Comparative effect of ginger (an anti-inflammatory medicinal herb) and aspirin (a non-steroidal anti-inflammatory drug) on cytoprotection and body weight changes in male albino Wistar rats

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat acute and chronic musculoskeletal problems (osteoarthritis, rheumatoid arthritis, and gout injuries), headaches, dental aches, surgical pains, and dysmenorrhea. Aspirin and other NSAIDs can cause ulcers. Ginger, a common spice, is anti-inflammatory. Ginger's anti-inflammatory qualities make it suitable for the herbal treatment of inflammatory diseases in numerous cultures. This study examined the effects of ginger, an anti-inflammatory plant, and aspirin, a NSAID, on animal stomachs and body weight. Both are used to treat inflammatory conditions in various nations. Given that aspirin has been linked to stomach ulcers, the study sought to determine if ginger had a larger stomach cytoprotective impact. The study hopes to determine if ginger, a medicinal herb, might reduce inflammation and weight. This study used 45 male Wistar rats. Three 15-rat groups—Control, Ginger, and Aspirin—were created. Five (5) rats in each group were utilized to assess mucus, ulcer scores, and pepsin secretion from the 15 rats in each group. Three animal groups received food and water daily. For four weeks, ginger group mice received 150 mg/kg aqueous ginger extract, while aspirin group animals received 150 mg/kg aspirin. At the end of the trial, ginger group animals had considerably lower mucus secretion than the control and aspirin groups. At 0.05 significance difference, the ginger group's pepsin secretion was significantly higher than the control and aspirin groups, although the ulcer score was significantly higher in the aspirin group. Ginger and aspirin groups had lower body weight changes than the control group at 0.05 significant differences. Ginger may treat inflammatory diseases, according to ulcer scores, and decrease weight gain.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin have been an important intervention in patient management and treatment of painful inflammatory conditions as well as fever.[1] However, these groups of drugs, including aspirin, have a high tendency to cause ulceration.[2]

Considering the high ulcerative index of aspirin, it becomes necessary to look for a herbal alternative that will be useful for treating inflammation. Consequently, folk medicine has attempted the use of herbs such as ginger in treating inflammation.

Ginger shows a broad and positive anti-inflammatory activity, hence it is suitable for the treatment of inflammation, fever, and headache.[3]

Ginger, scientifically called *Zingiber officinale* (Zingiberaceaae) is a plant native to Asia. Its root which is commonly available and used as a spice for several dishes is suitable for the treatment of arthritis, inflammation, and various types of infection.[4]

Following the high ulcerative index of aspirin and the cheap source of ginger, this study sets off to compare the ulcerative capacity of an alternative anti-inflammatory medicinal herb (ginger) with that of aspirin (a NSAID) in view of establishing which will offer a better cytoprotective effect on the stomach in treating inflammatory disorders using rats as experimental animals. The study further assessed these two anti-inflammatory therapies on the weight changes of the animals.

Materials and Methods

Collection, preparation, and storage of ginger aqueous extract

Ginger was procured from a trader from Jos, Nigeria. The ginger rhizome was peeled, rinsed in water, and air-dried at room temperature. The partially dried ginger rhizome was introduced into an oven to be completely dried at a temperature of 60°C for a period of one week. Dried ginger was ground to dust and soaked in 150 mL of water. The weight of the ground ginger was 150 g.

The supernatant was filtered using Whatman's filter paper (large) to remove all forms of debris. The filtered solution of ginger was again introduced into an oven at a temperature of 60°C for 3 days to get the dried extract of ginger.

The dried extract of ginger was an oily thick brown gummy paste. The extract was scrubbed and collected into a sample bottle. The weight of the extract was 90 g. It was refrigerated to be used at a standard concentration for the ginger group administration.

Determination of the LD_{50} of ginger (*Zingiber officinale*) in albino Wistar rats

The LD_{50} of *Zingiber officinale* (ginger) was determined following the method of Lorke.[5] One hundred (100) albino Wistar rats were divided into 5 different groups, n=20. Different doses (100, 400, 800, and 1200 mg/kg) of the *Zingiber officinale* extract were administered intraperitoneally to rats in their respective groups except for the control group that received normal saline. All animals were given access to feed and water at will.

The setup was left untouched for 48 hours but the rats were allowed free access to food and water. At the expiration of 48 hours, the rats in their respective cages were observed and the results of the death of animals in each group were recorded.

Experimental animals

Forty-five male albino Wistar rats weighing 80-120 g were the animals of choice in this study. The animals were made to acclimatize for two weeks. Thereafter, they were separated into three groups (control, ginger, and aspirin), each rat in separate cages. Fifteen rats (15) were assigned to each group. They were maintained in an animal house at a temperature of 28±2°C and, light-dark cycle 12-12 hours. The cages were always kept clean. Food and water intake were provided daily with the aid of an electronic weighing balance.

Calculation, storage, and administration of aqueous form of drug dose

After one week of acclimatization, 4.3 mg/kg doses of prepared solution of aspirin were administered to the aspirin group orally while 300 mg/kg doses of prepared solutions of ginger were administered to the ginger group orally, once daily for 28 days with the aid of an orogastric cannula. The aspirin dose was chosen based on the standard prescription of the drug for an adult of 70 kg which is 300 mg. The dose of the ginger extract was chosen based on the toxicity study (LD_{50}) carried out on the extract.

Determination of body weight

The body weight of the animals was determined by using a weighing balance. The initial weight of each rat was recorded after random grouping, before the commencement of administration. The rats were subsequently weighed daily until the end of the study. The weights were recorded and differences in weight were computed.

Measurement of gastric mucus

The adherent gastric mucus was measured by the method of Tan *et al.*[6] The animals fasted for 24 hours to completely empty their stomach of any food content. Thereafter, they were anesthetized with chloroform and their stomach was removed for sacrifice. The stomach was then opened along the greater curvature and pinned on a flat board. Using a spatula, the gastric mucus was scraped off the surface of the mucosa and introduced into a pre-weighed sterilized sample bottle containing 3 mL of distilled water. The sample bottle containing distilled water and the collected mucus was then weighed on an electronic balance. Mucus output was calculated as the difference in weights of the sample bottle containing water and the sample bottle containing water and mucus.

Determination of ulcer scores

Gastric ulcer score was assessed using the method of Alphin and Wards.[7] The rats were anesthetized using chloroform. Thereafter, an abdominal incision was made and the pylorus of the stomach was exposed. A pyloric incision was made and a cannula was inserted and held in place by tying with a thread. The stomach was infused with 1.5 mL of acid alcohol. The infusion was made via the pyloric incision. The stomach was surgically removed after an hour, washed, cut open along the greater curvature, and rinsed with normal saline. Pins were used to hold the tissue to the dissecting board. A magnifying lens and a vernier caliper were used to measure the extent of ulceration. Ulcer score was done according to the grading system in Table 1.

The ulcer score was calculated by multiplying each grade by its frequency of occurrence. The sum of all the values formed the ulcer score for each animal.

Table 1. Grading system of ulcer scores.

Grade	Interpretation
0.0	No lesion (normal stomach)
0.5	Pin size ulcer
1.0	2 or more hemorrhagic or small linear ulcers
2.0	Ulcer spots greater than 3 mm

Collection and analysis of pepsin

Gastric juice used for the analysis of pepsin was collected according to the method of Shay *et al.*[8] The animals were fasted for 36 hours to ensure that their stomachs were completely emptied but were given free access to water. Under chloroform anesthesia, a midline incision was made on the abdomen to expose the stomach. A pyloric ligature was made with the aid of a silk thread to prevent gastric emptying. The abdomen was then returned to its normal position and the abdomen was closed, abdominal wounds were cleaned carefully with normal saline.

The anesthesia was discontinued and all animals usually recovered consciousness within 10 to 15 minutes. Four hours later, all animals were again anesthetized and their abdomens were opened. The gastric contents were emptied into sample tubes and centrifuged for ten (10) minutes at about 3000 revolutions per minute (rpm). Thereafter, the supernatants were collected for analysis of pepsin. The determination of the proteolytic activity of gastric secretion was performed using casein as a substrate according to the method of Hawk *et al.*[5] Exactly 0.2 mL of centrifuged gastric juice +3 mL of casein 3% for each rat test was used in the preparation of the blank. Then, 10 mL of 6% trichloracetic acid was added to the blank to stop enzyme activity. Both blank and test tubes were incubated in a water bath at a temperature of 37°C for 30 minutes. Then, 10 mL of trichloracetic acid was added to test tubes, shaken well, and filtered. Proteolytic activity was determined spectrophotometrically by optical density measured at 280 nm.

Data presentation and statistical analysis

The results are presented as mean±standard error of the mean (SEM). The data were computed and analyzed using the 2020 version of the Microsoft Excel application package. The One-way Analysis of Variance (ANOVA) was used in the statistical testing. Values were considered significant at $p<0.05$ and $p<0.001$.

Results

Lethality study of aqueous ginger root extract and effective dose of administration

The lethality study to determine the LD_{50} of ginger at doses of 100, 400, 800, and 1200 mg/kg body weight gave a result of 496.17 mg/kg graphically (Figure 1). The LD_{50} of the ginger extract was determined from the graph of percentage mortality of the animals in probit plotted against the Log_{10} of the dose of the extract that was administered on the animals as shown in Figure 1. The percentage of mortality was determined from the number of deaths recorded in each group of animals used for the LD_{50} study. The effective doses are those below the LD_{50} as these doses are considered safe for the animals in carrying out the research. Exactly 300 mg/kg was the effective dose chosen for administration in this research work.

Comparison of body weight change of animals between the different experimental groups

The mean±SEM body weight changes of the control, aspirin, and ginger groups were 5.17±1.14, -7.50±1.61, and -17.00±3.53 g, respectively (Figure 2). Analysis of the result shows that there was a significant decrease in the body weight of rats in the aspirin group at $p<0.05$ and ginger group at $p<0.001$ compared to the control group. Also, body weight changes of ginger group animals was significantly lower than that of aspirin group at $p<0.001$.

Comparison of gastric mucus output between the different experimental groups

The mean±SEM gastric mucus output of the control, aspirin, and ginger group were 0.19±0.02, 0.08±0.00, and 0.06±0.01 g, respectively (Figure 3). The results show that there was a significant decrease in the gastric mucus output of rats in the aspirin and ginger groups when compared to that of rats in the control group ($p<0.001$).

Comparison of pepsin output between the control and test groups

The mean±SEM pepsin secretion of the control, aspirin, and ginger group were 0.50±0.01, 0.44±0.02, and 0.58±0.01 mg/mL respectively (Figure 4). Pepsin secretion in the ginger group treated rats showed a significant increase in comparison to that of rats

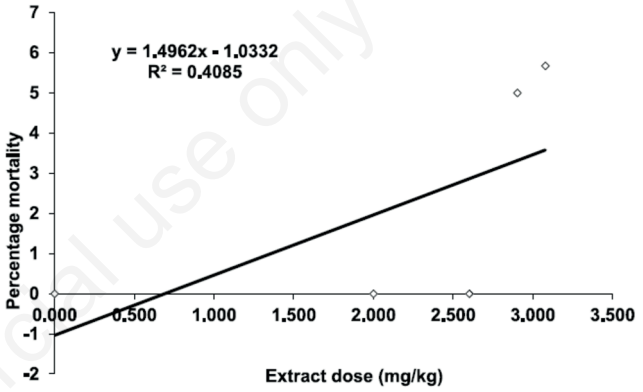

Figure 1. Lethality study of ginger extract. (LD_{50}=496.17mg/kg and the effective dose used for administration=300mg/kg).

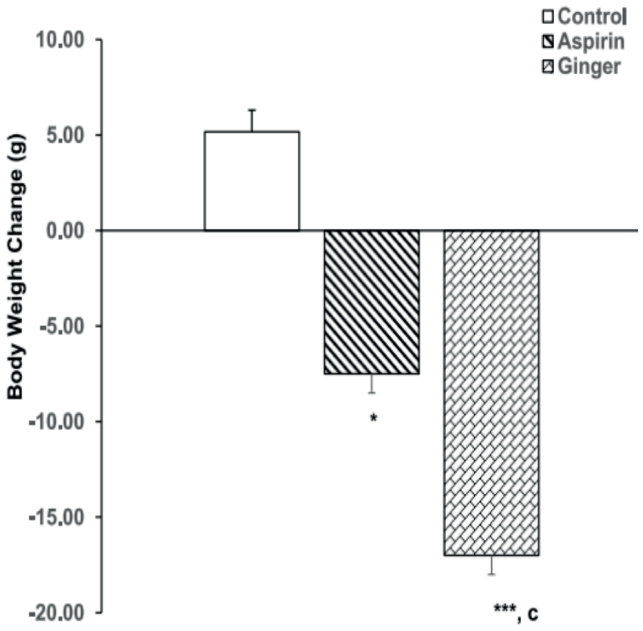

Figure 2. Body weight changes of experimental animals. *p<0.05 *vs* control; ***p<0.001 *vs* control; c, p<0.001 *vs* aspirin.

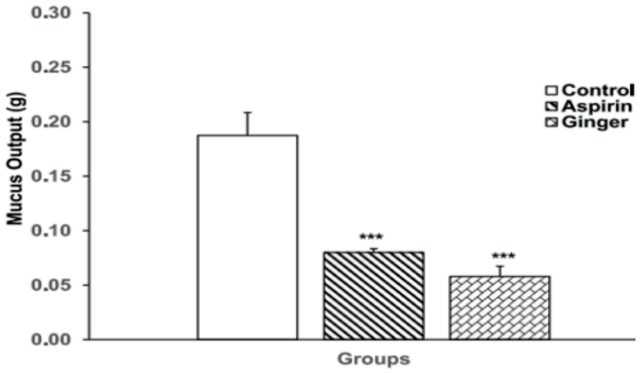

Figure 3. Gastric mucus output of experimental animals. ***p<0.001 *vs* control.

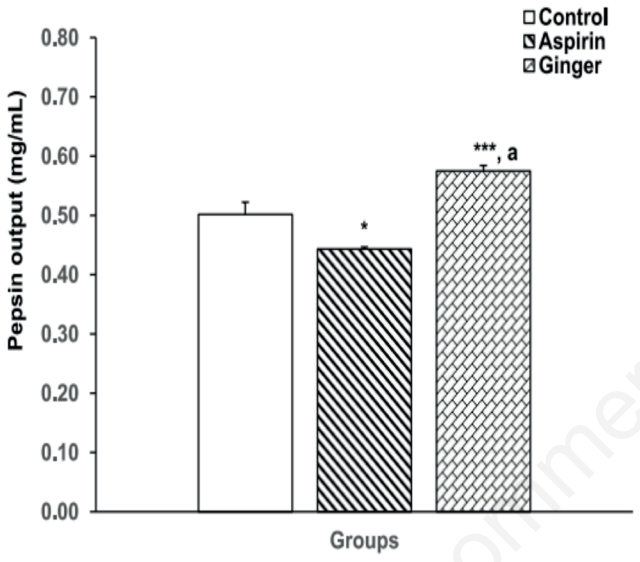

Figure 4. Pepsin output of experimental animals. *p<0.05 *vs* control; ***p<0.001 *vs* control; a, p<0.001 *vs* aspirin.

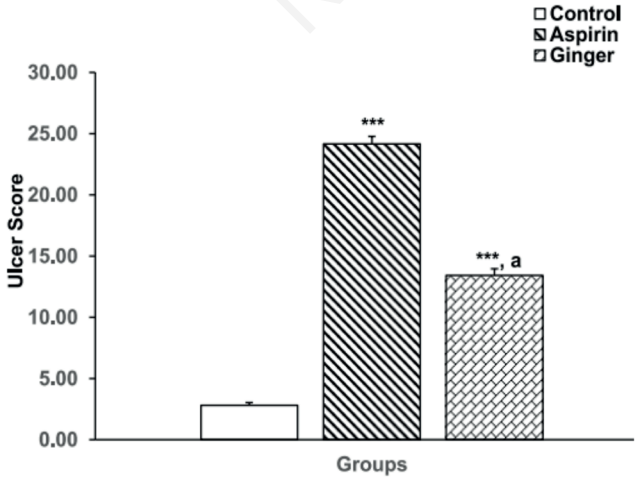

Figure 5. Ulcer scores of control and experimental groups. ***p<0.001 *vs* control; a, p<0.001 *vs* aspirin.

in the control and aspirin group at p<0.001. Also, pepsin secretion in aspirin group was significantly lower than that of the control groups at p<0.05.

Comparison of gastric ulcer scores between the control and test groups

The mean±SEM gastric ulcer scores of the control, aspirin, and ginger groups were 2.82±0.21, 24.17±0.60, and 13.42±0.54 (Figure 5). Comparison of ulcer scores in the aspirin and ginger groups showed a significant decrease in the ginger group at p<0.001. Also, comparison of ulcer scores in aspirin group and control group showed a significant increase in the aspirin group at p<0.001. Pictorial representation of ulcer scores in the aspirin, ginger, and control group (Figure 6) suggests that both anti-inflamma-

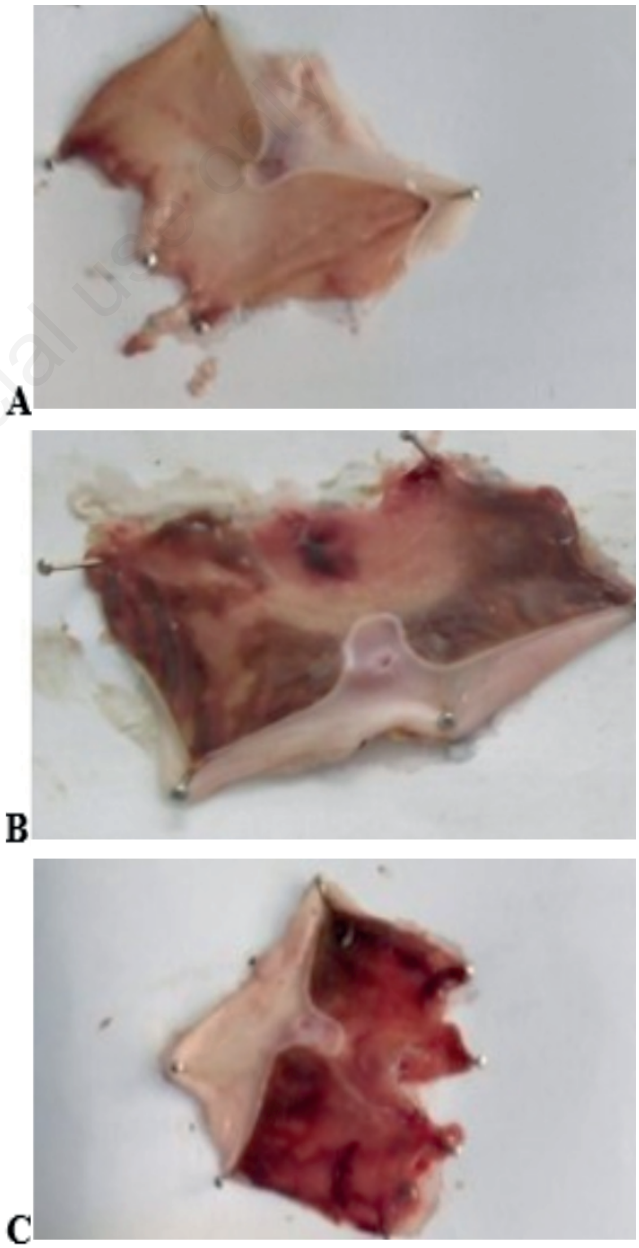

Figure 6. Pictorial representation of ulcer scores in aspirin, ginger, and control group. A, control; B, aspirin; C, ginger.

tory therapies (ginger and aspirin) possessed ulcerative properties. Nevertheless, counting the ulcer scores showed a reduced ulcerative effect in the ginger-treated group compared to the aspirin group as seen in Figure 5.

Discussion

The result of the body weight changes showed a significant decrease in the ginger and aspirin groups. This result is in line with an earlier report[9] which suggests that anti-inflammatory agents can trigger sustained weight loss. Inflammation is associated with weight gain and obesity.[7] Therefore, anti-inflammatory interventions such as aspirin and ginger might be a novel approach to the prevention of obesity and weight gain. Inflammation can lead to possible weight gain. Hence, the use of anti-inflammatory agents such as aspirin and ginger will be useful in reducing weight gain. According to Chen *et al.*,[9] NSAIDs are involved in reducing visceral body weight by reducing the fat deposits in the visceral. Ginger on the other hand has a lipid-reducing effect.[10] The lipid-reducing or cholesterol-lowering effect of ginger has been attributed to the presence of saponins.[11] Since cholesterol or fat deposits contribute to weight gain in an animal, the presence of saponins explains the reduction in weight of rats in the ginger group irrespective of their increased food intake.

The result of the gastric mucus output in the experimental animals showed a significant decrease in the ginger and aspirin group when compared to the control. Mucus protects the stomach wall from digestion by gastric enzymes. One of the mechanisms of cytoprotection is increased mucus secretion. Prostaglandins are one such substance known to increase mucus secretion in the stomach.[12] NSAIDs such as aspirin antagonize the prostaglandin system by its irreversible inhibition of the cyclooxygenase one (COX-1) enzyme. This irreversible inhibition of COX-1 explains the decrease in mucus secretion as seen in the aspirin group.

The phytochemical screening of ginger showed the presence of reducing sugar, flavonoids, alkaloids, and cardiac glycosides. Tannins, phlorotannins, anthraquinones, and anthranoids were absent in the phytochemical screening of ginger. Tannin is a phytochemical compound found in plants that is reported to act on protein towards forming a protective layer on the mucus membrane. Such a compound will likely reinforce the cytoprotective integrity of the stomach as it will increase the mucus layer of the stomach. Since the presence of tannin was not reported in the aqueous extract of *Zingiber officinale*,this could possibly be one of the reasons the ginger group has a low mucus content compared to the control and aspirin groups.[11]

The pepsin output result of the different experimental groups showed that there was a significant increase in pepsin secretion of rats in the ginger group when compared to those of the aspirin and control group. The pepsin secretion of rats in the aspirin group was significantly lower than those of rats in the control group. Increased peptic activity due to excessive secretion of pepsin causes peptic ulcers.[6] Following the result of this study, the ulcer scores of rats in the aspirin and ginger group were significantly higher than that of the control group. Ulcer scores of the rats in the aspirin group were significantly higher than that of the rats in the ginger group. Apart from excessive secretion of pepsin, other factors could lead to the damage of the gastric mucosal barrier such as inhibition of endogenous mediators (prostaglandins, epidermal growth factor, and sulfhydryls, etc.), decrease in gastric motility, increased mucosal blood flow and mucus secretion, etc.[13] Aspirin causes ulcers by the irreversible inhibition of cyclooxygenase one (COX-1) and modification of the enzymatic activity of cyclooxygenase-2 (COX-2) in the prostaglandin system. This, therefore, inhibits the production of prostaglandin and explains the increase of ulcerative effect seen in rats treated with aspirin when compared to the ginger and control group.[1]

It can therefore be concluded in this study that the use of ginger (an anti-inflammatory medicinal herb) as a therapy for treating inflammatory disorders is beneficial in terms of reducing ulcerative effects in the gastrointestinal tract as compared to the use of aspirin (a NSAID). Since ginger showed a significant decrease in the body weight changes of animals compared to the control group, it could be a useful measure to reduce weight gain in obese conditions.

References

1. Ghlichloo I, Gerriets V. Nonsteroidal anti-inflammatory drugs (NSAIDs). StatPearls Publishing; 2023.
2. Malik TF, Gnanapandithan K, Singh K. Peptic ulcer disease. StatPearls Publishing; 2023.
3. Ozkur M, Benlier N, Takan I, et al Ginger for healthy ageing: A systematic review on current evidence of its antioxidant, anti-inflammatory, and anticancer properties. Oxid Med Cell Longev2022:4748447.
4. Fletcher J. Why Is Ginger Good For You? Medical News Today; 2020. Available from: from www.medicalnewstoday.com/article/265990
5. Lorke, D. A new approach to practical acute toxicity testing. Arch Toxicol 1983;54:275-87.
6. Tan PV, Mezui C, Boda M. Anti-*Helicobacter* and gastroduodenal cytoprotective actions of the leaf aqueous extract of *Ocimum suave* (Lamiaceae). J Med Plants Res 2011;5:5958-66.
7. Alphin RS, Ward JW. An investigation of antihistaminic activity and gastric ulceration. Eur J Pharmacol 1969;6:61-6.
8. Shay H, Sun DC, Gruenstein M. A quantitative method for measuring spontaneous gastric secretion in the rat. Gastroenterology 1954;26:906-13.
9. Chen DM, Zhang ML, Shi ZQ, et al. Anti-inflammatory and anti-infectious dietary paradigms may be crucial for visceral weight reduction. Front Immunol 2019;10:422.
10. Thorpe W, Lamoreux K. 10 Natural Ways to Lower Your Cholesterol Levels. 2021. Available from:https://www.healthline.com/nutrition/how-to-lower-cholesterol
11. Osabor VN, Bassey FI, Umoh UU. Phytochemical screening and quantitative evaluation of nutritional values of *Zingiber officinale* (Ginger). Am Chem Sci J 2015;8:1-6.
12. Sembuligam K, Sembulingam P. Essentials of Medical Physiology: Jaypee Brothers Medical Publishers. New Delhi; 2012.
13. Gaskill 3rd HV, Sirinek KR, Levine BA. Prostacyclin-mediated gastric cytoprotection is dependent on mucosal blood flow. Surgery 1982;92:220-5.