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PHARMACOLOGICAL SCREENING OF ANTI-ULCER POTENTIAL OF HYDROALCOHOLIC LEAVES EXTRACT (HLE) OF *AZADIRACHTA INDICA*

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ABSTRACT

Stomach ulcer is also termed as gastric ulcer, are lesions that form on the stomach lining. In total, 23 patients who were diagnosed with UC, resulting in a 44.3% prevalence rate. This research focuses on pharmacological screening of anti-ulcer potential of hydroalcoholic leaves extract of *Azadirachta indica* in albino rats by following standard protocols. Leaves of *A. indica* were obtained from the Lucknow region. It will be identified and authenticated by a botanist. The leaves are washed making dust-free and dried at room temperature or shade. The dried leaves are rendered into coarse powders and then finally into fine ones. The powder is weighed and soaked into hydroalcoholic solution- Ethanol + distilled water (1:1) for fifteen days with gradual stirrings. Rats were divided into 4 groups. Pylorus ligation method was used to induce ulcer and different parameters were evaluated such as pH, volume of gastric content, UI and % Ulcer inhibition with histopathological studies. *A. indica* significantly exhibited anti-ulcer activity in all the models. In conclusion, *A. indica* has significant anti-ulcer potential at both the doses used 200mg/kg and 400mg/kg. It may prevent ulcer formation by the same action as ranitidine does. This study suggests to isolate and develop the suitable dosage form of concerning chemical constituents for the activity.

KEYWORDS: antiulcer, Azadirachata indica, neem, pylorus ligation.

INTRODUCTION

Stomach ulcer is also termed as gastric ulcer, are lesions that form on the stomach lining (Stomach Ulcer, NHS, 2022). The gastric ulcer consists of following and most common symptoms like Indigestion, Burning sensation in abdomen, Pain in tummy, Heartburn, Acid reflex and Feeling of sickness. In fact, men are more prone than women to suffer from duodenal ulcer. Gastric ulcer is 4 times as prevalent as intestinal ulcer (Datta & Roy, 2015). Peptic ulcer is acid-induced injury of intestinal system that is generally situated in stomach or upper segment of duodenum. It has been characterized by bared mucosa with imperfection stretching out into sub-mucosa (Zhang et al. 2014).

In total, 23 patients who were diagnosed with UC, resulting in a 44.3% prevalence rate. A year later, during a second trip to the same area, the incidence was estimated again and estimated to be 6.02/100,000. These findings show that UC is not uncommon in India. India has the largest disease burden among Asian countries, according to a comparison of prevalence and incidence with other countries (Kedia & Ahuja, 2017).

Neem is a tree that belongs to family- Meliaceae. It comprises of two species in the genus Azadirachta, and is native to India and Myanmar. It is susceptible to grow in tropical and semi-tropical regions/islands. It grows fast that can be developed in a height of 15-20 m and rarely to 35-40 m. Mostly, this tree seems as evergreen but under severe conditions it may shed most or nearly all of its leaves. Neem tree has wide spread branches. For several centuries, the beneficial properties of Neem (Azadirachta indica) have been well recognized in the Indian culture. Each part of the neem tree consists some medicinal property (Maithani et al. 2011). The holy Quran and Bible additionally upheld the the role of plants in disease prevention and their therapeutic effect. The Prophet Mohammed (PBUH) by the Islamic point of view confirms that several herbs and fruits are good sources for the treatment of illnesses (Alzohairy, 2016).

GC–MS investigations showed that greater part of these recognized mixtures in different unrefined concentrates contain ordinary hydrocarbons, phenolic compounds, terpenoids, alkaloids and glycosides (Hossain et al. 2013).

The chemical constituents were isolated from the leaves of neem as followings.

- nimbin
- nimbanene
- 6-desacetylnimbinene
- nimbandiol
- nimbolide
- ascorbic acid
- azadirachtin
- nimocinol
- naheedin
- mahmoodin
- n-hexacosanol and amino acid
- 7-desacetyl-7-benzoylazadiradione
- 7-desacetyl-7-benzoylgedunin
- 17-hydroxyazadiradione and nimbiol

But some toxicological effects such as allergic, genotoxic, cytogenetic and radio-sensitizing effects have also been seen in humans & some economic animals, particularly chicks and goats (Atawodi & Joy, 2009).

Previous studies give very limited data on screening of anti-ulcer potential of neem. So, this research focuses on pharmacological screening of anti-ulcer potential of hydroalcoholic leaves extract of *Azadirachta indica* in albino rats by following standard protocols.

MATERIALS AND METHODS

Experimental requirements

Hydroalcoholic leaves extract (HLE) of *Azadirachta indica*, Ranitidine (API), Water bath, digital balance, distilled water, albino rats (either sex), microscope, ethanol, diethyl ether.

Collection, Identification & Authentication of plant

Leaves of *A. indica* were obtained from the Lucknow region. It will be identified and authenticated by a botanist. The leaves are washed making dust-free and dried at room temperature or shade. The dried leaves are rendered into coarse powders and then finally into fine ones. The powder is weighed and soaked into hydroalcoholic solution- Ethanol + distilled water (1:1) for fifteen days with gradual stirrings. A rotating evaporator is used to dry the brownish, semisolid extract obtained under partial vacuum. The yield of the leaf extract is calculated as a percentage (Khan et al. 2020).

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

Preparation of animals

Animal House, Mahatma Gandhi Institute of Pharmacy (MGIP), Lucknow will provide albino rats of either sex weighing 150–200 g. The animals are kept in good health, with room temperatures of 25°C and a 12-hour light/dark cycle. The relative humidity is kept at 44–56 percent, and the rats are provided a regular rodent diet and free access to water. The animals will continue to fast but have free access to water until 1 hour before the ulcers are induced (Bhajoni et al. 2016).

Experimental protocols

All the rats are divided into 4 groups (n=6) as followings.

Group 1: Rats are administered only distilled water once a day for 15 days.

Group 2: Rats are administered Ranitidine (20mg/kg) orally, for 15 days.

Group 3: Rats are administered HLE of *A. indica* (200mg/kg) orally, for 15 days.

Group 4: Rats are administered HLE of *A. indica* (400mg/kg) orally up to 15 days.

Parameters

Pylorus ligation

Prior to pylorus ligation, all rats are kept in separate cages and starved for 24hr (water ad libitum). Coprophagy is avoided by keeping an eye on the animals. A mid-abdominal incision originating from xiphoid process is done under light ether anaesthesia (1cm). The pyloric ligation is performed using sterilized cotton thread while taking care not to disrupt the blood flow. The abdomen incision is completely washed with salt solution, dried, and wrapped with betadine-soaked cotton. The animals are slaughtered by cervical dislocation after 19hrs of pyloric ligation. By pinching the lower length of the oesophagus, the pyloric segment of the stomach is dissected out. The ulcer index is measured in the glandular part of the stomach (UI). The ulcer area is calculated by adding the width and length of each lesion, as well as the total area of every incision (mm2).

The PI is calculated by the below mentioned formula.

(PI) = [(UAcontrol − UAtreated) ÷ UAcontrol] X 100.

The gastric content is taken into tubes and after centrifugation it is used to test for different parameters-

- pH
- Volume of gastric content
- It is (content of gastric- lavage) titrated against 0.01N NaOH to determine free & total acidity (Dinda, 2012).

Evaluation parameters

Microscopical examination

All the groups of rodents are evaluated for deep haemorrhagic lesions of mucosal layer, submucosal oedema and leucocytes infiltration.

pH detection

Gastric content is taken out and kept in contaminated free petri-dish. After, the pH is easily measured by using

digital pH meter. It confirms about the acidity level in the rodent.

Gastric Volume determination

In this test, gastric content of stomach of rat is taken out and filled into measuring cylinder to confirm the actual volume of gastric fluid. It confirms about the level of acidity developed in the rat and effect of the drug.

Free & total acidity

In this procedure, firstly gastric content is taken out separately. It is (content of gastric- lavage) titrated against 0.01N NaOH to determine free & total acidity. It confirms the level of acidity and beneficial effect of drug given.

RESULTS AND DISCUSSION

Pylorus-ligation

In this method, ulcer index and percentage inhibition effect of HLE of *A. indica* was determined for all the groups (1-4). Group 1 was treated with distilled water (20mg/kg), Group 2 administered Ranitidine (20mg/kg) while Group 3 administered HLE of *A. indica* (200mg/kg) and Group 4 treated with HLE of *A. indica* (400mg/kg).

Group 1 exhibited UI score of 1.72±0.06** but PI was observed Nil. Whereas, group 2 (Ranitidine 20mg/kg) demonstrated UI as 0.27±0.04** and PI as 93.07 that is highly significant as it serves at standard drug. So, UI was achieved minimum after ranitidine administration and PI was noted maximum in terms of ulceration inhibition. Group 3 (n=6) given HLE of *A. indica* (200mg/kg) that reduced the ulcer index by up to 0.52±0.04** and PI was observed in ascending manner as 67.21.

Whereas, group 4 which was treated with HLE of *A. indica* (400mg/kg) was demonstrated the ulcer index as 0.47±0.06** and percentage inhibition was recorded as 82.02 which is near to standard drug treatment.

So, in this model the result indicates that HLE of *A. indica* in higher dose is much effective which is comparable to standard anti-secretory drug- ranitidine.

The following table confers the UI and PI effect of HLE of *A. indica* in pylorus ligation- induced ulcer model.

Table 1: Ulcer Index (UI) & Percentage Inhibition (PI) effect of HLE of *A. indica* in pylorus ligation- induced ulcer model.

Group	Treatment	UI	PI
1	Distilled water (20mg/kg)	1.72±0.06**	Nil
2	Ranitidine (20mg/kg)	0.27±0.04**	93.07
3	HLE of *A. indica* (200mg/kg)	0.52±0.04**	67.21
4	HLE of *A. indica* (400mg/kg)	0.47±0.06**	82.02

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

Microscopic studies

The microscopic studies of isolated stomach were done using 100× magnification power compound microscope. Firstly, the stomach was dissected washed thoroughly with saline solution to make it fat and gastric content free. After, it kept in saline solution until examination was completed by using high magnification power compound microscope. The following figure depicts the leucocytes infiltrations and streaking of ulcer perforations.

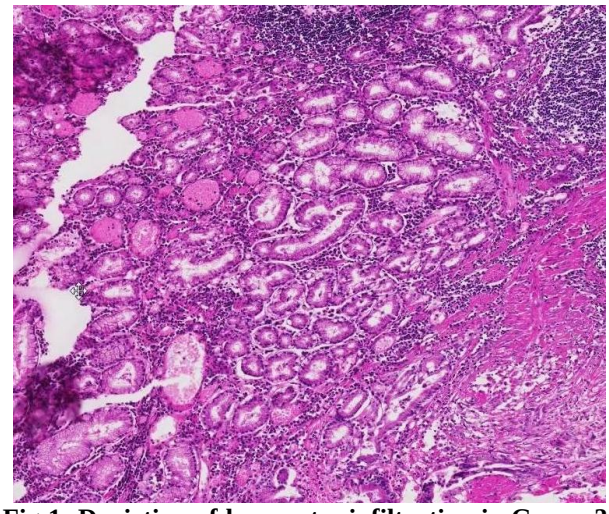

Fig 1: Depiction of leucocytes infiltration in Group 3- treated with HLE of *A. indica* (200mg/kg)

The following figure demonstrates the streaking and mucosal edema. It confirms the level of oedema in group 4.

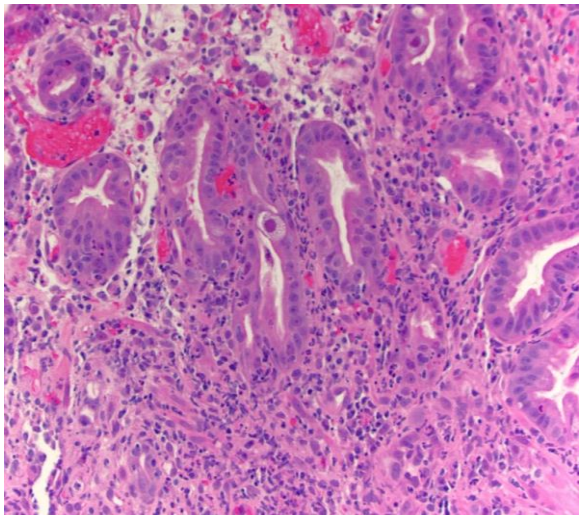

Fig. 2: Depiction of ulceration- mucosal edema of Group 4 treated with HLE of *A. indica* (400mg/kg)

All the rats were treated for continuously 15 days as per once-a-day schedule.
In all the microscopical studies it showed that minimal mucosal edema, leucocyte infiltration was recorded in group 4 that was administered HLE of *A. indica* in the dose of 400mg/kg.
So, the response observed in the dose dependent manner. Less anti-ulcer was seen in the group 3 treated with HLE of *A. indica* (200mg/kg).
The following table depicts the ulceration in the standard group which is minimal-

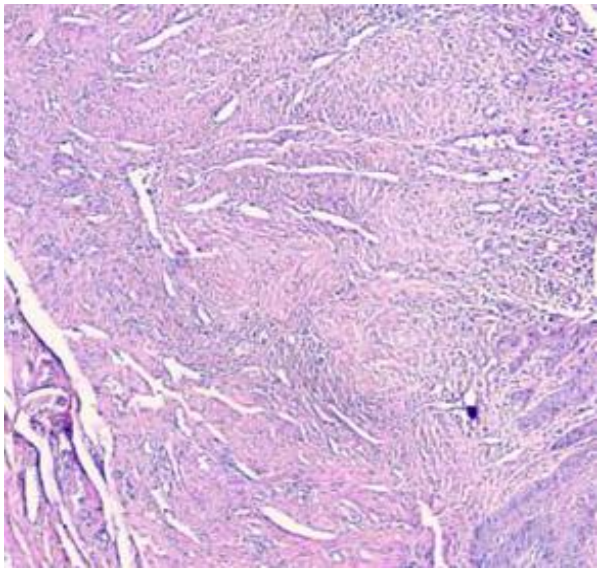

Fig 3: Depiction of ulceration- mucosal edema of Group 2 treated with Ranitidine (20mg/kg).

pH detection
The pH detection of HLE of *A. indica* was determined for all the groups (1-4). Group 1 was treated with distilled water (20mg/kg), Group 2 treated with Ranitidine (20mg/kg), Group 3 treated with HLE of *A. indica* (200mg/kg) and Group 4 treated with HLE of *A. indica* (400mg/kg).

Group 1 was administered distilled water (20mg/kg) and pH was observed as 2.23±0.20** after 15 days of continuous exposure of dosing frequency once per day. Group 2 given Ranitidine in the dose of 20mg/kg showed optimum and increased pH in the range of 4.51±0.21**. Group 3 administered HLE of *A. indica* for 15 days of continuous exposure, the pH was recorded as 2.91±0.17*** whereas Group 4 given HLE of *A. indica* once a day for 15 days and showed pH as 3.72±0.41**. Therefore, the maximum pH increasing effect was seen in higher dose when compared to standard drug-Ranitidine (20mg/kg).

The following table depicts the response of HLE of A. indica on pH.

Table 2: Response of HLE of *A. indica* on pH.

Group	Treatment	pH range
1	Distilled water (20mg/kg)	2.23±0.20**
2	Ranitidine (20mg/kg)	4.51±0.21**
3	HLE of *A. indica* (200mg/kg)	2.91±0.17***
4	HLE of *A. indica* (400mg/kg)	3.72±0.41**

Significance Level= *
Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

Volume of gastric content
The effect of volume of HLE of *A. indica* on gastric content was determined for all the groups (1-4). Group 1 was treated with distilled water (20mg/kg) and served as control, Group 2 treated with Ranitidine (20mg/kg) considered as reference group, Group 3 treated with HLE of *A. indica* (200mg/kg) taken as Test 1 and Group 4 treated with HLE of *A. indica* (400mg/kg) observed as Test 2.

After 15 days of continuous treatment, volume of gastric content for group 1 was obtained as 11.91±0.34*** ml. Group 2 administered with Ranitidine in the dose of 20mg/kg recorded for volume of gastric content as 5.67±0.30* ml. The volume of gastric content (ml) for Group 3 treated with HLE of *A. indica* was observed as 8.32±0.15** ml whereas group 4 exhibited and decreased level of secreted volume of gastric content as 6.11±0.24*** ml which is much significant and comparable to standard group.

The following table demonstrates the decreased volume of gastric content (ml)-

Table 3: Effect of HLE of *A. indica* on volume of gastric content (ml).

Group	Treatment	Volume of gastric content (ml)
1	Distilled water (20mg/kg)	11.91±0.34***
2	Ranitidine (20mg/kg)	5.67±0.30*
3	HLE of *A. indica* (200mg/kg)	8.32±0.15**
4	HLE of *A. indica* (400mg/kg)	6.11±0.24***

Significance Level= *
Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

Free acidity

The effect of HLE of *A. indica* on free acidity was determined for all the groups (1-4). Group 1 was treated with distilled water (20mg/kg) and served as control, Group 2 treated with Ranitidine (20mg/kg) considered as reference group, Group 3 treated with HLE of *A. indica* (200mg/kg) taken as Test 1 and Group 4 treated with HLE of *A. indica* (400mg/kg) observed as Test 2.

Group 1 administered with distilled water in the dose of 20mg/kg showed free acidity with value of 41.12±1.72** which is in-effective completely. Group 2 treated with ranitidine in the dose of 20mg/kg, all the animals exhibited mean value of free acidity as 22.63±2.12**. Animals treated with test drug- HLE of *A. indica* (200mg/kg) produced free acidity in the range of 32.72±1.22*** whereas group 4 treated with same in the dose of 400mg/kg showed 26.82±1.41** free acidity which is near to standard drug- ranitidine.

The following table depicts the response of HLE of *A. indica* on free acidity.

Table 4: Response of HLE of *A. indica* on free acidity (mEq/l).

Group	Treatment	Free acidity (mEq/l)
1	Distilled water (20mg/kg)	41.12±1.72**
2	Ranitidine (20mg/kg)	22.63±2.12**
3	HLE of *A. indica* (200mg/kg)	32.72±1.22***
4	HLE of *A. indica* (400mg/kg)	26.82±1.41**

Significance Level= *
Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

Total acidity

The effect of HLE of *A. indica* on total acidity was determined for all the groups (1-4). Group 1 was treated with distilled water (20mg/kg) and served as control, Group 2 treated with Ranitidine (20mg/kg) considered as reference group, Group 3 treated with HLE of *A. indica* (200mg/kg) taken as Test 1 and Group 4 treated with HLE of *A. indica* (400mg/kg) observed as Test 2.

Total acidity was noted as 73.43±1.32*** (mEg/l) in group 1 which was treated with distilled water (20mgkg). Group 2 treated with Ranitidine in the dose of 20mg/kg exhibited total acidity as 49.41±2.11**. Whereas, group 3 showed total acidity as 68.12±1.31*** that was administered HLE of *A. indica* in the dose of 200mg/kg for persistently 15 days. At last, group 4 given HLE of *A. indica* (400mg/kg) was evaluated for total acidity as observed as 56.23±1.22**.

The following table shows the response of HLE of *A. indica* on total acidity.

Table 5: Response of HLE of *A. indica* on total acidity (mEq/l).

Group	Treatment	Total acidity (mEq/l)
1	Distilled water (20mg/kg)	73.43±1.32***
2	Ranitidine (20mg/kg)	49.41±2.11**
3	HLE of *A. indica* (200mg/kg)	68.12±1.31***
4	HLE of *A. indica* (400mg/kg)	56.23±1.22**

Significance Level= *
Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

pH was determined as low as compared to compared and standard group. As hyperacidity is the first factor behind its anti-ulcerogenic potential so decreasing the pH is much supported treatment in ulcer as anti-ulcer agent. It makes act due to its neutralizing potential of acidity or increase release of some alkaline bicarbonate ion and others to raise the pH (alkaline). The highest pH decrease was recorded in the group 2 and group 4 treated with ranitidine (20mg/kg) and HLE of *A. indica* (400mg/kg) respectively.

Volume of gastric content was found less when compared to control group. However, the group 4

demonstrated a well and efficient role in lowering the volume of gastric content might be due to blocking property at Histamine receptors located in gastric wall. In turn, it decreases the release the gastrin and thus gastric juice. It was much comparable in the dose of 400mg/kg (HLE of *A. indica*) to standard group.

The free acidity was found decreased after the exposure to HLE of *A. indica* for continuously 15 days. It might give action to neutralize the free acid and thus to modulate the high and severe action of free acid to develop ulcer and perforation in gastric wall. It might be so due to long term exposure of plant extract to accommodate and produce pharmacological action.

Total acidity when observed was also found less after treatment among different group of animals. It demonstrated that total acidity was also get reduced in the group 2, 3 and 4. The mechanism behind this pharmacological activity is not known as such.

In all the protocols, *A. indica* showed a significant anti-ulcerogenic activity when compared to reference drug-Ranitidine. The response was observed as dose dependent.

When we compared the results obtained in pylorus ligation, cold- restraints and forced swimming test, it found that in all the groups anti-ulcer potential was recorded but effect was clear in pylorus ligation when studied microscopically.

Standard group of animals treated with ranitidine (20mg/kg) showed highest anti-ulcerogenic potential being the reference and well proved anti-ulcer as it decreases the release of gastrin in turn gastric juice (hydrochloric acid). Mucosal damage, infiltration of leucocytes was observed as negligible.

A. indica in the dose of 200mg/kg is also effective when compared with the control group of animals that were treated with distilled water in the same dose. Whereas, higher dose demonstrated a highly efficient anti-ulcerogenic activity when compared to standard drug administered group of rats.

CONCLUSION

To summarize, the leaves of A. indica have antiulcer properties and may work through a variety of mechanisms, including suppression of histamine-2 receptors/ H+/K+-ATPase, prostaglandin modulation, or antioxidation. The current investigation backs up the folklore belief that A. indica is a medicinal plant. In the treatment of PUD, it is successful.

In conclusion, A. indica has significant anti-ulcer potential at both the doses used 200mg/kg and 400mg/kg. It may prevent ulcer formation by the same action as ranitidine does. In all the parameters, it exhibited dose-dependent response in sub-siding ulcerogenic response. It selectively decreases gastric juice production and thus lowers pH of stomach.

This study suggests to isolate and develop the suitable dosage form of concerning chemical constituents for the activity.

SOURCE OF FUNDING

Nil.

CONFLICT OF INTEREST

None.

REFERENCES

1. Alzohairy M A. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evid Based Complement Alternat Med, 2016; 7382506.
2. Atawodi Sunday E, Joy C Atawodi. Azadirachta indica (neem): a plant of multiple biological and pharmacological activities. Phytochemistry Review, 2009; 8: 601-620.
3. Bhajoni P S, Meshram G G, Lahkar M. Evaluation of the Antiulcer Activity of the Leaves of Azadirachta indica: An Experimental Study. Integrative Medicine International, 2016; 3: 1-2.
4. Datta De D., Roychoudhury S. To be or not to be: The host genetic factor and beyond in Helicobacter pylori mediated gastro-duodenal diseases. World J. Gastroenterol, 2015; 21: 2883–2895.
5. Dinda S C. Anti-ulcer activity of aqueous and ethanolic leaf extract of neem (Azadirachta indica) in albino rats. Journal of Pharmacy Research, 2012; 5(3): 1571-1573.
6. Hossain M A, Wafa A S Al-Toubi, Afaf M W, Qasim A Al-Riyami, Jamal N. Al-Sabahi. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. Journal of Taibah University for Science, 2013; 7(4): 181-188.
7. Kedia S., Ahuja V. Epidemiology of Inflammatory Bowel Disease in India: The Great Shift East. Inflamm. Intest. Dis, 2017; 2: 102-115.
8. Khan Mohammad Asif, S B Tiwari, H Gupta, Huma Noor. Evaluation of anxiolytic and antidepressant potential of hydro-alcoholic leaves extract of Azadirachta indica in albino rats. Pharmacology Online, 2020; 3: 207-213.
9. Maithani A, Versha P, Geeta P, Ishan Dhulia, Deepak K. Azadirachta Indica (Neem) Leaf: A Review. Journal of Pharmacy Research, 2011; 4(6): 1824-1827.
10. Stomach Ulcer. 2022. Overview. www.nhs.uk.
11. Zhang B.B., Li Y., Liu X.Q., Wang P.J., Yang B., Bian D.L. Association between vac A genotypes and the risk of duodenal ulcer: A meta-analysis. Mol. Biol. Rep, 2014; 41: 7241–7254.