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RESEARCH ARTICLE

Investigation for Anti Ulcer Properties of Aqueous Extracts Prepared from the Leaves of Ocimum Sanctum in Rats

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ABSTRACT
Stress causes an ischemic condition in the gastric mucosa by activation of parasympathetic and sympathetic nervous system it indicates vasoconstriction. It causes free radical generation. Aspirin which is prostaglandin synthase inhibitor, it producing ulcers by preventing secretion of mucin and bicarbonate and impaired mucosal blood flow. Ranitidine is a H2 blocker which blocks the H2 receptors & inhibits the gastric acid secretion. The experimental studies an animal model confirmed the protective and curative activities of the *Ocimum Sanctum* against gastric ulceration compared with dose of ranitidine used as standard drug. *Ocimum Sanctum* of both doses 100 & 200mg/kg shows anti-ulcer activity but dose response observed at 200mg/kg and ranitidine results the similar to the plant dose. It significantly *Ocimum Sanctum* decreased gastric ulceration in aspirin induced model rats.
Keywords: *Ocimum Sanctum,* gastric ulceration.

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CONTENTS

1. Introduction

Stress has become a very common problem in every household and it leads to many diseases. One among them is peptic ulcer Psychological stress not only causes peptic ulcer but also can exacerbate it .Peptic ulcer impairs the

quality of life and is associated with increased morbidity and mortality hence its treatment is essential. Although there are many drugs available for the treatment of peptic ulcer they are associated with side effects hence there is always a need for a better drug. Plants are one of the most important sources of medicines and many drugs are derived from it. Ocimum sanctum commonly called as Tulsi grown easily in household is a medicinal plant used since ages for various properties. It has been used as antiasthamatic, antifungal, antiulcerogenic, antipyretic, antiviral, antibacterial, insecticidal and antimalarial. It possess antioxidant, Anti-inflammatory, Immunostimulant and antistressproperties. It is considered to be an adaptogen, balancing different processes in the body and helpful for adapting to stress.

2. Materials and Methods

Ocimum Sanctum was collected and its juice was extracted using cold extraction. Ranitidine was gifted by Zydus - cadila, Ahmadabad, India. Albino rats were weighing between 140-200gmswere collected and fed with rat chow and acclamatized for the surrounding environment.

Experimental procedure

Preliminary Phytochemical Investigation

Experimental animals

Albino rats were weighing between 140-200gms. Wister rats were taken into sainath agencies, bapujinagar, Hyderabad. They were exporting animals in an AC vehicle in water and food facility very caring to transporting. The animals acclimatized for seven days. In laboratory conditions of temperature 27 degrees centigrade $\pm$ 1 degree centigrade. 12: 12 hours light dark conditions for animal house.

Screening procedure

Aspirin induced antiulcer: Animals were divided into five groups, with each group containing six animals.

Group I – Normal control
Group II – Ulcer control
Group III - Standard
Group IV –test control group 1 (100mg/kg)
Group V –test control group 2 (200mg/kg)

The first group served as a control and was administered vehicle only, second group served as a positive control and were treated with standard drug ranitidine (20mg/kg) third group is the standard group fourth and fifth group served as test groups and were administered at the dose level 100 and 200mg/kg. Plant extract administered for 7 days. After seven days aspirin were administered 30 minutes before 200mg/kg per orally. After 6 hours rats will be sacrificed by anesthesia. Stomachs were dissected out for determination of gastric lesions, washed in tap water and examined ulcers with the help of microscope (10x). Gastric juice collected into centrifuge tubes and centrifuged at 1000 rpm for 10 minutes. The gastric juice volume will be noted. Gastric juice of P_H was recorded by P_H meter. Ulcer score for each animal called as ulcer index. Free and gastric acidity were analyzed.

Biochemical Parameters

Gastric acid volume:

Gastric acid present in stomach. After stomach dissecting gastric acid is collected into centrifuge tubes. Tubes were centrifuged at 1000 rpm per 10minutes. The gastric acid volume will be noted.

Determination of total acidity:

Take 50 ml of conical flask add 1ml of gastric juice with 1ml distilled water. To this solvent 2 drops of phenolphthalein indicator was added.0.01N NAOH was taken in to a burette. Titrant is titrated. A permanent pink color was observed. Burette reading was noted calculate the given formula

$$\text{Acidity} = \frac{\text{volume of NaoH} \times N \times 100\text{m Eq/l}}{0.1}$$

Determination of free acidity:

Gastric juice was titrated with 0.01N NAOH using topfer's reagent (dimethyl amino benzene) instead of phenolphthalein indicator. Canary color was observed. NAOH volume will be noted. Free acidity was calculated by same formula for total acidity.

Gastric acid P_H:

P_H range was estimated by using P_H strips. The P_H range is 2.0 – 4.5 and 5.0 – 8.5.

Determination of gastric ulceration:

After dissecting the stomach are opened, washed with tap water and normal saline solution. The sores of ulcers are measured by microscope (10X). Length was measured by each group determine by ulcer index.

Ulcer index:

Rats are dissected, after dissection of stomach along grater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant if any. Tissue were kept overnight in 10% formalin solution. Next day ulcers were examined by microscope (10X). Ulcer index of each animal was calculated by adding the values and their mean values were determined.

0– normal colored stomach
0.5– red color stomach
1– Spot ulcers
1.5-hemorrhagic
2 – Ulcers
3 –Perforations

Ulcer index formula: $UI = (UN + US + UP) \times 10^{-1}$

Histopathological Studies

After scarification of rats the stomach dissected out to remove any blood contaminants and washed with normal saline solution. Dissected stomach is placed in container fixed it 10% of formalin solution at least 48 hrs. These histopathological studies for each animal in a group. These stomachs are examined by histopathologists.

3. Results and Discussion

Results: The phytochemical screening carried out on ethyl extract of *Ocimum Sanctum* indicates the presence of phytoconstituents such as Alkaloids, flavonoids, tannins & phenolic compounds Carbohydrates.

Table 1 Preliminary phytochemical test of *Ocimum Sanctum* Ethanolic extract

Phytochemical test	Results
Carbohydrates	+
Alkaloids	+
Flavonoids	+
Steroids	–
Glycosides	–
Proteins	–
Phenols	+
Saponins	–
Tannins	+

(+) indicates presence, (-) indicates absence

Aspirin induced ulcer model in rats:

Table 2 Effect of ethanolic extract of *Ocimum Sanctum*on Ulcer index in Aspirin induced Ulcer models

Groups	Treatment	Ulcer index
1	Normal control	–
2	Ulcer control	2.89 ± 0.1
3	Standard	0.76 ± 0.05
4	*Ocimum Sanctum* (100mg/kg)	1.25 ± 0.06
5	*Ocimum Sanctum* (200mg/kg)	0.79 ± 0.06

All the values are expressed as mean ±SD; *P<0.05 vs ulcer control

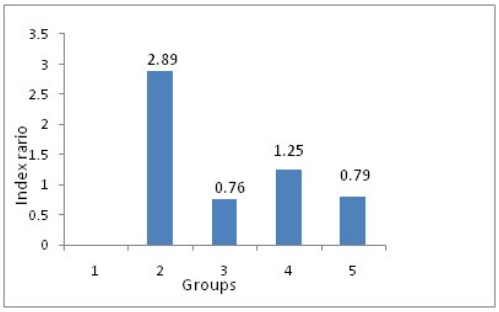


Fig 1: Graphical representation showing effect of ethanolic extract of *Ocimum Sanctum*on Ulcer index in Aspirin induced Ulcer models

Table 3: Effect of ethanolic extract of *Ocimum Sanctum*on Total acidity in Aspirin induced Ulcer models

Groups	Treatment	Total acidity
1	Normal control	11.4± 0.19
2	Ulcer control	48.50 ± 3.2
3	standard	20.20 ± 1.09
4	*Ocimum Sanctum*(100mg/kg)	38.60±1.98
5	*Ocimum Sanctum*(200mg/kg)	29.45 ± 1.98

All values are expresses as mean ±SD (n=4) *p,0.05vs ulcer control

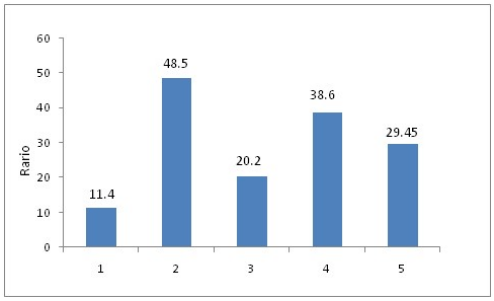


Fig 2 Graphical representation showing effect of ethanolic extract of *Ocimum Sanctum*on Total acidity in Aspirin induced Ulcer models

Table 4 Effect of ethanolic extract of *Ocimum Sanctum*on free acidity in Aspirin induced Ulcer models

Groups	Treatment	Free acidity
1	Normal control	8.0 ±0.9
2	Ulcer control	40.50 ± 2.4
3	Standard	15.8 ± 0.65
4	*Ocimum Sanctum*(100mg/kg)	19.6 ± 0.14
5	*Ocimum Sanctum*(200mg/kg)	16.80 ± 0.8

All the values are expressed as mean ±SD (n=4); *p<0.01vs ulcer control

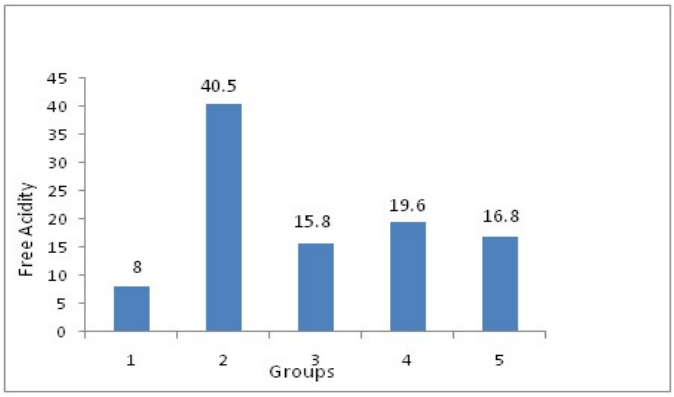


Fig 3: Graphical representation showing effect of ethanolic extract of *Ocimum Sanctum*on free acidity in Aspirin induced Ulcer models

Table 5 Effect of ethanolic extract of *Ocimum Sanctum*on Gastric volume in Aspirin induced Ulcer models

Groups	Treatment	Gastric volume
1	Normal control	2.0± 0.19
2	Ulcer control	3.95 ± 0.03
3	Standard	1.89 ± 0.05
4	*Ocimum Sanctum*(100mg/kg)	4.03± 0.03
5	*Ocimum Sanctum*(200mg/kg)	2.32 ± 0.01

All the values are expressed as mean ± SD (n=4); *p<0.05 vs ulcer control

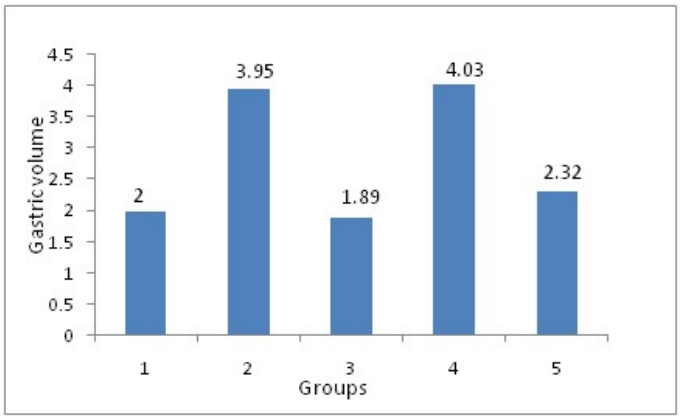


Fig 4. Graphical representation showing effect of ethanolic extract of *Ocimum Sanctum*on Gastric volume in Aspirin induced Ulcer models

Table 6 Effect of ethanolic extract of *Ocimum Sanctum*on Gastric pH in Aspirin induced Ulcer models

Groups	Treatment	Gastric pH
1	Normal control	1.84± 0.14
2	Ulcer control	1.84 ± 0.14
3	Standard	4.6 ± 0.04
4	*Ocimum Sanctum*(100mg/kg)	3.02 ± 0.03
5	*Ocimum Sanctum*(200mg/kg)	4.35 ± 0.01

All the values are expressed as mean ±SD(n=4) *p<0.05 vs ulcer control

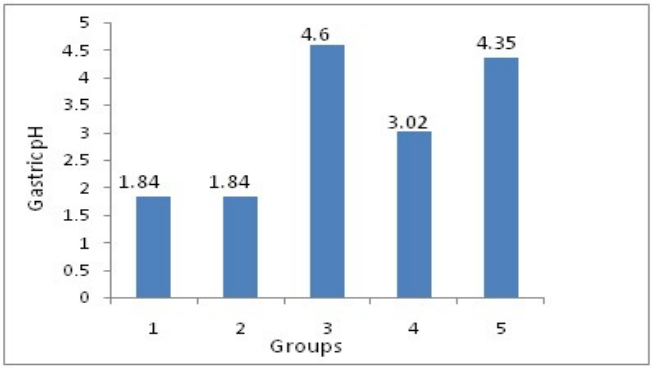
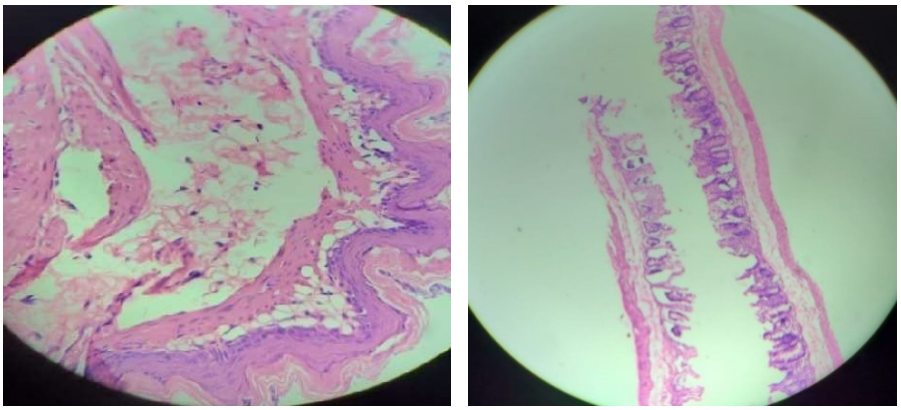


Fig 5. Graphical representation showing effect of ethanolic extract of *Ocimum Sanctum*on Gastric pH in Aspirin induced Ulcer models

Histopathology results:



Aspirin + Ranitidine Aspirin + *Ocimum Sanctum*

Fig 6. Histopathology results Aspirin + Ranitidine Aspirin + *Ocimum Sanctum*

Discussion

Gastric acid secretion is regulated by many factors including vagal activity, cholinergic, histaminergic and gastroenteric neurotransmissions, the activities of various post synaptic receptors and the proton pump inhibitors. Various therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, mucosal defense mechanisms by increasing mucosal invention, alleviating the surface epithelial cells. Anti-ulcer activity significantly reduced in gastric volume and total acidity. Tannins, reducing sugars, sterols, flavonoids are reported for anti-ulcer activity. Ethanolic extract of *Ocimum Sanctum* contain Tannins, carbohydrates, alkaloids, flavonoids, phenols. These subsidized for the anti-ulcer activity. Theethanolic plant extract not produce any toxic effects of mortality at the dose level 200mg/kg. It defines these drugs were safe for other pharmacological actions. According to the OECD-423 guide lines for acute toxicity studies. Aspirin induced ulcer model significant in ulcer index (2.89 ± 0.1), free acidity (40.50 ± 2.4), Total acidity (48.50 ± 3.2), Gastric pH (1.84 ± 0.14), Gastric volume (3.95 ± 0.03).Standard drug significantly condensed ulcer index (0.76 ± 0.05), Free acidity (15.8 ± 0.65), Total acidity (20.20 ± 1.09), Gastric pH (4.6 ± 0.04), Gastric volume (1.89 ± 0.05).Plant extracts 100mg/kg, 200mg/kg low and high reduced changes were occurred. Significantly ulcer index is (1.25 ± 0.06, 0.79 ± 0.06), Free acidity (19.6 ± 0.14, 16.80 ± 0.8), Total acidity (38.60±1.98, 29.45 ± 1.98), Gastric pH (3.02 ± 0.03, 4.35 ± 0.01,), Gastric volume (4.03± 0.03, 2.32 ± 0.01).The anti-ulcer effect of ethanolic extract of *Ocimum Sanctum* shows acid development of peptic ulcers.

The surface morphology of liposome granules and plain Phosphatidyl cholinegranules were examined by scanning electron microscopy. The surface morphology of liposome powder was different as compare to plain Phosphatidylcholine s powder as shown in SEM. From SEM photographs it is clear that, the surface of Phosphatidylcholine s were clear.

Viscosity measurement:

Viscosity of the gel was measured by Brookfield viscometer (LVDV II pro+). Viscosity of liposomal gel showed 1156cps at 100rpm.

P^H measurement:

The P^H of the developed formulation was in accordance with human skin P^H rendering them more acceptable. Therefore formulated liposomal gel was suitable for topical application. The P^H values of prepared liposomal gels were within the limits of 5.5 to 5.8.

Release kinetics: Various mathematical models were selected to evaluate the kinetics and mechanism of drug release from liposomal gel formulation. Best model was selected for release data which showed high correlation coefficient (r) value. In-vitro drug release over semi

permeable membrane and skin was performed and release kinetics was calculated.

Release kinetic graphs of optimized formulation: The mechanism of release for the optimized liposomal formulation based on regression coefficient (R^2) value. For most of the liposomal formulation the R^2 value nearer to1. Hence it can be concluded that the drug release follow peppasmodel. The n value of peppas model of the liposomal formulations are in the range of 0.1 to 0.5 which confirms that release of liposomal formulation was fickian diffusion.

4. Conclusion

Stress causes an ischemic condition in the gastric mucosa by activation of parasympathetic and sympathetic nervous system it indicates vasoconstriction. It causes free radical generation. Aspirin which is prostaglandin synthase inhibitor, it producing ulcers by preventing secretion of mucin and bicarbonate and impaired mucosal blood flow. Ranitidine is a H2 blocker which blocks the H2 receptors & inhibits the gastric acid secretion. The experimental studies an animal model confirmed the protective and curative activities of the *Ocimum Sanctum* against gastric ulceration compared with dose of ranitidine used as standard drug. *Ocimum Sanctum*of both doses 100 & 200mg/kg shows anti-ulcer activity but dose response observed at 200mg/kg and ranitidine results the similar to the plant dose. It significantly *Ocimum Sanctum* decreased gastric ulceration in aspirin induced model rats.

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