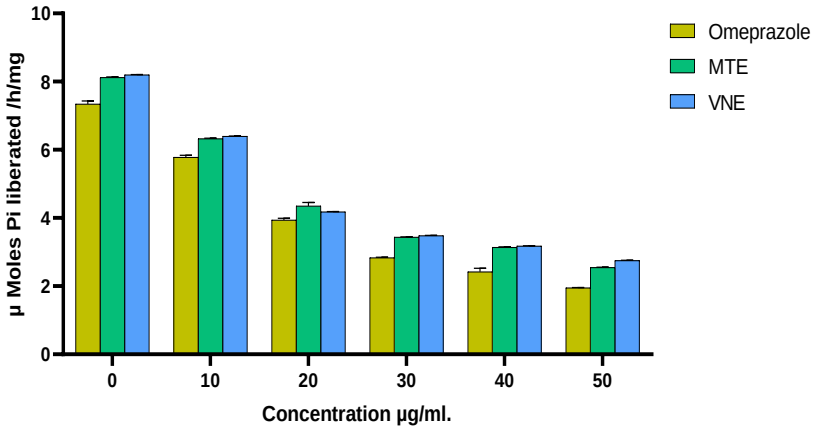

Figure 5. Effect of *Morinda tinctoria* & *Vitex negundo* and Omeprazole on Hydrogen Potassium ATPase activity

CONCLUSION

In comparison to omeprazole and Famotidine, MTE and VNE successfully protect the stomach mucosa against ulcers generated by different in vivo models. Both plants' alcoholic extracts have hydrogen potassium ATPase (in vitro) inhibitory action, the extract showed significant proton pump inhibition comparison to the standard. Such findings revealed that both plants' alcoholic extracts had antiulcer properties however, when comparing the two plants, we can conclude that MTE is an efficient proton pump inhibitor with good antiulcer activity.

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ETHICS STATEMENT: None

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