STUDY OF ANTI-ULCER ACTIVITY OF NUTRACEUTICALFORMULATION NC20 IN SPRAGUE DAWLEY RATS.

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Abstract

Anti-ulcer medicines, also known as antisecretory agents, are medications that reduce the release of gastric acid in the stomach. Anti-ulcer medicines are now used to treat a variety of gastrointestinal illnesses. Currently, the goal of peptic ulcer treatment is to relieve pain and prevent ulcer recurrence using nutraceutical formulations with anti-ulcerproperties. The formulation was evaluated using an ethanol-induced ulcer with characteristics of mucosal prostaglandin inhibition. Acid secretion regulation. At 200mg/kg and 400mg/kg body weight doses. The results revealthat the action is statistically significant and dose dependent. The results, however, were in comparison to the normalOmeprazole medicine (20mg/kg, p.o.). The formulation will be evaluated further in future investigations.

Key words: Evaluation, anti-ulcer activity, nutraceutical formulation, ethanol-induced ulcer.

1) Introduction

A peptic ulcer is a sore or hole in the stomach or duodenum lining, and it is a phrase used to describe a lesion in the esophagus, stomach, or duodenum (7). A more precise word is used to characterize an ulcer that is positioned in a specific location. Peptic ulcers in the duodenum (the first part of the small intestine) are more common than other varieties (7). Peptic ulcers are caused by excessive hydrochloric acid and pepsin production,which erodes the GI mucosal lining.

The most common cause of peptic ulceration is an imbalance between the rate of gastric juice secretion and the degree of protection provided by the gastroduodenal mucosal barrier and gastric acid neutralization by duodenalfluids. All areas normally exposed to gastric juice are well supplied with mucous glands, beginning with compound mucous glands in the lower esophagus plus the mucous cell coating of the stomach mucosa, the mucous neck cells of the gastric glands, the deep pyloric glands, which secrete primarily mucus, and, finally, the Brunner glands of the upper duodenum, which secrete a highly alkaline mucus. The duodenum is protected by alkalinity in addition to mucus protection of the mucosa (7). Pancreatic output is especially significant because it contains substantial amounts of sodium bicarbonate, which neutralizes the hydrochloric acid in gastric juice, inactivating pepsin and inhibiting mucosal digestion. Furthermore, considerable levels of bicarbonate ions are present in the secretions of the big Brunner's glands in the first few centimeters of the duodenum, as well as in bile from the liver.

Causes include: high acid and peptic content, irritation, poor blood supply, and mucus secretion. Infection, Helicobacter Pylori (9).

Peptic ulcers are remitting, relapsing lesions that are most commonly identified in middle-aged to older adults, but they can appear as early as adolescence. They frequently arise without evident triggering factors and may recover after weeks to months of active disease. Even after healing, the proclivity to form peptic ulcers continues. As a result, obtaining accurate data on the prevalence of active disease is difficult. According to best estimates, approximately 2.5% of males and 1.5% of females in the United States suffer peptic ulcers. In the United States,the lifetime risk of getting peptic ulcer disease is roughly 10% for both men and women.Animal models of stomach ulcers caused by alcohol are frequently used to study potential novel ulcer medications. The administration of ethanol lowers the release of bicarbonate, gastric mucus, and nitric oxide while also causing stomach necrotic damage and subsequent inflammatory cell infiltration. Additionally, ethanol decreases stomach blood flow and causes oxidative stress by lowering glutathione production and increasing malondialdehyde formation.

Nutraceuticals:

Any item that is a food or a component of a food and offers medical or health advantages, including the prevention and treatment of disease, is referred to as a nutraceutical. Health-related necessities including nutrition and pharmaceuticals used to treat illnesses or injuries. Nutraceuticals, a type of preventive medicine, are a combination of pharmaceuticals and nutrition. The majority of food items include nutraceuticals in variable amounts. Maintaining a healthy body weight, regular exercise, stress management, and a diet high in nutraceuticals will improve health and lower illness risk (8).

The role of nutrients in ulcer disease:

The most prevalent gastrointestinal condition in the world, peptic ulcer, can have harmful side effects such liver and kidney damage when treated for a long time. Since they are naturally occurring substances with little adverse effects, nutrients play a safer and better function in treating peptic ulcer disease. Additionally, it offers the additional benefits of vitamins, flavonoids, and antioxidants on peptic ulcers to maintain acid management. (10)

2) Methodology: -

Procedure: The NF02 nutraceutical formulation makes use of the flowing processes.

Purchasing high-quality raw materials:
Getting safe and high-quality raw materials is the most crucial step in obtaining good items when sourcing raw materials.

Disturbance:
Technical techniques are used to separate products from contaminants or other products in the raw material supply chain. Dust can be separated from recyclable materials as part of separation. removal of any foreign objects that may be present.

Weighing:
Weighing Think on each component separately. Transfer to appropriate containers, such as plastic, steel, aluminium, or pin-go ones.

Soaking/Dipping:
This technique involves moistening and softening the seeds to make them easier to remove. To start the germinal process in the nucleus, water must be consumed. By adding a precise amount of solvent 5 to the water, soak the materials. midnight to 24 hours.

Drying
Drying is a mass transfer procedure that entails the evaporation of water. Using a filter, such as a steel strainer, meat cloth, or coconut cloth, drain the solvent.

Spreading:
To dry or air dry the material, spread it out evenly over a large cloth. Every 3-6 hours, turn the material over and let it dry. Regularly check the humidity. Collect all items in suitable containers once they have dried completely.
The materials should be heated to the proper temperature. Weigh the material before adding it to a heating device with a low flame. Place in an appropriate container and allow to cool.

Grinding or pulverizing

Weigh the stuff, then grind it until it resembles coarse powder. We then sift them collectively, gather, and weigh the unprocessed powder. then place them in an airtight container.

Phytochemical Evaluation:

Using a variety of established phytochemical techniques, including the alkaline reagent test, lead acetate test, molish's test, Benedict's test, xanthoproteic test, and ninhydrin test, the NF02 formulation was examined for the presence of phytoconstituents such as antioxidants, flavonoids, vitamins, carbohydrates,proteins, alkaloids, tannins, and glycosides.

3) Experimental work:

- **Experimental animal**

From VAB BIOSCIENCES in Hyderabad, we got adult albino SD rats weighing 200–300gm. At Teena Biolabs Pvt. Ltd. in Hyderabad, they were housed in polypropylene cages. ad libitum access to regular rat food and water while living in a temperature-controlled environment (25 2 °C) with 50–70% relative humidity and 12-hour light–dark cycles. They spent a week getting used to the lab environment before being randomly assigned to one of four experimental groups. The Committee for the Control and Supervision of Experiments on Animals' criteria were followed in all experimental methods. The Committee for the Control and Supervision of Experiments on Animals (CPCSEA) (registration number: 177/PO/RcBi/2000/CPCSEA) criteria were followed in all experimental procedure.

- **Acute toxicity studies**

According to OECD-423 recommendations, a study on acute oral toxicity was conducted. Four groups of four rats each were created at random (n=4). Prior to the dose, animals were fasted (water but not food was withheld overnight). Each animal's fasting body weight was identified, and the dose was computed based on body weight. The oral doses of the NC20 nutraceutical formulation were 5, 50, 300, and 2000 mg/kg body weight. For the first four hours, the ani0mals were regularly monitored for any unfavorable signs such constipation, pain, diarrhea, nausea, and vomiting.

- **Selection of dose**

Based on investigations of acute toxicity, extracts were determined to be safe at dosages of 200 mg and 400 mg per kilogram of body weight, respectively, in accordance with OECD 423 standards (HAEAL).

- **ethanol induced peptic ulceration**

Animals were given ethanol dissolved in normal saline orally to develop peptic ulcer disease.

- **Experimental protocol**

Twenty SD rats were used in this investigation, and they were separated into five groups, each with four rats. The therapy lasted for 28 days. The following is how the protocol was created.

Table3:1 Treatment schedule

S. no	GROUP	TREATMENT	PURPOSE
1	Normal	Standard chew of pellets and drinking water *ad libitum.*	To research the typical rat liver physiology
2	Control	1ml of 96%ethanol controlled to cause a stomach ulcer	Both to research the preventive effects of the test compounds under study and to serve as disease control.
3	Test:200 mg/kg	200mg/kg of NC20was administered every day on day one	To understand the nutraceutical formulation's ability to treat peptic ulcers brought on by ethanol.

4	Test:(400 mg/kg)	400mg/kg of NC20was administered every day on day one	To understand the nutraceutical formulation's ability to treat peptic ulcers brought on by ethanol.
5	Standard: (Omepra zole)	From the first day omeprazole (20mg/kg b.wt) was administered	To assess NF02s nutraceutical formulation's ability to prevent ethanol-induced peptic ulcers.

4) Results: -

Evaluation studies: -
1.Bulk density: - 0.466 gm/ml
2.Tapped density: - 0.496 gm/ml
3.Carr's consolidation index: - 29.7
4.Porosity: - 54.3%
According to the porosity value it will show high dissolution rate.
And carr's index says about that the powder will have poor and passable flow property.

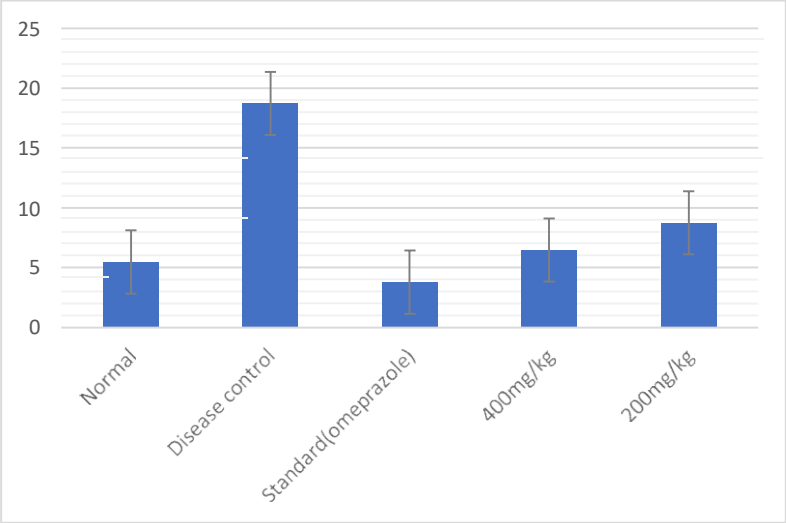
Table 4.1 Effect of Nutraceutical formulation on ethanol induced ulcers

Groups	Ulcer Index	% Protection
Group 1 – normal	5.46±0.18	--
Group II- Control	18.71±0.10	--
Group III - Standard (Omeprazole)	3.79±0.07***	79.7 %
Group IV – Test (High dose) 400mg/kg	4.67±0.08**	65.41%
Group V – test (low dose) 200mg/kg	7.74±0.08**	53.28 %

All values represent Mean ± SEM, n=4 in each group. ***p<0.001, **p<0.01, Control group (Group II) is compared with standard and Formulation doses.Datawere analyzed by one-way ANOVA followed by Dunnett's test.

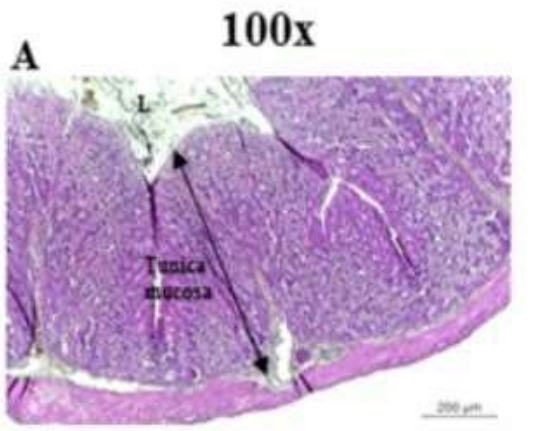
GRAPHICAL REPRESENTATION

4.1 Effect of Nutraceutical formulation on ethanol induced ulcers



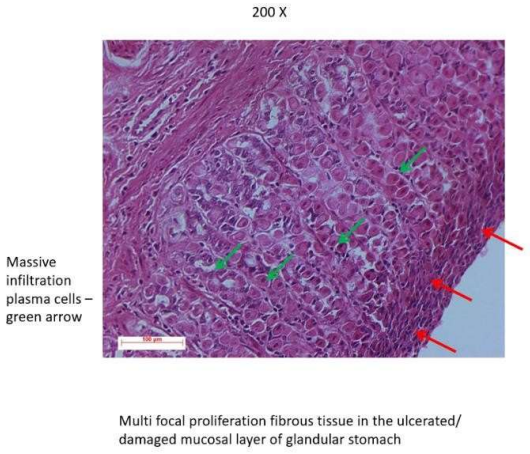
4.2 HISTOPATHOLOGICAL STUDIES OF ETHANOL INDUCED ULCER

Normal: -



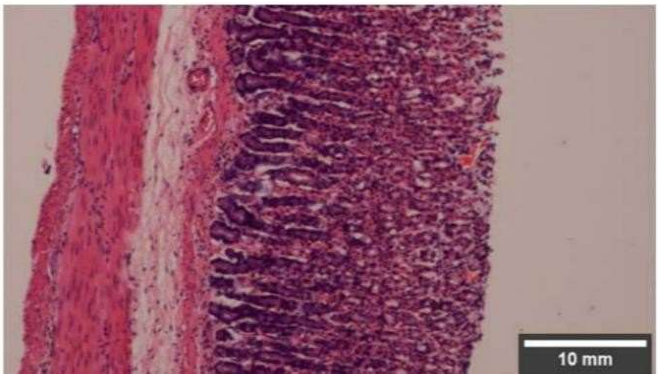
No focal proliferation of fibrous tissue in mucosal layer of glandular stomach

Disease control:



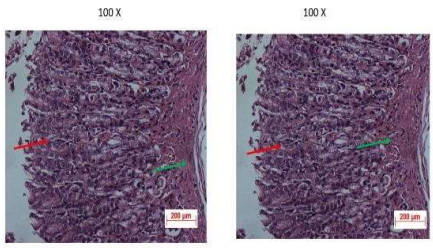
Multi focal proliferation fibrous tissue in the ulcerated/ damaged mucosal layer of glandular stomach

Standard: -



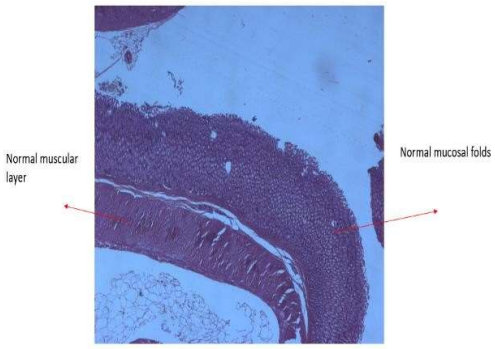
Normal morphology of sub mucosal and mucosal layerOf glandular stomach was observed

Test: Low dose (20mg/kg)



Normal morphology of mucosal [Red arrow], sub mucosal [green arrow] and muscular layer [yellow arrow] of glandular stomach was observed

Test: High dose (40mg/kg)



Normal morphology of glandular stomach

Table No4. 3 Effect of nutraceutical formulation, free acidity and total acidity on ethanol inducer ulcer model.

Group	Gastric Volume	pH	Free Acidity	Total Acidity
Normal	2.37±0.01	4.28±0.08	36.82±0.26	48.36±0.47
Control	5.56 ± 0.10	1.5 ± 0.08	65.06 ± 1.19	70.75 ± 1.21

Std(omeprazole)	1.46±0.07***	4.31±0.07***	28.95±0.96***	36.99±0.97***
Test (High dose) 40mg/kg	2.14±0.07**	3.84±0.08***	35.71 ± 0.88**	44.04±1.11***
Test (Low Dose)20mg/kg	3.48 ± 0.01**	2.45 ± 0.09**	53.19 ± 0.54*	63.03±1.15***

All values represent Mean ± SEM, n=6 in each group. ***p<0.001, **p<0.01, *p<0.05. Control group is compared with standard and extract doses. Data was analyzed by one-way ANOVA followed by Dunnett's test.

By reducing stomach volume, free acidity, total acidity, and ulcer index after oral administration of Nutraceutical formulation, table 3's antisecretory mechanism is demonstrated. At dosages of 200 and 400 mg/kg, respectively, nutraceutical formulation, shown a dose-dependent inhibition percentage of 27.25 and 40.77 (p 0.001). Omeprazole, a common medication, has an inhibition percentage of 61.54. Comparing the extract and standard groups to ulcer control. The results are shown in table 4.1,4.2,4.3

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5) Discussion of results: -

In the current study, a formulation including particular functional foods thought to work as antacids and ulcer preventives was created and tested for antiulcer activity in rats using an ulcer model caused by ethanol. For the prevention and treatment of peptic ulcers, herbalists and native healers have long employed these. By adhering to endothelial cells and blocking capillaries, this decreases the secretion of bicarbonates and mucus while increasing neutrophil infiltration into the gastric mucosa, which leads to endothelial cell damage through the release of protease, leukotrienes, and oxygen free radicals. We have been able to conduct in-vivo experiments thanks to this factor using SD rats. The NF02 contains a variety of vitamins, fatty acids, carbohydrates, proteins, flavonoids, and antioxidants. Their pharmacological effects may be brought on by the presence of flavonoids. In order to employ this formulation as an ingredient in foods and medications that promote health, it must first be recognized as promising in terms of its pharmacological properties and availability in nature.

Additionally, nutraceutical formulation demonstrated significant antioxidant activity in the study, possibly as a result of its ability to scavenge free radicals. Therapy with nutraceutical formulation significantly reversed the histopathological alterations seen in animals given ethanol therapy. As a result, nutraceutical formulation might be thought of as a potential treatment option for a number of peptic ulcer disease.

6) Conclusion: -

The present study shows the nutraceutical formulation was protect gastric mucosal damage induced by ethanol. The protection was found to be dose dependent. Since the ingredients used acts by various mechanisms and also possess nutritional value, the present formulation can be considered as an alternative, cost effective, safer and from the presentstudy it can be concluded that the formulation developed could significantly potential as medicine to treat gastric ulcers. Due to the increasing popularity with health-conscious consumers, functional food science is becoming popular and creating new interest in marketing the products. It is hoped that the study to serve to develop cost effective, safer and potential formulation for peptic ulcer.

7) Acknowledgement: -

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8) References: -

1. Astin J. Why patients use alternative medicine? Results of a national survey. JAmer Med Associated. 1998; 279: 1548-1553

2. REVIEW OF Horse gram-an underutilized nutraceutical pulse crop DOI 10.1007/s13197- 014-1312-z 12.

3 Nutraceutical value of finger millet [Eleusine coracana] and their improvement using omicsapproaches doi:10.3389/fpls.2016.00933

4. New concepts in nutraceuticals as alternatives for pharmaceuticals rafieian@yahoo.com.

5. REVIEW OF Medicinal and biological potential of pumpkin doi:10.1017/S095442210000107 https://www.researchgate.net/publication/49638808

6. https://www.sciencedirect.com/science/article/pii/S2405844022024471#bib37

7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6100067/#sec6-molecules-23-01751title.

8. "**ana-jana.org.** American nutraceutical association. [Online] "ana-jana.org., 03 19,2011.

9. Antiulcer Agents: From Plant Extracts to Phytochemicals in Healing Promotion https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4458761/

10. Preparation and Evaluation of Nutraceutical Formulation for Ulcer Healing. http://impactfactor.org/PDF/IJCPR/7/IJCPR,Vol7,Issue4,Article9.pdf

11. Sherwood: Human physiology. Thomson books., 6th edition, 591-593.

12. Anti-Ulcer Activity of Essential Oil Constituents, https://www.mdpi.com/1420-3049/19/5/5717

13. Narayan Das Prajapati, SS Purohit and Tarun Kumar. "A Hand Book of medicinal plants".

14. Kulkarni SK. Hand book of experimental pharmacology, 3rd edition, New Delhi, vallabhprakasham, 199,142-147.

15. REVIEW OF Medicinal uses of cashew nut [Anacardium occidentale] Zarqa et. Al

16. Antioxidant and radical scavenging properties of curcumin doi:10.1016/j.cbi.2008.05.003

17. Bandyopadhyay U, Das D, Banerjee RK, Reactive oxygen species: Oxidative damage andpathogenesis. *urrent science*, 77 (1999) 10