Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

ORIGINAL ARTICLE

Gastroprotective Effect of *Trichosanthes cucumerina* fruit, Hydro-Alcoholic Extract on Experimentally Induced Gastric Ulceration in Albino Wistar Rats.

Maneesh Kumar Pandey[1] Praveen Sharma[2]

⁕*1 and 2, Department of Pharmacy Oriental University, Indore Sanwer Road, Jakhya Opposite Revati Range Gate No.1, Indore, Madhya Pradesh, 453555, India.*

⁎ *Corresponding author: maneyp@gmail.com (Maneesh Kumar Pandey).*

(Received: 11 June 2024 Revised: 16 July 2024 Accepted: 10 August 2024)

KEYWORDS

Gastroprotective; *Trichosanthes cucumerina* Linn.; Aspirin plus pylorus ligation; Antioxidant enzymes;

ABSTRACT:

Objectives: The objective of the present study is scientifically validate the gastro-protective effect and derive the possible mechanistic activity of *Trichosanthes cucumerina* Linn. fruit, hydro-alcoholic extract against aspirin plus pylorus ligation, ethanol plus HCl induced, acetic acid induced and cold restraint stress induced ulcer models in wistar albino rats.

Methods: The gastroprotective effect of TCHAE (125, 250 & 500 mg/kg, p.o.) was evaluated on both the chemical and physical (Aspirin plus pyloric ligation, ethanol plus HCl, Acetic acid, and Cold restrain stress,) ulcerogens induced ulceration in wistar rats. The mechanistic activity of TCHAE was tested on various gastric-ulcer parameters, namely gastric pH, volume, total and free acidity, output, total protein level, level of mucoprotein and glycoprotein content, antioxidant markers level like LPO, SOD and CAT. Ulcer index and % ulcer protection was assessed to determine the gastroprotective potential.

Results: TCHAE (250-500 mg/kg, p.o.) significantly decreases the ulcer index ($p<0.0001$) against all the four tested gastric-ulcer models. In the aspirin plus pylorus ligation model exhibited % protection of 67.56 and 74.66, respectively. In ethanol plus HCl induced ulcer model, 250 mg/kg and 500 mg/kg displayed % protection of 56.68 and 70.32, respectively. In acetic acid induced chronic ulcers model 250 mg/kg and 500 mg/kg displayed % protection of 50.60 and 68.83, respectively. Cold restraint stress (CR Stress) induced ulcer model, 250 mg/kg and 500 mg/kg displayed % protection of 63.28 and 73.29, respectively. A decrease in aggressive parameters like acid volume, LPO and an increase in protective parameters like total carbohydrate and total protein ratio, SOD, CAT were observed during the estimation of biochemical parameters justifying its protective effects.

Conclusions: The present study indicates that the *Trichosanthes cucumerina* Linn. fruit, hydroalcoholic extract possesses significant gastroprotective property through the defensive mechanism.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

1. INTRODUCTION

Peptic ulcer was one of the most important causes of morbidity worldwide until the last decade of the 20th century (Palle, Kanakalatha, and Kavitha 2018). It was found that the highest incidence (56.5%) of peptic ulcer in India is among the semiskilled workers and the lowest (2.5%) in managerial and professional groups (Tarique et al. 2016).

The health and lifespan of human strongly depend on the harmony of the digestive system. Gastric ulcer and oesophageal reflux have common symptoms including nausea, heart burn and discomfort that affect the overall quality of life (Fass 2007). Recently, the connection between modern lifestyles and gastrointestinal issues has been acknowledged. Although a direct link has not been confirmed between contemporary sedentary behaviors and dietary patterns (like smoking, drinking coffee, consuming fast food, and experiencing stress) and symptoms of gastric ulcer, there is proof that eating fast food deteriorates gastrointestinal function and worsens ulcers (Thapa, Ghatane, and Rimal 2009). Arguably, the most effective way to address stomach issues is to focus on restoring gastric balance instead of just treating symptoms with medication.

Healthy individuals have strict regulation of the secretion of gastric acid and digestive enzymes like pepsin, rennin, and lipases. The stomach's contents are sterilized by gastric acid and create an optimal environment for enzymes to work (Kong and Singh 2009), while mucus and enzymes are released in a precise way to protect the stomach from its acidic contents and maintain proper enzymatic activity for digestion. Food needs to remain in the stomach for about an hour for optimum digestion, allowing for pre-digestion and the activation of gastric acids and pepsin through gastric reflexes (Schubert and Peura 2008). Additional enzymes that are active and come from foods like meat, vegetables, and fruits can also be found in the stomach. Nonetheless, for people whose diet lacks foods containing these enzymes regularly, the entire digestive process may be affected. Eating fast, processed, and overcooked foods without essential digestive enzymes causes the stomach to produce varying levels of acid to compensate, leading to negative effects on gastroesophageal reflexes and promoting the growth of aggressive bacteria like Helicobacter pylori in the stomach. H. pylori and prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) are two of the top reasons for developing gastric ulcers (Mehmood et al. 2010). NSAIDs work by calming the gastric receptors and preventing the production of prostaglandins that play a key role in protecting the stomach lining. This causes the stomach to become more sensitive to overindulgence in coffee and cigarettes, which contain chemicals that can further irritate the stomach due to existing weaknesses in the gastroesophageal sphincter. This irritation leads to an increase in the production of gastric acid, elevated levels of stress hormones, and a weakened immune system. Coffee and smoking are associated with numerous predisposing factors that worsen the occurrence of gastric ulcers. The best method to rebalance the proteolytic ability of the stomach, influenced by hydrochloric acid, pepsin, and other enzymes, and the protective mucin layer is by stimulating the stomach to secrete enough mucus to cover the lining during important digestion phases. However, it is crucial to constantly replenish gastric acid in order to keep gastric function at its best (Salama et al. 2016).

The extensive range of medications available to treat gastric ulcers requires ongoing research for alternative antiulcer treatments due to their harmful side effects. The search for new drugs to treat ulcers has been crucial, leading to an exploration of herbal remedies with better protection and fewer side effects. Plant-based drugs are gaining popularity and are currently under investigation for various disorders. In many developing nations, traditional medicine serves as the primary source of medical care for the majority of the population, rather than just an alternative option. The World Health Organization (WHO) reported that 75% of Malians rely on traditional medicines and the popularity of traditional medicine is increasing.

The plant, *Trichosanthes cucumerina* Linn. is often referred to as "snake tomato, snake gourd, viper gourd, or lone tomato" and is part of the Cucurbitaceae family (Okonwu and Muonekwu 2019). It is commonly found in the wild throughout South and Southeast Asia, such as India, Bangladesh, Nepal, Pakistan, Sri Lanka, Indonesia, Malaysia, Myanmar (Burma), and southern China. It is also considered indigenous in northern Australia (Kumar 2018). The entire plant is utilized in

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

the Ayurveda medicine system to address hepatic and digestive system issues. The fruits of *Trichosanthes cucumerina* Linn. have medicinal uses as a laxative, purgative, antipyretic diabetes, hepatoprotective, controlling fertility, acting as an antioxidant (Adebooye,2008) and antibacterial agent. The fruits are rich in ascorbic acid, lycopene, phenols, flavonoids, alkaloids, tannins, and saponins. Further exploration into the plant's active constituents, which are responsible for its therapeutic effects, could be beneficial (Kumar 2018).

2. OBJECTIVES

The current research aimed to assess how *Trichosanthes cucumerina* Linn. fruit hydro-alcoholic extract can protect the stomach against peptic ulcers caused by Aspirin pylorus ligation induced gastric ulcer, Acetic acid induced chronic ulcer, HCl plus ethanol induced ulcer acute ulcer and Cold-restraint stress induced ulcer Experimental model.

3. MATERIALS AND METHODS

3.1 Collection & Authentication of Test drug

We have purchased unripe fruit of Trichosanthes cucumerina Linn. (10 kg) from Shivpur Sabji market of Varanasi Uttar Pradesh India. Plant sample were authenticated taxonomist by of Prof. N.K. Dubey Centre of Advanced Study in Botany Institute of Science Banaras Hindu University Varanasi with Voucher specimen No.- Cucurbita 2022/01 and were kept in their herbarium.

3.2 Preparation of extract

The unripe fruit (10 kg) *Trichosanthes cucumerina Linn*. were cut into small pieces and air-dried under controlled shade conditions and powdered coarsely (1300 g). Coarsely powdered fruits of 1300 g were exhaustively at room temperature for 3 consecutive days with 50% v/v ethanol (da Silva et al., 2015) (Danai et al. 2021).

The extract was filtered with Whatman filter paper No. 1 in a circular bottom flask and centrifuge at the rotation on at 10000 rpm then concentrated it using vacuum rotatory evaporator and dried using lyophilizer under reduced pressure to obtained solid residue (Reddy et al. 2012). The dried material kept in desiccators for further analysis and in research.

3.1 Preliminary phytochemical screening of the extract

Phytochemical screening of TCHAE in favor of carbohydrates (Benedict's test), protein (Million's reagent test and Ninhydrin reagent test), alkaloid (Maeyer's test and Dragendorff's test), saponins (Foam test), phenolics (ferric chloride test) and flavonoids (Shinoda test and Lead acetate test) was carried out according to standard methods (Tarique et al. 2016) (Trease and Evans 1983).

3.2 Experimental animals

Wistar albino rats weighing 150- 200 g of either sex (8-10 weeks of age) were procured from the Pinnacle Biomedical research institute (PBRI), Bhopal, Madhya Pradesh, India and these were kept in its departmental animal house under standard husbandry conditions (temp 23 ± 2°C, relative humidity 55 ± 10%, and 12-hour light-dark cycle). Animals were fed with standard laboratory food ad libitum during the study period. All experiments were approved by the Institutional Ethical Committee (IAEC) of Pinnacle Biomedical research institute (PBRI), Bhopal, (Reg. No. 1824/PO/Ere/S/15/CPCSEA) with Protocol Approval Reference No.PBRI/IAEC/PN-23194 and following the standard guidelines for the use of laboratory animals.

3.3 Acute toxicity test

In the acute toxicity study, using up and down procedure, hydroalcoholic extract of *Trichosanthes cucumerina* Linn. was administered orally to Wistar rats of either sex selected by random sampling weighing 150–200 g (OECD. 1994). This analysis was conducted according to OECD test guidelines 423. Hydroalcoholic extract of fruits *Trichosanthes cucumerina* Linn. (TCHAE) was given in varying doses from 100 to 4000 mg/kg p.o., dissolved in 1 % carboxy methyl cellulose in rats and continuously monitored for 48 hrs. for behavioral and other indications of toxicity or mortality up to 14 days.

3.4 Experimental design

Animals were randomly divided into six group, Vehicle group/Normal control, Negative control group/Ulcer control, Positive control/Std. drug control groups and three Treatment group TCHAE 125 mg/kg, TCHAE 250 mg/kg & TCHAE 500 mg/kg group which receive

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

TCHAE extract of dose 125, 250, 500 mg/kg body weight (Sakat, Tupe, and Juvekar 2012) respectively by oral route. Each group (vehicle group, negative control group, positive control groups and three treatment group) containing six Wistar albino rats. In our study 1 % carboxy methyl cellulose p.o. use as vehicle and Ranitidine 50 mg/kg body weight by p.o. as standard drug. Following four antiulcer activity models were employed for their research work.(Wang et al. 2018; Kern et al. 2012)

3.5 Model 1: Aspirin plus pylorus ligation induced gastric ulcer in rats

Ulceration in rats will be produced by administration of aspirin (a dose of 200 mg/kg suspended in 1% carboxy methyl cellulose orally) as described by (Ingale, Pinnelli, and Rajendran 2016) (Kommavari et al. 2019) is Negative control, group second. Vehicle group- group first (normal saline + 1 % carboxy methyl cellulose p.o.). Treatment group- group fourth receive Aspirin 200 mg/kg, p.o. + extract of TCHAE 125 mg/kg body weight p.o., group fifth receive Aspirin 200 mg/kg, p.o. + extract of TCHAE 250 mg/kg body weight p.o. and group sixth receive Aspirin 200 mg/kg, p.o. + extract of TCHAE 500 mg/kg body weight orally. Positive control/ Std. drug control - group third receive ranitidine (50 mg/kg suspended in 1% carboxy methyl cellulose) orally 30 min before each aspirin treatment. The rats were fasted (food-withdrawn, water given ad libitium) 36 hours before the pylorus ligation, during which they were housed singly to prevent cannibalism and coprophagy. On the fifth day pylorus part was ligated under light ether anaesthesia. The animals were sacrificed with an over dose of ether after 4 hrs. of pylorus ligation. The stomach was opened and the percentage inhibition of ulcer was determined (Shay et al., 1945).

3.5.1 Estimation of ulcer index

The stomach was opened along with the larger curvature, cleaned with water to remove gastric contents and blood clots, and examined for ulcer formation using a 10 x magnifying lens. As a result, the ulcer index was determined. 0.0 = Normal colored stomach, 0.5 = Red coloration, 1.0 = Spot ulcers, 1.5 = Hemorrhagic streaks, 2.0 = ulcers>3 but < 5, 3.0 = ulcers> 5.

Ulcer index in each group was calculated by the formula: UI = UN + US + UP*10-1

Where, UI = Ulcer Index;

UN = Average of number of ulcer per animal;

US = Average of severity score and

UP = Percentage of animal with ulcer.

Percentage ulcer protection was calculated using following formula: (Abebaw, Mishra, and Gelayee 2017).

$$\text{Percentage Ulcer Protection} = \frac{\text{UI in Control - UI in Test}}{\text{UI in control}} \times 100$$

Where, UI = Ulcer Index;

3.6 Model 2: Ethanol plus HCl induced ulcer

Rats were randomly allotted into six groups of six animals each and fasted for 24 hrs. with free access to water prior to the experiment. Sixty minutes after oral administration of Hydroalcoholic extract TCHAE (125, 250 and 500 mg/kg) and the standard medicine ranitidine 50 mg/kg for treating gastric lesions, all groups were orally treated with 0.2 mL of a solution containing 60% ethanol-0.15 M HCl (ethanol-HCl) for gastric lesion induction with some modification(Sahin et al. 2018). Animals were sacrificed with anaesthesia, 1hrs. after administration of HCl–ethanol mixture and then stomach was excised. Ulcer score and ulcer index was calculated as described above like pylorus method. Percentage ulcer inhibition was calculated for each group on comparison with vehicle control group(Tan et al. 2000).

3.7 Model 3: Acetic acid induced chronic ulcers

Chronic gastric ulcer was induced by serosal application of acetic acid gastric mucosal ulcers, in this model became chronic within 2 – 3 days, and healed completely within 14 – 21 days without perforation or penetration to the surrounding organs (Okabe and Pfeiffer 1972). After an overnight fasting and under anesthesia (ketamine, 100 mg/kg and chlorpromazine, 0.75 mg/kg), the stomach was gently exteriorized through a small incision and The barrel of a 3-ml

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

syringe was placed on the serosal surface of the stomach the glandular portion of stomach 1 cm above from the pyloric end. Acetic acid 80 % v/v, 0.5 ml was emptied on corpus area approximately 6.0 mm^2 for 1 min contact. The area exposed to acid was gently rinsed with saline and the incision was closed. Animal of six group similar surgical procedures were performed, but a saline-containing barrel of a 3-ml syringe was placed on the serosal surface of the stomach of vehicle group/normal control (Kolgazi et al. 2017). TCHAE was given in the dose of 125, 250, 500 mg/kg on day first, orally twice daily, 4 hrs. after application of acetic acid and continued up to 7 to 10 days. On the 10th day and 18 hours after the last injections, the rats were sacrificed after 18 hrs. by with an over dose of ether. The stomach was opened and area (mm^2) and diameter of the ulcers were measured using a ruler. Ulcer index was calculated based upon the lesion diameter (mm/rat) of ulcer. No visible change-0, Hyperemia at sites-1, Lesions having diameter l mm or less-2, Lesions having diameter 2 mm or less -3, Lesions having diameter 3 mm or less -4, Lesions having diameter 4 mm or less -5, and continued. The ulcer index and % inhibition was determined according to method described previously by (Ajayi and Olaleye 2020).

3.8 Model 4: Cold restraint stress (CR Stress) induced ulcer

Rats of either sex weighing 150-200 gm were deprived of food but not water, for about 18 hrs before the experiment. Animals were divided into six groups of six animals each. Group 1- Un-stressed group rats did not receive any stress or treatment. Group 2- Negative control rats did not receive any treatment. Group 3- Positive control rats receive ranitidine (50 mg/kg suspended in 1% carboxy methyl cellulose p.o.). Groups 4, 5, and 6 rats received 125 mg/kg p.o., 250 mg/kg p.o. and 500 mg/kg TCHAE, respectively. Stress was induced in all rats, except the group first, by strapping the fore and hind limbs on a flat wooden plank, and transferred into the refrigerator (4–6^0C) cited by (Ali et al. 2022). The rats were sacrificed after two hours by cervical dislocation and stomach was incised along the greater curvature and the lumen was rinsed with normal saline. Ulcer index and preventive index was calculated as described above like pylorus method. Then, the fundic part of the stomach was homogenized for other analysis like super oxide dismutase (SOD), Catalase (CAT) and Lipid Peroxidation (LPO).

3.9 Gastric Secretion And Gastric Mucosal Studies

The effects of Hydroalcoholic extract of fruits *Trichosanthes cucumerina* Linn. (TCHAE) on biochemical parameters such as gastric volume, pH, free acidity, total acidity, total protein, hexoses, hexosamine, fucose and sialic acid was studied in aspirin plus pylorus ligation model.

3.9.1 Estimation of Gastric volume

Gastric juice collected from each animal was centrifuged at 3000 rpm for 10 min, decanted and squeezed into10 ml measuring cylinder. The amount of gastric juice in the stomach was measured (Abebaw, Mishra, and Gelayee 2017).

3.9.2 Determination of pH

The pH of the supernatant of gastric content was calculated using a pH meter after an of 1 mL gastric juice was diluted with 1 mL of distilled water (Abebaw, Mishra, and Gelayee 2017).

3.9.3 Estimation of total and free acidity

The supernatant of gastric content (1 mL) was mixed with 10 mL of purified water. With the aid of a digital pH meter, the pH of the solution was noted. Using Topfer's reagent as an indicator, the solution was titrated against 0.01 N sodium hydroxide until all signs of red color vanished and the solution color turned yellow. The amount of sodium hydroxide added was noted. The volume of the alkali used, which correspond to the free acidity was noted. Then 2 - 3 drops of phenolphthalein were applied and titration was repeated until a pink hue was obtained. The total added alkali volume was noted once again. The volume was proportional to total acidity (Manchala 2019). Free or total Acidity is determined by using the formula:

$$\text{Acidity} = \frac{\text{Vol of NaOH} \times \text{N of NaOH}}{0.1} \times 100$$

Where, Vol = Volume; N= Normality;

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

3.9.4 Preparation of homogenate

In ice cold (50 mM) phosphate buffer at pH 7.4, 10% stomach tissue homogenate (w/v) was made. Homogenate was extracted and then centrifuged at 4000 rpm for 10 min at 4 ◦C (Danai et al. 2021).

3.9.5 Estimation of total proteins

The standard protocol was used to measure the total protein content (Lowry et al. 1951). Gastric juice (1 mL) was mixed with 9 mL of 95 % ethanol and shaken properly. To extract the precipitate, it was centrifuged at 3000 *g* for 15 min. The collected supernatant was dissolved in 1 mL of 0.1 N NaOH and was added to this 0.9 mL of purified water. In another test tube, 0.4 mL of this solution was added, along with 3 mL of alkaline reagent, and the mixture was allowed to react for 10 min. Then 0.3 mL of phenol reagent was added and allowed to stand in the dark at room temperature for 30 min to produce color. The results were compared to a blank test tube which only contained distilled water. Absorbance against the blank was measured at 610 nm. The amount of protein in the gastric juice was determined using the standard curve made with BSA and measured in terms of µg /mL of gastric fluid.

3.9.6 Estimation of Total Carbohydrates

3.9.6.1Estimation of total hexoses

A mixture of orcinol reagent (3.4 ml) and 0.4 ml of hydrolysate was heated for 15 min. The mixture was then cooled and the intensity of the color was read in a Spectronic-20 absorptiometer set at 540 nm. The total hexoses content was estimated from the standard curve of galactose-mannose and expressed as µg/ml in gastric juice or µg/100 mg gastric mucosa (Satish Narra, Nisha, and Nagesh 2015).

3.9.6.2Estimation of hexosamine

A mixture containing 0.5 ml of the hydrolyzed fraction and 0.5 ml of acetyl acetone reagent was heated for 20 min. A 1.5 ml of 95% alcohol was added to the above mixture after cooling. A 0.5 ml of Ehrlich's reagent was added and set aside for 30 min for completion of the reaction. Color intensity was measured on the Spectronic-20 absorptiometer set at 530 nm. The hexosamine content was expressed as µg/ml in gastric juice or µg/100 mg of gastric mucosa (Dische and Borenfreund 1950).

3.9.6.3Estimation of fucose

To 0.4 ml of the hydrolysate, 1.8 ml of 6:1 H_2 SO_4 was added to the tubes placed in ice-cold water. This mixture was then heated on the boiling water bath for exactly 3 min and then cooled. After 90 min, the absorbance was measured at 396 and 430 nm. True optical density for fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 nm and 430 nm (Dische and Shettles 1948).

3.9.6.4Estimation of sialic acid

Hydrolysate of 0.5 ml obtained in 0.1 N H_2SO_4 was added 0.2 ml of sodium periodate and thoroughly mixed by shaking. A time of 20 minutes allowed elapsing before addition of 1 ml of sodium arsenite solution to the mixture. The brown color was made disappear by shaking. Then 3 ml barbituric acid was added and mixture was heated in boiling water bath for 15 minutes. After taking out and cooling the tubes, 4.3 ml of cyclohexanone was added and a through shaking for 15 minutes was done till all color was taken up by the cyclohexanone supernatant. The mixture was centrifuge to get a clear pink layer of cyclohexanone. This supernatant was peppered out and it color intensity was measured in Colorimeter at 550 nm. The sialic acid content of the sample was found from the standard curve of sialic acid and content expressed as µg/ml in gastric juice or µg/100 mg gastric mucosa (Warren 1959).

3.10 Estimation Of Free Radical Generation

Estimation of super oxide dismutase (SOD), Catalase (CAT) and Lipid Peroxidation (LPO) was studied in aspirin plus pylorus ligation model, ethanol plus HCl induced chronic ulcers, and Cold restraint stress (CR Stress) induced ulcer model.

3.10.1 Determination of super oxide dismutase (SOD)

The SOD activity was assessed using NBT as a substrate according to the standard protocol (Flohe 1984). About 1 mL of the homogenated tissue was placed in the test tube, along with 1 mL of sodium carbonate (50 mM), 1 mL of NBT (24 µM) and 1 mL of EDTA (0.1 mM). To initiate the reaction, 1 mL of 1 mM hydroxyl amine hydrochloride was used. Then the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

reaction mixture was then incubated for 5 min at 25^0C. The blank was prepared without homogenate and the absorbance was recorded at 560 nm. The activity of the enzyme was measured in unit /mg of protein (Danai et al. 2021).

3.10.2 Determination of catalase (CAT)

The standard protocol, with minor modifications, was used to predict catalase (CAT) behavior (Das and Roy 2012). In 5 mL of ice-cold 0.1 M phosphate buffer (pH 7.4) tissue samples were homogenized. The homogenates were then centrifuged for 10 min at 3000 rpm. The precipitate was then mixed with 15 mL of ice-cold 0.1 M phosphate buffer and held cold with continuous shaking. Three times the shaking process was carried out. In 9 mL of H_2O_2, 1 mL of the sample was added. The concentration of H_2O_2 decomposition was quantified spectrophotometrically at 350 nm. The inhibitory effect of CAT was quantified using a percentage inhibition unit.

3.10.3 Determination of lipid peroxide (LPO)

The total amount of lipid peroxidation (LPO) in tissue homogenates was measured using a modified version of the standard method(Ohkawa, Ohishi, and Yagi 1979). In the test tubes, 1 mL of homogenized tissue was added to 1 mL of SDS (8 %), 1 mL of acetic acid (20%), and 1 mL of TBA (0.8 %). In the water bath, the test tubes were held for 1 hrs. The tubes were cooled under running tap water after removing the test tubes from the water bath, and 1 mL of purified water and 5 mL of *n*- butanol and pyridine mixture (15:1, v/v) were applied to each test tube and continuously shaken for 2 min. The test tubes were left undisturbed at room temperature for 10 min. The organic layer absorbance was measured at 532 nm.

3.11 Statistical analysis

Data were analysed as mean ± SEM using one-way analysis of variance (ANOVA) by Dunnet's test using Graph Pad prism software version 10.3.0 where $P < 0.001$, and $P < 0.0001$deemed statistically significant.

4. RESULTS AND DISCUSSION

4.1 Extraction

The hydro-alcoholic extractive value of *Trichosanthes cucumerina* Linn. fruit was found to be 8.16 % w/w. The preliminary phytochemical screening showed the presence of the phytoconstituents carbohydrates, proteins, alkaloids, steroids, flavonoids, saponins and phenolic compounds in hydro-alcoholic *Trichosanthes cucumerina* Linn. fruit.

4.2 General behavior and acute toxicity studies

The graded doses of TCHAE extract from 100 mg/kg up to 4000 mg/kg did not cause any mortality in experimental rat. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behaviour etc. during the period of observation.

4.3 Anti-ulcer study

4.3.1 Effect of TCHAE on Aspirin plus Pylorus ligation induced ulcer

A significant gastroprotective effect was observed in the animals treated with 250 mg/kg and 500 mg/kg *Trichosanthes cucumerina* Linn. The doses of 125 mg/kg, 250 mg/kg and 500 mg/kg showed an ulcer index of 13.08 ± 1.45, 5.82 ± 0.54 and 4.55 ± 0.47 respectively and the percentage protection of TCHAE in ulcer induced by aspirin plus pylorus model was showed ulcer reduction at different doses respectively 125 mg/kg 27.1 %, 250 mg/kg 67.56 % and in 500 mg/kg 74.66 % while standard drug showed 80.24 % (Table1and Fig.1).

Table 1- The Effect of TCHAE extract on ulcer index and percentage protection in Aspirin plus Pylorus ligated rats.

Group No.	Group Name	Treatment	Dose (mg/kg b.w)	Ulcer Index	Percentage Protection
1	Normal control	CMC	1ml/kg		
2	Ulcer control	CMC+Aspirin	200	17.95 ± 2.95	
3	Positive control	Ranitidine	50	3.54 ± 0.38	80.24
4	Sample conc. 1st	TCHAE	125	13.08 ± 1.45	27.10
5	Sample conc. 2nd	TCHAE	250	5.82 ± 0.54***	67.56

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

6	Sample conc. 3rd	TCHAE	500	4.55 ± 0.47***	74.66

The values are presented as mean ± SEM, with n = 6 in each group. Dunnett's test was used to compare TCHAE-treated and control group, *** P< 0.001.

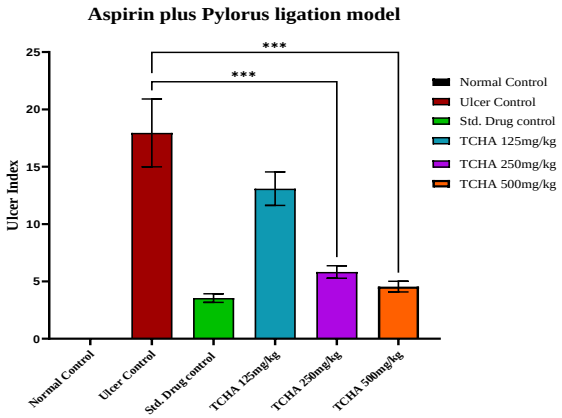

Fig.1- Effect of TCHAE extract on ulcer index in Aspirin plus Pylorus ligated rats.

4.3.2 Effect of TCHAE on Ethanol plus HCl Induced Acute Gastric Lesions

The mixture of ethanol and HCl administration caused severe hemorrhagic lesions that appeared as elongated bands (streaks) parallel to the long axis of the glandular stomach in control rats. Whereas the control group showed a ulcer index of 7.02 ± 0.53, the ulcer index of rats treated with TCHAE at doses of 125, 250 and 300 mg/kg were reduced 5.42 ± 0.38 (not significant), 3.04 ± 0.82 and 2.08 ± 0.38 ($p < 0.0001$), respectively (table 2 and Fig. 2). The positive control, ranitidine (50 mg/kg), also significantly reduced the ulcer index to 2.17 ± 0.33 ($p < 0.0001$).

Table 2- The Effect of TCHAE extract on ulcer index and percentage protection in Ethanol plus HCl Induced rats.

Group No.	Group Name	Treatment	Dose (mg/kg b.wt.)	Ulcer Index	Percentage Protection
1	Normal control	Distilled water	1 ml		
2	Ulcer control	HCl + Ethanol	0.2 ml	7.02± 0.53	
3	Positive control	Ranitidine	50	2.17±0.33****	69.14
4	Sample conc. 1st	TCHAE	125	5.42±0.38	22.85
5	Sample conc. 2nd	TCHAE	250	3.04±0.82****	56.68
6	Sample conc. 3rd	TCHAE	500	2.08±0.38****	70.32

Values are presented as mean ± SEM, with n = 6 in each group. Dunnett's test was used to compare TCHAE-treated and control group, **** P< 0.0001.

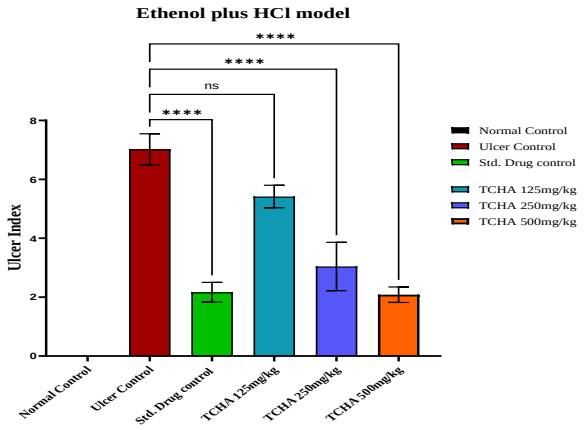

Fig. 2- The Effect of TCHAE extract on ulcer index in Ethanol plus HCl rats.

4.3.3 Healing Effect of TCHAE on Acetic Acid-Induced Chronic Gastric Ulcer

The high dose (500 mg/kg) of TCHAE was selected for the acetic acid gastric ulcer model, as it was more effective than the low dose (125 and 250 mg/kg) in reducing EtOH/HCl-induced gastric lesions. The instillation of acetic acid resulted in chronic gastric ulcer in control rats, whereas the wounds of TCHAE-treated rats were markedly healed. Treatment with TCHAE at 125, 250 and500 mg/kg significantly reduced the gastric lesion area 24.51 ± 0.66, 20.74 ± 1.27 and12.76 ± 1.3 mm2, and gastric ulcer index 40.92 ± 1.99, 28.83 ± 2.14 and 19.36 ± 2.66 compared to the control group gastric lesion area 4.84 ± 0.87 and

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

ulcer index 14.13 ± 1.37, respectively (Table 3 and Fig. 3). Reference drug also significantly reduce gastric lesion area and ulcer index compared to the control rats.

Table 3- The Effect of TCHAE extract on lesion index and percentage protection in Acetic Acid-Induced rats.

Group No.	Group Name	Treatment	Dose (mg/kg b.w)	Gastric lesion area(mm2)	Ulcer Index	Percentage Protection
1	Normal control	Distilled water	1 ml/kg			
2	Ulcer control	Acetic Acid		30.65±1.15	58.37±2.12	
3	Positive control	Ranitidine	50	4.84±0.87***	14.13±1.37***	75.79
4	Sample conc. 1st	TCHAE 125	125	24.51±1.66**	40.92±1.98***	29.89
5	Sample conc. 2nd	TCHAE 250	250	20.74±1.27***	28.83±2.14***	50.60
6	Sample conc. 3rd	TCHAE 500	500	12.76±1.38***	19.36±2.66***	66.83

Values are presented as mean ± SEM, with n = 6 in each group. Dunnett's test was used to compare TCHAE-treated and control group, *** P< 0.001.

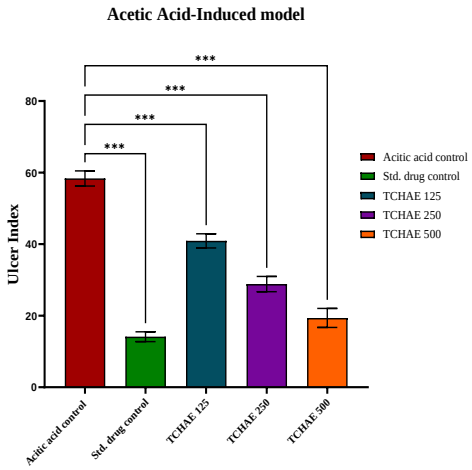

Fig. 3- The Effect of TCHAE extract on ulcer index in Acetic Acid-Induced rats.

4.3.4 Effect of TCHAE on ulcer index and percentage protection in Cold plus Stress-Induced Acute Gastric Lesions

Table 4. and Fig. 4. shows that TCHAE pre-treatment among the ulcerative rats significantly ($p < 0.001$) reduced the ulcer index as compared to the ulcer control group(11.03 ± 0.43) and normal control group. Similarly, the positive control drug treated group (3.58 ± 0.40) also had a significantly ($p < 0.001$) lower the ulcer index compared to the Ulcer control (11.03 ± 0.43). The percentage of inhibition was 67.50 %, 63.28 % and 73.29 % of ranitidine 50 mg/kg, TCHAE 250 mg/kg and TCHAE 500 mg/kg respectively. The TCHAE 500mg/kg shows more percentage inhibition (73.29 %) of ulcer to the std. drug control which exhibited 67.50 % inhibition.

Table 4- Effect of TCHAE extract on ulcer index and percentage protection in Cold plus Stress-Induced rats.

Group No.	Group Name	Treatment	Dose (mg/kg b.w)	Ulcer Index	Percentage Protection
1	Normal control	Distilled water	1ml/kg		
2	Ulcer control	Stress + cold		11.03±0.43	
3	Std. drug control	Ranitidine	50	3.58±0.40***	67.50
4	Sample conc. 1st	TCHAE 125	125	8.01±0.83***	27.36

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

5	Sample conc. 2nd	TCHAE 250	250	4.05±0.39***	63.28
6	Sample conc. 3rd	TCHAE 500	500	2.94±0.24***	73.29

Values are presented as mean ± SEM, with n = 6 in each group. Dunnett's test was used to compare TCHAE-treated and control group, *** P< 0.001.

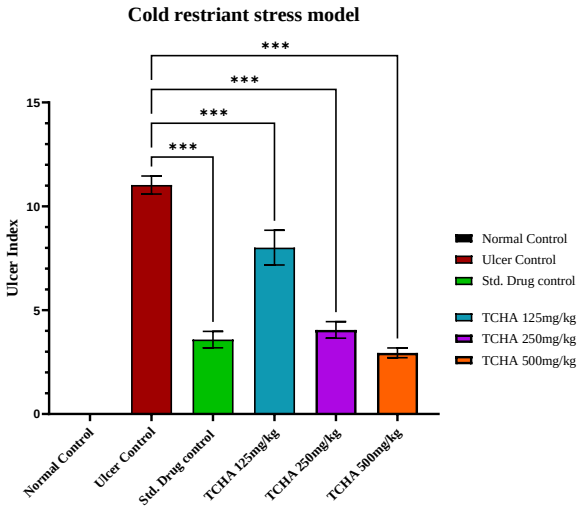

Fig. 4- Effect of TCHAE extract on ulcer index in Cold plus Stress-Induced rats.

4.4 Gastric secretion study

4.4.1 Effect of TCHAE on gastric volume, pH, free acidity, total acidity and acid output

In pylorus-ligated rats, TCHAE treatment affects the gastric secretion parameters, including volume, pH, free acidity, total acidity and acid output of gastric juice shows in Table 5. A significant effect was observed at 250 and 400 mg/kg of TCHAE on acid parameters compared to ulcer control but 125 mg/kg of TCHAE did not show significant effect only on gastric volume and free acidity. Acid volume, total acidity, free acidity and acid output were decreased whereas pH of the gastric juice was increased in animals treated with TCHAE extract.

Table 5- Effect of TCHAE on gastric volume, pH, free acidity, total acidity and acid output in aspirin plus pylorus ligation induced ulcer.

Group Name	Treatment	Gastric Vol. (ml)	Gastric pH	Acidity (µEq/ml)		Acid Output (µEq/4 h)
				Free acidity	Total acidity	
Ulcer Control	CMC+Aspirin 200mg/kg	5.08± 0.10	1.92± 0.05	70.24± 0.65	144.85± 1.08	736.40± 16.44
Std. Drug Control	Ranitidine 50 mg/kg	2.27± 0.04****	5.11± 0.06****	25.53± 0.28****	56.98± 0.28****	129.14± 2.25****
TCHAE 125	TCHAE 125 mg/kg	4.82± 0.18ns	2.77± 0.20***	68.98± 0.81ns	133.57± 1.09****	643.77± 26.18***
TCHAE 250	TCHAE 250 mg/kg	3.58± 0.09****	3.02± 0.13****	65.23± 0.26****	125.48± 0.26****	449.60± 11.45****
TCHAE 500	TCHAE 500 mg/kg	2.83± 0.07****	4.07± 0.07****	41.67± 0.25****	89.43± 0.60****	253.39± 6.63****

Values are presented as mean ± SEM, with n = 6 in each group. Dunnett's test was used to compare ulcer group to TCHAE-treated and std. drug control, **** P< 0.0001, and ns: Non-significant.

4.4.2 Effect of TCHAE on total hexose, hexosamine, fucose, sialic acid and total protein in aspirin plus pylorus ligation induced ulcer

The result shows an increase in total hexoses present in gastric juice of TCHAE treated animals. The concentration of total hexoses at the dose of 125 mg/kg, 250 mg/kg and 500 mg/kg group was found to be 275.17 ± 30.45 µg/ml, 298.83 ± 18.25 and 327.33 ± 12.8 µg/ml which show nonsignificant increase when compared to the ulcer control group (247.43 ± 4.29 µg/ml). The concentration of hexosamine at the dose of

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

125 mg/kg, 250 mg/kg and 500 mg/kg group was found to be 211.33 ± 29.44 µg/ml, 192.67 ± 20.62 µg/ml and 239.35 ± 9.03 µg/ml. It shows a nonsignificant increase in hexosamine content when compared to the ulcer control group (196.33 ± 20.49 µg/ml). The concentration of fucose at the dose of 125 mg/kg, 250 mg/kg and 500 mg/kg group was found to be 145.83 ± 29.15 µg/ml, 147.84 ± 15.99 µg/ml and 188.37 ± 14.46 µg/ml. This shows a nonsignificant increase of fucose content when compared to the ulcer control group (126.76 ± 14.76 µg/ml). The concentration of sialic acid at the dose of 125 mg/kg, 250 mg/kg and 500 mg/kg group was found to be 26.02 ± 2.37 µg/ml, 3.42 ± 1.52 µg/ml and 36.33 ± 1.41 µg/ml. This shows a dose of 250 mg/kg and 500 mg/kg group significant increase of sialic acid content when compared to the ulcer control group (23.72 ± 1.03 µg/ml). Total carbohydrate concentration at the dose of 125 mg/kg, 250 mg/kg and 500 mg/kg group was found to be 658.35 ± 50.60 µg/ml, 669.65 ± 36.10 µg/ml and 791.42 ± 12.84 µg/ml. 500 mg/kg group shows a significant increase of total carbohydrate concentration content when compared to the ulcer control group (601.55 ± 32.76 µg/ml). The concentration of total protein at the dose of 125 mg/kg, 250 mg/kg and 500 mg/kg group was found to be 430.43 ± 29.21 µg/ml, 344.17 ± 16.90 µg/ml and 310.17 ± 19.59 µg/ml. 250 mg/kg and 500 mg/kg group shows the significant increase of protein content when compared to the control group (439.17 ± 27.46 µg/ml). Total carbohydrate and total protein concentration ratio of dose 125 mg/kg, 250 mg/kg and 500 mg/kg TCHAE was found to be 1.55 ± 0.13, 1.93 ± 0.10 and 2.62 ± 0.21. Dose 500 mg/kg TCHAE shows the significant increase of total carbohydrate and total protein concentration ratio when compared to the ulcer control group (1.39 ± 0.10) (Table 6).

Table 6- Effect of TCHAE on gastric secretion total hexose, hexosamine, fucose, sialic acid, total carbohydrate, total protein and TC/TP ratio in 4 hrs. aspirin plus pylorus ligation induced ulcer in rat for 5 day.

Treatment	Mucoprotein (µg/ml)						TC/TP Ratio
	Total hexose	Hexosa-mine	Fucose	Sialic acid	Total carbohydrate	Total proteins	
Ulcer control	254.83± 18.13	196.33± 20.49	126.67± 14.76	23.72± 1.03	601.55± 32.76	439.17± 27.46	1.39± 0.10
Ranitidine 50 mg/kg	350.67± 11.53	223.33± 8.88	140.67± 11.78	37.18± 1.15 $^{\alpha}$	751.85± 23.12 $^{\gamma}$	308.33± 16.11 $^{\beta}$	2.48± 0.19 $^{\alpha}$
TCHAE 125 mg/kg	275.17± 30.45	211.33± 29.44	145.84± 29.15	26.02± 2.37	658.35± 55.60	430.34± 29.21	1.55± 0.13
TCHAE 250 mg/kg	298.83± 18.25	192.67± 20.62	147.83± 15.99	30.42± 1.52 $^{\gamma}$	669.65± 36.10	344.17± 16.90 $^{\gamma}$	1.96± 0.10
TCHAE 500 mg/kg	327.33± 12.08	239.35± 9.03	188.37± 14.46	36.33± 1.41 $^{\alpha}$	791.42± 12.84 $^{\beta}$	310.17± 19.59 $^{\beta}$	2.62± 0.21 $^{\alpha}$

All results are presented as mean ± SEM, with n = 6 in each group. Dunnett's test was used to compare ulcer group to TCHAE-treated and std. drug control (***)$^{\alpha}$, (**)$^{\beta}$, and (*)$^{\gamma}$ P< 0.001, and ns: Non-significant.

4.4.3 Effect of TCHAE on gastric mucosal glycoprotein in aspirin plus pylorus ligation induced ulcer

Oral administration of TCHAE dose 125,250 and 500 mg/kg at three separate doses resulted in substantial increetions in mucosal total hexose, are significant when compared to the ulcer control group (782.34 ± 79.74 µg/100 mg). Hexosamine, fucose and sialic acid, as well as an increase in gastric mucosa, at the TCHAE dose of 125, 250 and 500 mg /kg and standard

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

(ranitidine) 50 mg /kg, in aspirin plus pylorus ligation induced model. The effect on total carbohydrate, total protein and their ration in mucosa are significantly increases when compared to ulcer control, (Table 7) summarizes the all findings.

Table 7- Effect of TCHAE on mucosal total hexose, hexosamine, fucose, sialic acid, total carbohydrate, total protein and TC/TP ratio in 4 hrs. aspirin plus pylorus ligation induced ulcer rats for 5 day.

Treatment	Glycoprotein (µg/100 mg)						TC/TP Ratio
	Total Hexose	Hexosamine	Fucose	Sialic acid	Total Carbohydrate	Total Proteins	
Ulcer control	782.34± 79.74	1597.17± 238.96	175.67± 28.59	95.66± 18.63	2650.83± 263.58	3786.50± 138.81	0.71± 0.09
Ranitidine 50 mg/kg	2068.17± 102.20 $^{\alpha}$	2267.83± 165.87	210.17± 28.10	148.67± 17.80	4694.83± 195.37 $^{\alpha}$	2912.34± 134.33 $^{\alpha}$	1.62± 0.45 $^{\alpha}$
TCHAE 125 mg/kg	1559.83± 101.96 $^{\alpha}$	1950.00± 106.01	182.17± 24.87	106.84± 18.04	3798.84± 120.18 $^{\alpha}$	3632.83± 115.80^{ns}	1.05± 0.39 $^{\alpha}$
TCHAE 250 mg/kg	1882.82± 93.26^{ns}	2100.67± 81.83	192.66± 19.28	141.84± 17.87	4318.00± 80.65^{ns}	3414.17± 90.32^{ns}	1.27± 0.03 $^{\alpha}$
TCHAE 500 mg/kg	2019.34± 72.94 $^{\alpha}$	2215.67± 73.29	208.17± 19.08	156.67± 15.22	4599.83± 94.39 $^{\alpha}$	3078.83± 68.48 $^{\alpha}$	1.50± 0.03 $^{\alpha}$

All results are presented as mean ± SEM, with n = 6 in each group. Dunnett's test was used to compare ulcer group to TCHAE-treated and std. drug control (***)$^{\alpha}$, (**)$^{\beta}$, and (*)$^{\gamma}$ P< 0.001, and ns: Non-significant.

4.5 Antioxidant study

4.5.1 Effect on lipid peroxidation (LPO), Superoxide dismutase (SOD) and Catalase (CAT) activities in aspirin plus pylorus ligation induced ulcer.

The level of tissue lipid peroxidation was found to be increased significantly (Table 8) in the case of ulcer control group (245.67 ± 3.12) than that of normal group (123.67 ± 3.39). Ranitidine (50 mg /kg) showed the decrease of lipid peroxidation (160.17 ± 2.82) than the ulcer control group (table 8). TCHAE dose of 125, 250 and 500 mg/kg treatment also significantly decreased the level of LPO (208.67 ± 3.37), (174.83 ± 2.54) and (142.16 ± 2.52) respectively compare with ulcer control (Table 8 and Fig 5). The antioxidant enzyme activity of SOD was significantly lowered in the ulcer control group (53.83 ± 2.70) with respect to normal group (123.83 ± 2.90) as shown in Table 8. Ranitidine (50 mg /kg) showed effective antioxidant activity (91.67 ± 2.32) significantly increase in the SOD level compare to ulcer control group. The significant increase in activity was also seen in TCHAE 125 mg /kg (71.17 ± 2.59), TCHAE 250 mg/kg (81.50 ± 2.57) and TCHAE 500 mg /kg (100.50 ± 2.36) doses for SOD as compared to ulcer control group (Table 8, Fig 6). The CAT level shows significantly higher in normal control (33.17 ± 2.10) then the ulcer control (15.67 ± 1.99). Ranitidine 50 mg/kg (29.47 ± 1.70) also shows significant increase in the CAT level with respect to the control group as shown in Table 8 and Fig. 7. The significant increase in CAT was also observed in the TCHAE 500 mg /kg (26.83 ± 1.86) and non-significantly increase at doses 125 mg/kg, 250 mg/kg respectively (19.33 ± 2.09) and (21.5 ± 1.75) compare with ulcer control (Table 8, Fig 7).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

Table8 - Effect of TCHAE on lipid peroxidation (LPO), Catalase (CAT) and Superoxide dismutase (SOD) activities in aspirin plus pylorus ligation induced ulcer

Treatment (mg/kg b.w.)	**LPO (MDA, nmol/g tissue)**	**SOD (units/g tissue)**	**CAT (units/g tissue)**
Normal Control	123.67±3.93	123.83±2.90	33.17±2.10
Ulcerated Control	245.67±3.12$^{\alpha}$	53.83±2.70$^{\alpha}$	15.67±1.99$^{\alpha}$
Ranitidine 50 mg/kg	160.17±2.82***	91.67±2.32***	29.47±1.70***
TCHAE 125 mg/kg	208.67±3.37***	71.17±2.59***	19.33±2.09 ^{ns}
TCHAE 250 mg/kg	174.83±2.54***	81.5±2.57***	21.5±1.75 ^{ns}
TCHAE 500 mg/kg	142.16±2.52***	100.50±2.36***	26.83±1.86**

Values are represented as mean ±SEM, (n = 6) in each group. Control group was compared with normal group $^{\alpha}$P< 0.001; Drug treated groups were compared with control group using Dunnett's test, ***P< 0.001.

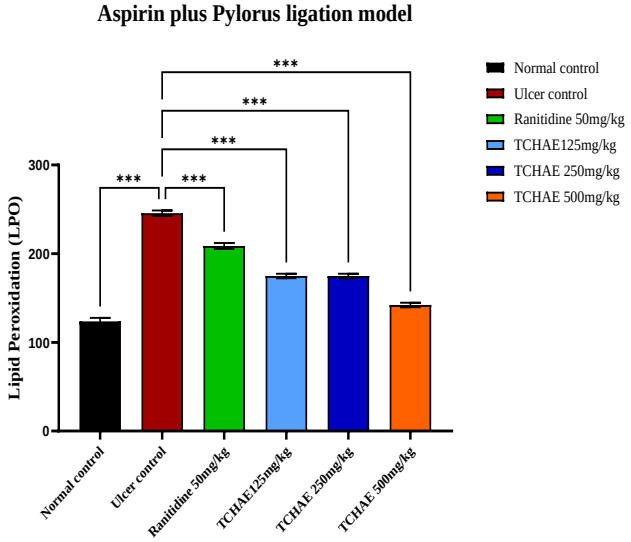

Fig. 5- Level of lipid peroxidation (LPO) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in aspirin plus pylorus ligation induced gastric ulcers. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and TCHAE treated groups were compared with ulcer control group.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

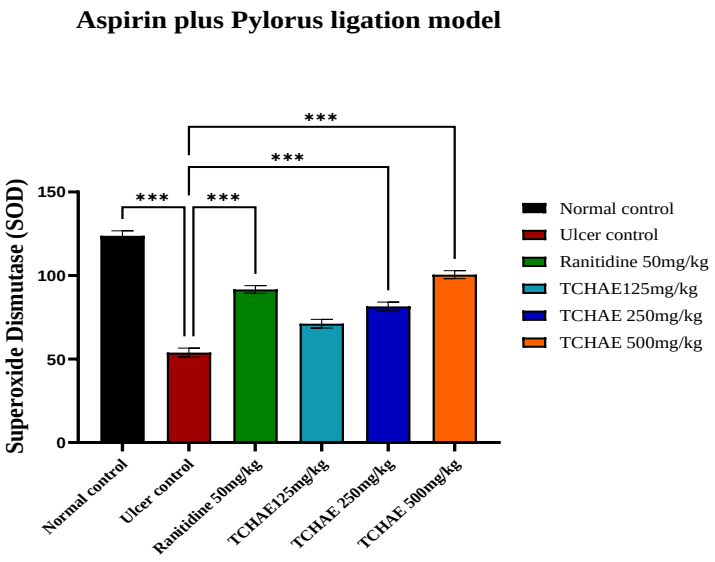

Fig. 6- Level of Superoxide Dismutase (SOD) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in aspirin plus pylorus ligation induced gastric ulcers. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and TCHAE treated groups were compared with ulcer control group.

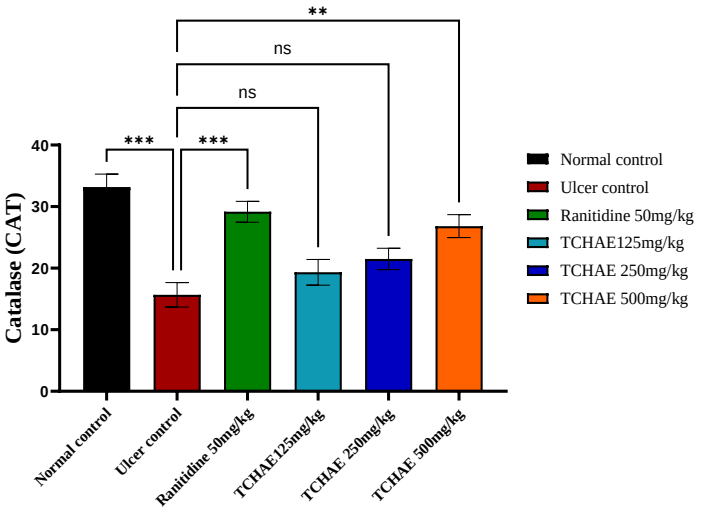

Fig. 7- Level of Catalase (CAT) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in aspirin plus pylorus ligation induced gastric ulcers. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and TCHAE treated groups were compared with ulcer control group.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

4.5.2 Effect on level of lipid peroxidation (LPO), Catalase (CAT) and Superoxide dismutase (SOD) activities in ethanol plus HCl induced ulcer.

Administration of ethanol and HCl in the ulcer control showed an increased level of LPO by 18.67 ± 0.61 and a decreased level of SOD (0.89 ± 0.03) and CAT (14.47 ± 1.02), when compared with the normal control group LPO (6.84 ± 0.60), SOD (3.37 ± 0.27) and CAT (36.83 ± 0.95) respectively significant (Fig 8,9,10). Pre-treatment with TCHAE graded doses and standard ranitidine significantly maintains the antioxidant enzyme activities leading to a decrease in the LPO levels and increase SOD & CAT showed in Table 9 as well as in Fig 8,9,10.

Table 9- Effect of TCHAE on antioxidant parameters in ethanol plus HCl induced ulcer rat for 5 day.

Treatment (mg/kg b.w.)	LPO (MDA, nmol/g tissue)	SOD (units/g tissue)	CAT (units/g tissue)
Normal Control	6.84±0.60	3.37±0.27	36.83±0.95
Ulcerated Control	18.67±0.61$^{\alpha}$	0.89±0.03$^{\alpha}$	14.47±1.02$^{\alpha}$
Ranitidine 50 mg/kg	9.17±0.70***	3.83±0.69***	37.42±0.69***
TCHAE 125 mg/kg	14.67±0.84**	1.55±0.22 ^{ns}	25.84±0.95***
TCHAE 250 mg/kg	9.33±0.80***	2.63±0.26***	31.77±0.60***
TCHAE 500 mg/kg	5.87±0.70***	4.29±0.24***	38.14±0.74***

Values are represented as mean ±SEM, (n = 6) in each group. Control group was compared with normal group $^{\alpha}$P< 0.001; Drug treated groups were compared with control group using Dunnett's test, ***P< 0.001 and ns: Non-significant.

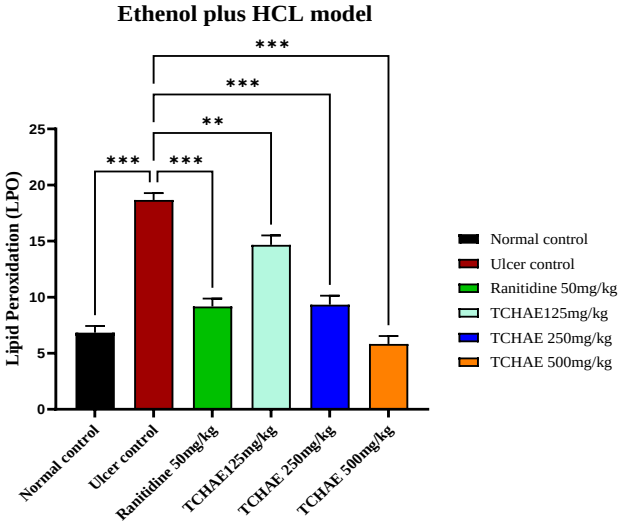

Fig. 8- Level of lipid peroxidation (LPO) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in EtOH plus HCl induced gastric ulcers. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and TCHAE treated groups were compared with ulcer control group.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

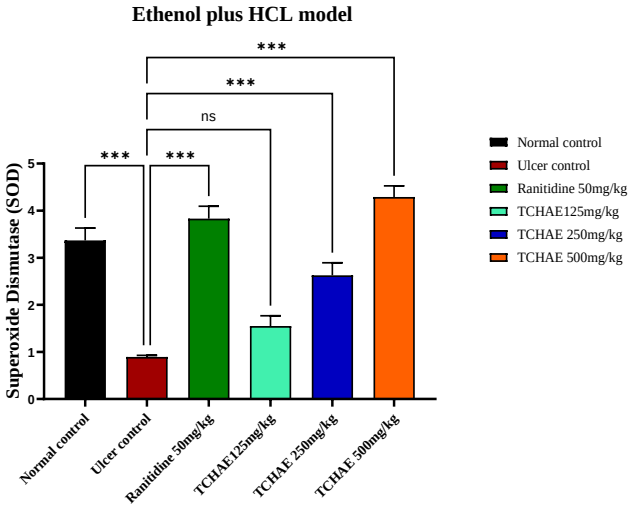

Fig. 9- Quantification of Superoxide Dismutase (SOD) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in EtOH plus HCl induced gastric ulcers. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and Drug treated groups were compared with ulcer control group.

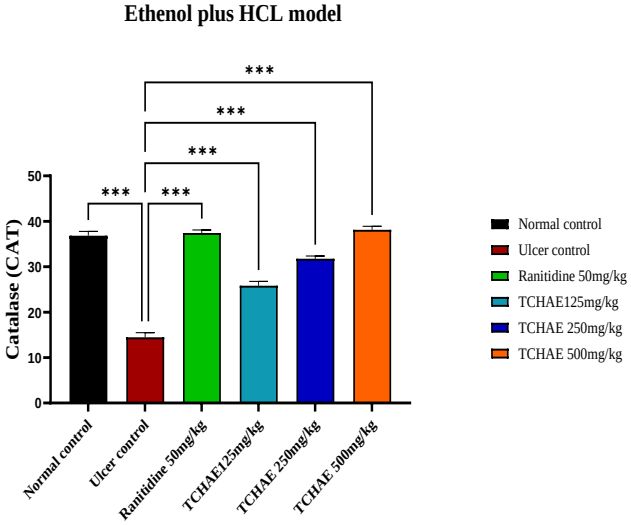

Fig. 10- Level of Catalase (CAT) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in EtOH plus HCl induced gastric ulcers. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and Drug treated groups were compared with ulcer control group.

4.5.3 Effect on level of lipid peroxidation (LPO), Superoxide dismutase (SOD) and Catalase (CAT) activities in cold resistant stress induced ulcer.

Administration of ethanol and HCl in the ulcer control showed an increased level of LPO by 0.59 ± 0.03 and a decreased level of SOD (81.82 ± 5.36) and CAT (25.83 ± 0.94), when compared with the normal control LPO (0.37 ± 0.12), SOD (109.34 ± 5.74) and CAT (36.83 ± 0.94) respectively significant (Fig 11, 12, 13). Pre-treatment with TCHAE graded doses (125 mg/kg, 250 mg/kg and 500 mg/kg) and standard ranitidine (50 mg/kg) significantly maintains the antioxidant enzyme

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

activities leading to a decrease in the lipid peroxidation (LPO) levels and increase superoxide dismutase (SOD) & catalase (CAT) [except 125 mg/kg dose of TCHAE only in CAT] showed in Table 10 as well as in Fig 11, 12,13.

Table10- Effect of TCHAE on lipid peroxidation (LPO), catalase (CAT) and superoxide dismutase (SOD) activities in cold resistant stress induced rat for 7 days.

Treatment (mg/kg b.w.)	**LPO (MDA, nmol/g tissue)**	**SOD (units/g tissue)**	**CAT (units/g tissue)**
Normal Control	0.37±0.12	109.34±5.74	36.83±0.94
Ulcer Control	0.59±0.03$^{\alpha}$	81.82±5.36$^{\beta}$	25.83±0.94$^{\beta}$
Ranitidine 50mg/kg	0.40±0.01 ***	164.03±3.85 ***	37.90±1.50 ***
TCHAE 125 mg/kg	0.36±0.02 ***	119.11±7.91 ***	30.57±2.01 ^{ns}
TCHAE 250 mg/kg	0.26±0.01 ***	166.82±4.49 ***	34.49±2.83 *
TCHAE 500 mg/kg	0.25±0.22 ***	152.83±4.36 ***	40.55±2.71 ***

Values are represented as mean ±SEM, (n = 6) in each group. Control group was compared with normal group $^{\alpha}$P< 0.001, $^{\beta}$P< 0.002; Drug treated groups were compared with control group using Dunnett's test, ***P< 0.001 and ns: Non-significant.

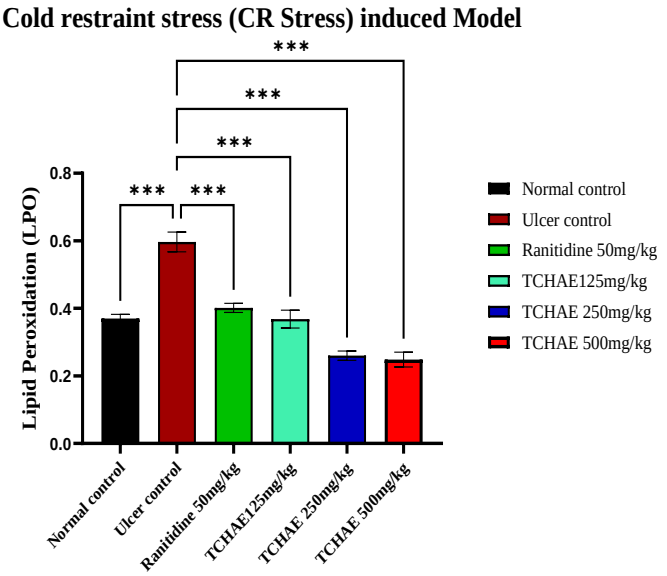

Fig. 11- Level of lipid peroxidation (LPO) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in Cold restraint stress (CR Stress) compared with ulcer control group.induced ulcer Model. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and TCHAE treated groups were compared with ulcer control group.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

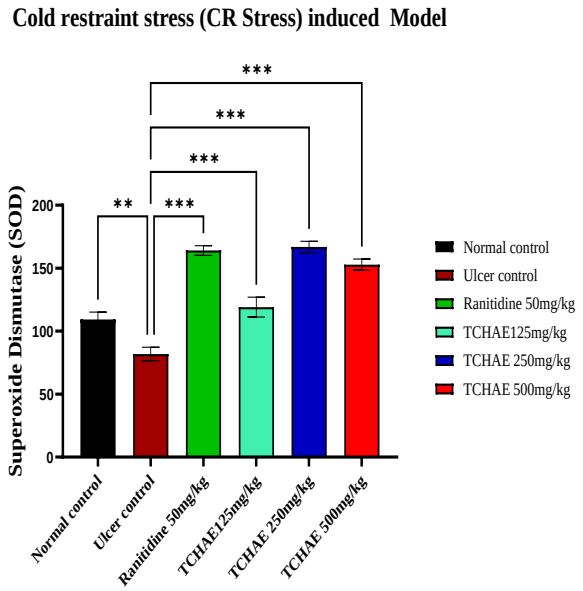

Fig. 12- Level of Superoxide Dismutase (SOD) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in Cold restraint stress (CR Stress) induced ulcer Model. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and TCHAE treated groups were compared with ulcer control group.

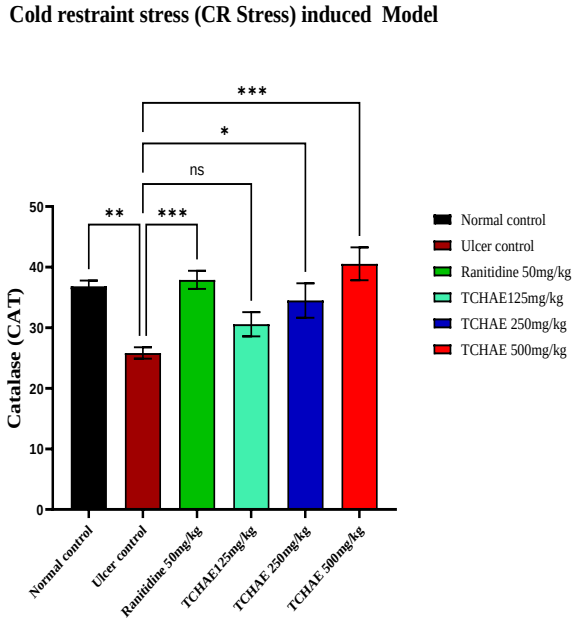

Fig. 13- Level of Catalase (CAT) in gastric mucosa of rats treated with TCHAE (125, 250, 500 mg/kg, p.o.), ranitidine (50 mg/kg) in Cold restraint stress (CR Stress) induced ulcer Model. Statistical comparison was analyzed by a one-way ANOVA followed by Dunnett's multiple comparison tests. P< 0.001, statistically significant as ulcer control group was compared with normal group and TCHAE treated groups were compared with ulcer control group.

5. CONCLUSION

Ulcer formation is currently viewed as an interactive process resulting from an imbalance of aggressive gastric juice and defensive mucosal factors, causing a break in the line of gastrointestinal mucosa. The hydro-alcoholic extract of fruits was studied in different gastric ulcer model in rats at a dose of 125, 250, and 500 mg/kg body weight p.o. for 5 days Aspirin plus pylorus ligation induced gastric ulcer, 5 days HCl plus ethanol induced ulcer, 10 days for acetic acid induced gastric ulcer and 7 days cold restraint stress induced ulcer, twice daily. Results of this study provided preliminary data for the hydroalcohalic extract of *Trichosanthes cucumerina* fruits may possess significant dose dependent reduction in ulcer score and gastric secretion also involve damage by reactive oxygen species apart from acid and pepsin related factor. Increased level of LPO is due to increase in generation of reactive species during ulcer leading to oxidative damage. SOD converts the reactive super oxide radicals to H_2O_2, which if not scavenge by CAT can by itself cause lipid peroxidation by generation of hydroxyl radicals. Hence decrease in CAT levels has lead to increase in accumulation of these reactive species and thus has caused increased lipid per oxidation and tissue damage TCHAE significantly reverses these oxidative changes in ulcer. These observed effects of TCHAE may be linked with its antioxidant effect due to the presence of bioactive compounds like flavonoids, saponins, phenol and tannins in it, which is attributed to its defensive property, namely, strengthening the mucosal defensive barrier and also by acting as potent antioxidants.

Thus the present study confirms the use of TCHAE fruit in the traditional management of peptic ulcer disease.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1259-1278 | ISSN:2251-6727

REFRENCES

Abebaw, Mastewal, Bharat Mishra, and Dessalegn Asmelashe Gelayee. 2017. 'Evaluation of anti-ulcer activity of the leaf extract of Osyris quadripartita Decne.(Santalaceae) in rats', *Journal of experimental pharmacology*: 1-11.

Ajayi, Ayodeji F, and Babafemi S Olaleye. 2020. 'Age-related changes in haematological parameters and biochemical markers of healing in the stomach of rats with acetic acid induced injury', *Toxicology Reports*, 7: 1272-81.

Ali, Kholoud A, M Mona, Alaa F Bakr, Mohamed Y Mahmoud, Essam M Abdelgawad, and Mohammed Y Matoock. 2022. 'The dual gastro-and neuroprotective effects of curcumin loaded chitosan nanoparticles against cold restraint stress in rats', *Biomedicine & Pharmacotherapy*, 148: 112778.

Danai, Priyanka, Saraswati Patel, Varsha Pandey, Purendra Singh, Govind Yadav, Amit Kumar, and Teena Agarwal. 2021. 'Antiulcerogenic activity of Anogeissus pendula hydroalcoholic extract on pylorus ligated induced gastric ulcers in Albino Wistar rats', *Phytomedicine Plus*, 1: 100127.

Das, Shyamal K, and Chandan Roy. 2012. 'The protective role of Aegle marmelos on aspirin–induced gastro-duodenal ulceration in albino rat model: a possible involvement of antioxidants', *Saudi Journal of Gastroenterology*, 18: 188-94.

Dische, Zacharias, and Ellen Borenfreund. 1950. 'A spectrophotometric method for the microdetermination of hexosamines', *Journal of Biological Chemistry*, 184: 517-22.

Dische, Zacharias, and Landrum B Shettles. 1948. 'A specific color reaction of methylpentoses and a spectrophotometric micromethod for their determination', *Journal of Biological Chemistry*, 175: 595-603.

Fass, Ronnie. 2007. 'Symptom assessment tools for gastroesophageal reflux disease (GERD) treatment', *Journal of clinical gastroenterology*, 41: 437-44.

Flohe, L. 1984. '[10] Superoxide dismutase assays.' in, *Methods in enzymology* (Elsevier).

Ingale, Anand M, Venkata Bharat Kumar Pinnelli, and Vijaya Rajendran. 2016. 'Experimental evaluation of the anti-ulcer activity of the ethanolic extract of grape (Vitis vinifera) seed in wistar albino rats against aspirin plus pylorus ligation induced gastric ulcer model', *Int J Basic Clin Pharmacol*, 5: 722-7.

Kern, Matthias, Nora Klöting, Heiko G Niessen, Leo Thomas, Detlef Stiller, Michael Mark, Thomas Klein, and Matthias Blüher. 2012. 'Linagliptin improves insulin sensitivity and hepatic steatosis in diet-induced obesity', *Plos one*, 7: e38744.

Kolgazi, MELTEM, ZN Ozdemir-Kumral, C Cantali-Ozturk, EK Demirci, M Yuksel, SERAP Sirvanci, and BC Yegen. 2017. 'Anti-inflammatory effects of nesfatin-1 on acetic acid-induced gastric ulcer in rats: involvement of cyclo-oxygenase pathway', *J Physiol Pharmacol*, 68: 765-77.

Kommavari, Chandra Sekhar, Nagaraju Bandaru, Manish Shaik, and Prasad Konduri. 2019. 'Anti-ulcer activity of methanolic extract of Polycarpea aurea in asprin plus pylorus ligation-induced ulcer model in rats', *Journal of Pharmaceutical Sciences and Research*, 11: 2689-93.

Kong, Fanbin, and R Paul Singh. 2009. 'Modes of disintegration of solid foods in simulated gastric environment', *Food biophysics*, 4: 180-90.

Kumar, Anurag. 2018. 'An experimental evaluation of gastro protective effect of trichosanthes cucumerina against chronic ulcers in rats', *european journal of Pharmaceutical and medical research*: 384-90.

Lowry, Oliver H, Nira J Rosebrough, A Lewis Farr, and Rose J Randall. 1951. 'Protein measurement with the Folin phenol reagent', *J biol Chem*, 193: 265-75.

Manchala, P. 2019. 'Evaluation of anti-ulcer activity of Lagenaria siceraria chloroform extracts in pylorus ligated rats', *Electron J Biol*, 15: 27-37.

Mehmood, Arshad, M Akram, Afzal Ahmed, Khan Usmanghani, Abdul Hannan, E Mohiuddin, and M Asif. 2010. 'Helicobacter pylori: An introduction'.

OECD. 1994. *OECD Guidelines for the Testing of Chemicals* (Oecd).

Ohkawa, Hiroshi, Nobuko Ohishi, and Kunio Yagi. 1979. 'Assay for lipid peroxides in animal tissues by

thiobarbituric acid reaction', *Analytical biochemistry*, 95: 351-58.

Okabe, Susumu, and Carl J Pfeiffer. 1972. 'Chronicity of acetic acid ulcer in the rat stomach', *The American journal of digestive diseases*, 17: 619-29.

Okonwu, K, and JE Muonekwu. 2019. 'Potentials of underexploited seed of Trichosanthes cucumerina Linn', *Journal of Applied Sciences and Environmental Management*, 23: 791-97.

Palle, Suresh, A Kanakalatha, and Ch N Kavitha. 2018. 'Gastroprotective and antiulcer effects of Celastrus paniculatus seed oil against several gastric ulcer models in rats', *Journal of dietary supplements*, 15: 373-85.

Reddy, V Prasanth, G Sudheshna, SK Afsar, S Saran, S NELSON Kumar, C Raja Ram, and K Ravindra Reddy. 2012. 'Evaluation of anti-ulcer activity of Citrullus colocynthis fruit against pylorus ligation induced ulcers in male wistar rats', *Int J Pharm Pharm Sci*, 4: 446-51.

Sahin, Hasan Huseyin, Alev Cumbul, Unal Uslu, Zeynep Yilmaz, Feriha Ercan, and Inci Alican. 2018. 'The effect of 1, 25 dihydroxyvitamin D3 on HCl/Ethanol-induced gastric injury in rats', *Tissue and Cell*, 51: 68-76.

Sakat, SS, Preeti Tupe, and Archana Juvekar. 2012. 'Gastroprotective effect of Oxalis corniculata (whole plant) on experimentally induced gastric ulceration in Wistar rats', *Indian journal of pharmaceutical sciences*, 74: 48.

Salama, Suzy M, Nura Suleiman Gwaram, Ahmed S AlRashdi, Shaden AM Khalifa, Mahmood A Abdulla, Hapipah M Ali, and Hesham R El-Seedi. 2016. 'A zinc morpholine complex prevents HCl/ethanol-induced gastric ulcers in a rat model', *Scientific Reports*, 6: 29646.

Satish Narra, Satish Narra, KS Nisha, and HS Nagesh. 2015. 'Evaluation of antiulcer activity of hydroalcoholic fruit pulp extract of Cucumis sativus'.

Schubert, Mitchell L, and David A Peura. 2008. 'Control of gastric acid secretion in health and disease', *Gastroenterology*, 134: 1842-60.

Tan, Paul V, Veronique B Penlap, Barthelemy Nyasse, and Joseph DB Nguemo. 2000. 'Anti-ulcer actions of the bark methanol extract of Voacanga africana in different experimental ulcer models in rats', *Journal of Ethnopharmacology*, 73: 423-28.

Tarique, Mohd, Hefazat H Siddiqui, Mohd Khushtar, and Md Azizur Rahman. 2016. 'Protective effect of hydro-alcoholic extract of Ruta graveolens Linn. leaves on indomethacin and pylorus ligation-induced gastric ulcer in rats', *Journal of Ayurveda and integrative medicine*, 7: 38-43.

Thapa, K, S Ghatane, and Surya P Rimal. 2009. 'Health problems among the street children of Dharan municipality', *Kathmandu University Medical Journal*, 7: 272-79.

Trease, George Edward, and William Charles Evans. 1983. *Pharmacognosy*.

Wang, Xiao-Yin, Jun-Yi Yin, Ming-Ming Zhao, Shi-Yu Liu, Shao-Ping Nie, and Ming-Yong Xie. 2018. 'Gastroprotective activity of polysaccharide from Hericium erinaceus against ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer, and its antioxidant activities', *Carbohydrate Polymers*, 186: 100-09.

Warren, Leonard. 1959. 'The thiobarbituric acid assay of sialic acids', *J biol Chem*, 234: 1971-75.