



## Preventive and curative effect of *Lagenaria vulgaris* in NSAID induced ulcer model

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### ABSTRACT

*Lagenaria vulgaris* is a popular vegetable grown almost all the year round. The fresh fruit juice has been used to treat acidity and ulcers in folklore medicines. In recent years, there has been phenomenal rise in the interest of scientific community to explore the pharmacological actions or to confirm the veracity of claims made about herbs in the official books of Ayurveda. The main objective of present work is to investigate the antiulcer activity of the fresh juice of *Lagenaria vulgaris*. Chemical analysis confirmed the presence of Vitamin A & C and Tannins. Male albino rats were used for the study and were divided into six different groups. Gastric ulcers were induced by NSAID (Diclofenac sodium).  $\beta$  – carotene was used as the standard drug for comparison. *L. vulgaris* was administered in a dose of 1ml and 2ml/rat. Levels of Glutathione and Ulcer Index were two major parameters. The fresh juice from the fruits of *L. vulgaris* exhibited both protective as well as healing effect on NSAID induced ulcers. It produced better recovery than  $\beta$  –carotene as far as both ulcer index and GSH levels were concerned.

**Keywords:** *Lagenaria vulgaris*; NSAID induced ulcer; Vitamin A & C; Glutathione; Ulcer Index.

### INTRODUCTION

*Lagenaria Vulgaris* Ser.; *Lagenaria siceraria* (Molina) Standley syn. *L. leucantha* Rusby; (Family: Cucurbitaceae) is an excellent fruit in the nature having composition of all the essential constituents that are required for normal and good health of humans. *L. vulgaris* is a popular vegetable grown almost all the year round, particularly in frost free areas. Young and tender fruits are eaten as vegetable. The flesh is soft, spongy and insipid when young; it is somewhat bitter when old (A.S.H.Rahman, 2003). Fruits of this plant are traditionally used for their cardioprotective, cardiotonic, general tonic, aphrodisiac, diuretic and nutritive properties (K.R. Kirtikar, 2001). Fruits are also used in treatment of pain, ulcer, fever, pectoral cough, asthma and other bronchial disorders (Anonymous, 1962; C. P. Khare, 2004).

The fruits are edible and considered as good source of vitamin C,  $\beta$ -carotene, vitamin B-complex, pectin and also contain highest choline level- a lipotropic factor (A.S.H.Rahman, 2003; W.C. Evans, 1996; S. Sonja, 2000). Modern phytochemical screening methods showed the presence of triterpenoid cucurbitacins B, D, G, H (W.C. Evans, 1996; S. Sonja, 2000) fucosterol,

campesterol and flavone C-glycosides (Shirwaikar, 1996).

The fresh fruit juice has been used to treat acidity and ulcers in folklore medicines (Anonymous, 1962). However there is paucity of data available on the effect of *L. vulgaris* fruits on antiulcer potential in animals. Therefore, the present study was undertaken to investigate the antiulcer activity of *L. vulgaris* fruits in rats.

### MATERIALS AND METHODS

**Drugs & Chemicals:** All the chemicals (AR grade) used were purchased from S. D. fine chem. limited, Mumbai unless specified. Diclofenac sodium was obtained as a gift sample from Lupin Limited, Mumbai. DTNB,  $\beta$  – carotene and Glutathione were purchased from Sigma-Aldrich co., USA.

#### Preparation of *Lagenaria vulgaris* Juice:

*L. vulgaris* was first grated, followed by squeezing of the grated material. This squeezed juice of *L. vulgaris* was administered immediately. Considering the dose recommended by the Ayurvedic physicians in human beings, the dose was extrapolated in rats as 1 ml/rat and 2 ml/rat.

Chemical analysis of this juice was done by Ramkrishna Bajaj – CFBP consumer education & testing centre, Mumbai, India.

### EXPERIMENTAL

Various groups formed for this experiment were as follows:

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Group I – Control (18 animals)

Group II – Negative control (NSAID only) 20mg/kg (18 animals)

Group III – NSAID (20mg/kg) + *L. vulgaris*(1 ml) (18 animals)

Group IV – NSAID (20mg/kg) +  $\beta$  – carotene (20mg/kg) (18 animals)

Group V – *L. vulgaris* (1 ml) + NSAID (20mg/kg) (6 animals)

Group VI –  $\beta$  – carotene (20mg/kg) + NSAID (20mg/kg) (6 animals)

Study was carried out in two parts to study the recovery from ulcer (Group III & IV) and the protection offered to ulcers (Group V & VI).

a. Male Wistar rats (180-230g) supplied by the Haffkine Biopharmaceuticals, Mumbai were used.

b. They were allowed to acclimatize one week before experimentation; in the departmental animal house. The protocol was approved by IAEC before experimentation (UICT/PH/IAEC/0206/18).

c. Group I & II animals were kept for fasting for 24 hours. Then group I & II animals were orally administered with water and NSAID (Diclofenac sodium, 20mg/kg) respectively. The oral feeding of water and NSAID were continued for 3 days. No feed was provided during this period while water was provided *ad libitum*.

d. On 4<sup>th</sup> day 6 animals from group I and group II were dissected. The stomach was stretched on wax. Then total stomach area was measured (cm<sup>2</sup>) and the area of any ulcer produced was also measured. Ulcer index was calculated from these values. Then Glutathione was estimated from small pieces of stomach mucosa (approx. 200mg).

e. The aforementioned step was repeated for the same groups I & II again on 7<sup>th</sup> day and 10<sup>th</sup> day.

f. Group III & IV animals were kept fasting for 24 hours and then treated with NSAID, followed by oral administration of *L. vulgaris* (1ml) &  $\beta$ -carotene (20mg/kg) respectively (after 3hours of oral administration of NSAID). The oral administration of NSAID was continued for 3 days along with administration of *L. vulgaris* and  $\beta$ -carotene respectively.

g. On 4<sup>th</sup> day 6 animals from both the groups were dissected. The stomach was stretched on wax. Then total stomach area was measured (cm<sup>2</sup>) and the area of any ulcer produced was also measured. Ulcer index was calculated from these values. Levels of Glutathione were estimated from small pieces of stomach mucosa (approx. 200mg).

h. The aforementioned step was repeated for both the groups again on 7<sup>th</sup> day and 10<sup>th</sup> day.

i. Group V & VI were treated with *L.vulgaris* (1ml) &  $\beta$ -carotene (20mg/kg) respectively for 14 days, after which the animals were kept for fasting 24 hours. They were orally administered NSAID for next 3 days. During these 3 days no feed was provided, water was provided *ad libitum*.

j. Then all of them (6 animals in each group) were dissected followed by aforementioned procedure.

k. All values are presented as Mean + SEM. Data were analyzed by student's t-test. Differences below the 0.01 level (P<0.01) were considered as statistically significant. Values of test juice & standard were compared with negative control.

#### Calculation of Ulcer Index (UI) (Majumdar B, 2003):

First measure the total area of stomach in cm<sup>2</sup> using graph paper. Also measure the area of ulceration in cm<sup>2</sup>. Ulcer index can be given as,

$$\text{Ulcer index} = \frac{\text{Area of ulcer}}{\text{Total stomach area}} \times 100$$

#### Data Analysis

Values are expressed as mean  $\pm$  SEM. Statistical differences between means were determined by performing one-way ANOVA followed by Dunnett's test. P<0.05 was considered as significant.

#### RESULTS & DISCUSSION

Gastric ulcer is the ulceration of mucosa coming in contact with acid and pepsin with consequent inflammation of the underlying and surrounding tissues (Green RJ, 2000). Gastric ulcer is one of the common gastrointestinal disorders in clinical practice. Excessive histamine release can cause ulcers by increasing gastric acid secretion. Mucus secretion by the gastric mucus cells combined with epithelial bicarbonate secretion contributes to a barrier that prevents gastric acid and pepsin from damaging the gastric mucosa (Satoskar RS, 1997).

These ulcers are the result of imbalance between aggressive and defensive factors involved in the stomach tissue. The healthy mucosa withstands acid attack and prevents back diffusion of H<sup>+</sup> in to the stomach. The specific biological mechanisms involved in mucosal defense include a mucus coat provided by surface epithelial cells that secrete mucus and bicarbonate, maintenance of adequate blood flow and production of prostaglandins by mucosa. Among the aggressive factors, it is generally accepted that increased gastric secretion, reduced gastric mucosal blood flow, increased gut motility, degranulation of mast cells and inhibition of prostaglandin biosynthesis are important factors which cause gastric erosions.

The reactive oxygen species generated by metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells may contribute to gastric mucosal

damage. Therefore, by scavenging free radicals, the reactive oxygen metabolites will protect the gastric mucosa from oxidative damage or even heal the gastric ulcers.

Anti inflammatory drugs like NSAID have long been to known to cause gastric ulceration in animals and man, which is attributed to the mucosal prostaglandin synthesis deficiency caused by inhibition of key enzyme cyclooxygenase (Satoskar RS, 1997). Due to reduced prostaglandin synthesis NSAIDs reduce the gastric mucosal blood flow, mucus and bicarbonate secretion. Several studies have confirmed that NSAIDs interfere with energy metabolism due to trapping of drug anion within cells of mucosa (Rainsford KD, 1978).

As can be seen from table 1, the different samples of juice exhibit similar content of Vitamin A, Vitamin C and tannins. Thus it was decided to use fresh juice

Similarly for ulcer index, control group showed mild ulceration ( $26.97 \pm 1.17$ ), which could be attributed to acidic content of stomach, as no feed was provided. This self recovered in next 7 days; while for negative control ulcer index was  $69.16 \pm 1.23$ , which showed maximum ulceration on day 4. After next 7 days, moderate ulceration was observed in group II ( $35.34 \pm 1.04$ ) indicating no good recovery. When animals were treated with fresh juice of *L. vulgaris*, they showed comparative results as that of control group ( $23.33 \pm 1.3$ ). These values were significantly different ( $P < 0.001$ ) than negative control. On day 10, animals showed almost negligible ulcers ( $3.96 \pm 1.35$ ), with treatment of *L. vulgaris*. Administration of fresh juice of *L. vulgaris* exhibited better potential for recovery of ulcer index than  $\beta$ -carotene.

For protective effect, GSH content of control and nega-

**Table 1: Chemical analysis of *Lagenaria vulgaris***

No.	Parameters	Results			
		Sample 1	Sample 2	Sample 3	Average
1	Vitamin A (IU/100g)	502.01	508.91	505.19	505.37
2	Vitamin C (mg/100g)	5.91	6.03	5.94	5.96
3	Tannins (%)	0.000301	0.000327	0.000319	0.000316

**Table 2: Recovery effect of *Lagenaria vulgaris* on gastric ulcers**

Groups		I	II	III	IV
Day 4	GSH ( $\mu\text{g/ml}$ )	$3.78 \pm 0.06^{**}$	$1.25 \pm 0.05$	$2.15 \pm 0.06^{**}$	$1.87 \pm 0.06^{**}$
	U.I. (%)	$26.97 \pm 1.17^{**}$	$69.16 \pm 1.23$	$23.33 \pm 1.3^{**}$	$47.97 \pm 1.8^{**}$
Day 7	GSH ( $\mu\text{g/ml}$ )	$3.85 \pm 0.11^{**}$	$1.65 \pm 0.06$	$2.75 \pm 0.06^{**}$	$2.07 \pm 0.07^*$
	U. I. (%)	$12.2 \pm 1.17^{**}$	$54.68 \pm 1.24$	$11.76 \pm 1.84^{**}$	$35.99 \pm 1.4^{**}$
Day 10	GSH ( $\mu\text{g/ml}$ )	$3.95 \pm 0.11$	$2.18 \pm 0.09$	$3.31 \pm 0.08^{**}$	$2.78 \pm 0.07^{**}$
	U. I. (%)	$2.16 \pm 0.98^{**}$	$35.34 \pm 1.04$	$3.96 \pm 1.35^{**}$	$12.11 \pm 1.77^{**}$

GSH – Glutathione; U.I. – Ulcer Index.

**Table 3: Protective effect of *Lagenaria vulgaris* juice on gastric ulcer**

Groups		I	II	V	VI
Day 18	GSH ( $\mu\text{g/ml}$ )	$3.78 \pm 0.06^{**}$	$1.25 \pm 0.05$	$3.15 \pm 0.08^{**}$	$2.62 \pm 0.16^{**}$
	U. I. (%)	$26.97 \pm 1.17^{**}$	$69.16 \pm 1.23$	$8.77 \pm 1.6^{**}$	$15.1 \pm 1.96^{**}$

without any further standardization as per the instructions of the Ayurvedic physician. It was decided to use 1ml of dose per rat, as preliminary experiments carried out at UICT revealed excellent activity produced by 1ml/rat dose better than even 2ml/rat dose.

For recovery effect of *L. vulgaris* against gastric ulcers produced due to NSAID are shown in table 2. On 4<sup>th</sup> day control showed normal GSH content ( $3.78 \pm 0.06$ ) while negative control (group II) showed significant decrease in GSH content ( $1.25 \pm 0.05$ ) ( $p < 0.001$ ) due to ulcer production. Group III ( $2.15 \pm 0.06$ ) & IV ( $1.87 \pm 0.06$ ) showed significantly ( $P < 0.001$ ) increased GSH content than group II on 4<sup>th</sup> day. For day 7 & 10, GSH content of group III ( $3.31 \pm 0.08$ ) & IV ( $2.78 \pm 0.07$ ) was significantly ( $P < 0.001$ ) higher than group II ( $2.18 \pm 0.09$ ). This shows faster recovery of GSH content than self recovery observed by animals of negative control.

negative control were considered same as for group I ( $3.78 \pm 0.06$ ) & II ( $1.25 \pm 0.05$ ) on day 4 respectively. Similarly, for ulcer index of control and negative control were considered same as for group I ( $26.97 \pm 1.17$ ) & II ( $69.16 \pm 1.23$ ) on day 4 respectively (table 3). For GSH content maintenance, group V ( $3.15 \pm 0.08$ ) showed significant ( $P < 0.001$ ) protection against NSAID produced change as compared to negative control, which was better than  $\beta$ -carotene. For ulcer index, both group V ( $8.77 \pm 1.6$ ) & VI ( $15.1 \pm 1.96$ ) showed better protection than control. In control, acidic condition without feed caused ulceration while group V & VI received drug treatments, which showed protection against ulcers, produced due to acid and even due to NSAID.

The protective and regenerative potential of fresh juice of *L. vulgaris* is due to the presence of antioxidant sub-

stances such as Vitamin A, Vitamin C and tannins present in juice (Rainsford KD, 1994; Ross, A.C, 1999; Ann E Hagerman). These substances scavenge the free radicals generated on administration of NSAIDs. The same is responsible for increasing the cellular defense such as reduced glutathione levels. (Various preliminary in-vivo experiments indicated excellent antioxidant potential of the fresh juice of *L. vulgaris*, confirming its antioxidant activity in-vivo.)

Gastric ulcers have been known to develop in critically ill patients, secondary to physiological stress since the 19th century. Stress ulcer prophylaxis has become an established routine practice in the intensive care unit. Numerous terms have been used to describe stress ulcers, but stress-related mucosal disease (SRMD) is commonly used terminology to denote such stress induced ulcers. Significant morbidity and mortality in critically ill patients is caused by SRMD and related bleedings (Sesler, 2007). Many options, including new pharmacologic advances, are available for the treatment and prophylaxis of stress-related ulcers; therefore, all critically ill patients should receive prophylaxis, even if they do not require treatment. Nutrition may play a significant role in the future in preventing stress-related ulcers. By improving stores of critical elements such as antioxidants, vitamins, and minerals before surgery, patients may lower the risk of developing stress ulcers (Flannery, 2002).

Considering the excellent protection exhibited by fresh juice of *L. vulgaris* against NSAID induced ulcers, it can be further explored and used as a dietary health supplement in critically ill patients as well as in physiologically stressed patients as a prophylaxis to lower the risk of SRMD.

## CONCLUSIONS

Fresh juice of *Lagenaria vulgaris* showed protective as well as regenerative potential as an anti-ulcer agent. It produced better recovery than  $\beta$ -carotene; for both parameters namely ulcer index and GSH. Fresh juice of *L. vulgaris* can be explored further using different models for evaluation of anti-ulcer activity of a compound. The fresh juice can be definitely used as a nutritional supplement against the various SRMDs.

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