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Evaluation of the Gastro-protective Potentials of Methanol Leaf Extract of *Nymphaea lotus* on Induced-Gastric Ulceration in Wistar Rats

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Abstract

Nymphaea lotus is a plant used traditionally for the treatment of many ailments including gastric ulcer without any scientific validation of its pharmacological action and safety. This study was undertaken to evaluate the possible gastro-protective potential of methanol extract of *N. lotus* leaves on gastric ulcers induced by different ulcerogens. Thirty rats (160-180 g) were divided into five groups: Group 1 was the normal control, group 2 was negative control, group 3 was administered 25 mg/kg Omeprazole (standard drug), groups 4, 5 and 6 were administered 200, 400 and 600 mg/kg methanol extract of *N. lotus* leaves, respectively. After two weeks of oral administration, gastric ulcer was induced in all the rats except group 1 using various ulcerogens such as 95% ethanol, indomethacin and hypothermic stress to access the gastro-protective potential of the extract. Phytochemical and anti-microbial analyses of the freshly prepared extract were carried out followed by histological studies of the gastric tissues. *Nymphaea lotus* leaves extract significantly ($p<0.05$) prevented peptic ulcers in a dose-dependent manner. The extract at 600 mg/kg showed a protective effect comparable to that of Omeprazole. Compounds having cyto-protective effect were detected in the extract. From the histological examination, pre-treatment with methanol extract at different doses preserved the functional structure of the whole mucosa, causing less pathological alterations in comparison to Omeprazole. Microorganisms (*Klebsiella pneumonia, Escherichia coli*, *Staphylococcus aureus, Salmonella typhi, Aspergillus niger and Pseudomonas aeruginosa*) tested in this study were inhibited by the methanol extract. The above findings indicate that methanol extract of *Nymphaea lotus* leaves has gastro-protective activities.

Keywords: *Nymphaea lotus*, phytochemicals, gastro-protective effect, ethanol, ulcer, Omeprazole

Introduction

Peptic Ulcer disease is considered one of the major forms of gastrointestinal disorder affecting over 14.5 million people worldwide. It is characterized by disruption in gastric mucosal infiltration through the muscularis mucosa (Garg *et al*., 2022; Rao *et al*, 2004). The pathophysiology of peptic ulcers caused by multiple etiologies involving an imbalance between invasive factors (pepsin, bile salt *Helicobacter pylori* and acid,) and protective factors (prostaglandin, nitric oxide, bicarbonate, growth factors and mucin,) (Hoogerwerf and Pasricha, 2001).

Many factors predispose an individual to peptic ulcer disease, and these include *Helicobacter pylori* infections, excessive use of anti-inflammatory drugs like NSAIDs, smoking, stress, family history, alcohol consumption and lower socio-economic status (Okwuosa *et al*., 2006). It should be noted that ulcerative disease is not fatal but could lead to serious complications, such as perforations, gastric outlet obstruction, gastrointestinal bleeding and ulceration that invades adjacent organs (Yau *et al*., 2017: Garg *et al*., 2022).

To achieve steady-state, various treatments, including herbal extracts are used to halt the secretion of excess gastric acid or stimulate mucosal protective mechanisms. These enhance mucus secretion, interfere with the synthesis of prostaglandins or stabilize surface epithelial cells (Dharmani and Palit, 2006). Synthetic drugs currently used to treat ulcers include proton pump inhibitors, prostaglandin analogues, antacids, and H_2 receptor blockers. Even though these drugs are very effective, numerous negative effects have been observed. Therefore, scientific screening of herbal medicine is of great importance for the development of safer and more potent anti-ulcer and anti-secretory agents (Garg *et al*., 2022).

Nymphaea lotus Linn (family: *Nymphaeaceae*) is a herbaceous aquatic plant whose leaves are either submerged or float in water (Aliyu *et al*., 2017). It is called water lily in English and *Bado* in Hausa. This plant prefers clear, warm and slightly acidic water and it is localized to Southern and central Europe, the Middle East, Asia, Northern Africa and West Africa, especially in Nigeria. According to rural dwellers, *Bado* leaves are used for the treatment of many ailments such as skin disease, diabetes, nervous ailment and ulcer. The medicinal benefits of water lily are attributed to its large number of pharmacological and nutritional components (Aliyu *et al.,* 2018).

However, the effectiveness of this therapeutic approach has not been established and this issue arises considering the areas where anti-ulcer drugs are not easily accessible. *Nymphaea. lotus* leaves have been reported to possess antioxidant potential by virtue of the abundance of antioxidant compounds in them that are capable of scavenging free radicals. (Aliyu *et al*., 2018).

Furthermore, to the best of our knowledge, no studies have been carried out to evaluate the gastro-protective activity of *N lotus* leaves. Therefore, this study was conducted to evaluate gastro-protective potential of methanol extract of *Nymphaea lotus* leaves against gastric ulcer induced by different ulcerogens including indomethacin, ethanol and hypothermic stress in rats.

Materials and Methods

Ethical Approval

The following research methodology and its ethics have been fully reviewed and approved by the Departmental Academic Board,

Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma, Katsina State, Nigeria.

Collection and Identification of plant material

Nymphaea lotus leaves were obtained from Guzu-Guzu Dam in Kabo Local Government Area, Kano State of Nigeria in February 2021 and identified by the taxonomist of Department of Biological Science, Federal University Dutsinma. The voucher specimen with reference number MA004 was deposited in the departmental herbarium.

Preparation of plant extract

Methanol extraction: Powdered *Nymphaea lotus* leaves (100 g) were soaked for 48 hrs. in 2 litres of 80% methanol. The extract was sieved through Whatman filter paper No. 1. The filtrate was evaporated to dryness using a rotary evaporator (Model type 349/2, Corning Ltd). The dried residue was kept at 4±2°C. The percentage yield of the methanol extraction obtained was 20.8 %. To obtain the concentration of extract required for the study, a known quantity of the methanol extract was dissolved in a 3% aqueous suspension of Tween 80.

Phytochemical tests

The phytochemical analyses of the methanol extract of *Nymphaea lotus* leaves were carried out using standard protocol (Harbour, 1998; Evans, 2009).

Animals

Healthy Wistar rats (weighing 120 to 150 g) of both sexes were purchased from Veterinary Institute Vom, Jos and kept in the animal house of the Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma. The animals were kept in cages for two weeks to allow for acclimatization before the commencement of the study. The rats were fed with water and standard rat food throughout the period of the study. The study was done according to OECD guidelines (EOCD, 2001).

Acute toxicity study

Acute oral toxicity study of aqueous and methanol extracts of the *Nymphaea lotus* leaves was carried out to determine the quantity of an ingested substance that kills 50 percent of a test sample (LD_{50}) by adopting a dosing schedule as per OECD guideline no. 425. Female albino mice (160-180 g) were used for the study. The rats were observed continuously for 12 hours to notice changes in behavioural or autonomic responses. Mortality for 24hours was observed.

Evaluation of the gastro-protective effect of methanol extract of *N. lotus* against gastric lesion induced by different Models.

The methanol extract of *N. lotus* was tested for it gastro-protective potential on ulcers induced by different ulcerogens in rats.

Ethanol-induced Gastric lesion

30 healthy albino rats (160-180 g) were used for the anti-ulcer activity study. The rats were divided into six (6) groups of five rats (5) each. Group 1 (normal control) received only standard rat food and water without pre-treatment. Group 2 (negative control) served as ulcer-induced control. Group 3 (standard drug group) received 25 mg/kg Omeprazole. Group 4, 5 and 6 received 200, 400 and 600 mg/kg methanol extract of *N. lotus* leaves, respectively. All the groups were given single dose of treatment for two weeks (14 days) via the use of gavage. After pre-treatment with the extract and the standard drug (Omeprazole) for two weeks, all the rats underwent 24 hours fast but with free access to water. Water was withdrawn two hours prior to ulcer induction. Gastric ulcer was induced by oral administration of 2 ml of 95% ethanol to each animal after 24 hours fasting (Hawkey, 1993). The rats were sacrificed through cervical dislocation two hours after the gastric ulcer was induced, and the stomach was removed after dissection. Following the removal of gastric contents, the stomach was rinsed with 0.9% saline, pinned onto a pliable board and ulcer lesions were measured using a ruler and hand lens magnified 10x. Scoring of the ulcer was performed as described by Okewumi and Oyeyemi (2013) with some modifications. Going by the method, 1mm or less was scored as 1 (erosion), 1 to 2 mm was scored as 2 and more than 2mm was recorded as 3. The ulcer index was calculated by dividing the overall score by a factor of 10 (Panda and Khambat, 2014).

$$\text{Ulcer Index} = \frac{\text{Ulcerated Area}}{\text{Total Stomach Area}} \text{ X } 100$$

The percentage of ulcer protection was calculated using the formula below (Djahanguiri,1969):

$$\text{Ulcer Protection (\%)} = \frac{(\text{Mean ulcer index of Ulcer control} - \text{Mean ulcer index of test}}{\text{Mean ulcer index of control}} \text{ X } 100$$

Gastric ulcers induced by Indomethacin

The gastro-protective activity of methanol extract of *N. lotus* leaves on gastric lesions in rats was evaluated using indomethacin, a well-known ulcerogen. The rats (160-180 g) were randomly divided into groups of five animals each and pre-treated as in the ethanol-induced ulcer. Rats were deprived of food for 24 hours before the commencement of the treatment. After pre-treatment with extract and the standard drug (Omeprazole) for two weeks, all the rats underwent 24 hours fast but with free access to water. Water was withdrawn two hours prior to ulcer induction. Indomethacin (100 mg/kg) was administered orally. After 2 hours, the rats were sacrificed through cervical dislocation two hours after the gastric ulcer was induced, and the stomach was removed after opening the body cavity. Following the removal of gastric contents, the stomach was rinsed with 0.9% saline, pinned onto a board and ulcer lesions. The degree of ulcer protection (Djahanguiri,1969) was also calculated as in the ethanol-induced model.

Gastric ulcer Induced by Hypothermic restraints stress.

Albino rats (of either sex) were randomly divided into groups of five animals each and pre-treated as in ethanol-induced ulcer. The rats then fasted for 24 hours but with free access to water. One (1) hour after treatment with extracts and drug, the rats were individually immobilized in retraining cages at a temperature of 4°C in a refrigerator for 2 hours. The rats were sacrificed, the stomach rinsed with 0.9% saline, pinned onto a pliable board and the ulcer lesions measured. Scoring of the ulcer was performed as described by Okewumi and Oyeyemi (2013) with some modifications. The Percentage of ulcer protection per animal was also calculated

(Djahanguiri,1969; Akah and Nwafor,1999) as in the ethanol-induced ulcer model.

Histopathological Examination

For histopathological studies, stomach fragments from each group were fixed in 10% formalin, embedded in paraffin wax, and stained with hematoxylin-eosin (Rtibi *et al.,* 2015).

Screening of microbial organisms

Helicobacter pyroli is a well-known enteric microbe involved in the development of peptic ulcer. Due to some challenges in culturing this organism, it was excluded in this antimicrobial study. However, other gastropathogenic microbes were used. The microorganisms considered in our study were *Klebsiella pneumonia, Escherichia coli*, *Staphylococcus aureus, Salmonella typhi, Aspergillus niger* and *Pseudomonas aeruginosa*. The strains of these organisms were obtained from the Federal Medical Centre Katsina, cultured and sub-cultured in the Laboratory of the Department of Microbiology, Federal University Dutsin-Ma. The organisms were sub-cultured and incubated at 25°C (for yeast and fungi) and 37°C (for bacteria). The method described by Lovian (1980) was employed in this study.

Results

Phytochemical analysis

Phytochemical analysis showed the presence of reducing sugars, tannins, alkaloids, saponins, cardiac glycosides, terpenoids, and flavonoids as shown in Table 1.

Acute toxicity study

Acute toxicity screening (LD_{50}) showed that both the aqueous and methanol extracts had an oral $LD_{50} > 6000$ mg/kg in rat.

Antiulcer activity of aqueous and methanol extracts of *N. lotus* Leaves

After 95% ethanol (2mL) was administered orally, the rats developed ulcers after two (2) hours and the results are shown in Table 2. All the concentrations (300 mg/kg and 600 mg/kg) of methanol extract of *N. lotus* leaves offered significant ulcer protective potencies against ethanol-induced ulceration with mean ulcer indices of 1.62 ± 0.06 and 1.20 ± 0.04 respectively when compared with the ulcer index of the negative control (3.85 ± 0.08). The methanol extracts of *N. lotus* had less ulcer protective potency compared with the positive control (Omeprazole). Table 2 also indicated that the two concentrations (300 mg/kg and 600 mg/kg) of aqueous extract of *N. lotus* leaves had a significantly lower anti-ulcer activity when compared with methanol extract at the concentration of 600 mg/kg. The methanol extract of *N. lotus* leaves at the concentration of 600 mg/kg had comparable potency with the standard control drug.

Gastro-protective effect of methanol extract of *N. lotus* on ethanol-induced gastric ulcers

The gastro-protective effect of methanol extract is presented in Table 2. The result showed that rats pre-treated with Omeprazole and different doses of methanol extract of *N. lotus* leaves significantly ($p<0.05$) reduced the gastric lesions as compared to the negative control in a dose-dependent manner. Methanol extract at all doses (200, 400 & 600 mg/kg) produced gastro-protective effects by as much as 54.15, 63.40 and 74.22%, respectively, comparable to the reference drug, Omeprazole (83.37%).

Gastro-protective effect of methanol extract of *N. lotus* leaves against Indomethacin-induced gastric lesion

The methanol leaves extract of *N. lotus* (200, 400 and 600 mg/kg) at all doses showed significant ($p<0.05$) dose-dependent protection of rats against ulcers induced by Indomethacin and is presented in Table 3. The gastro-protective effect after pre-treatment with methanol extract of *N. lotus* at 200, 400 and 600 mg/kg is up to 55.01, 63.23 and 71.97% from indomethacin-induced ulceration compared to the effect displayed by reference drug (Omeprazole).

Gastro-protective activity of extract on hypothermic restraint stress-induced gastric ulcer

The result of hypothermic restraint stress ulcer model is presented in Table 4. The pre-treatment with methanol extract of *N. lotus* remarkably ameliorated the ulcer index in the cold stress-induced ulcers in rats. The oral administration of Methanol Extract of *Nymphaea lotus* (MENL) (200, 400 and 600 mg/kg) significantly ($p<0.05$) reduced the gastric ulcer indices to 1.17± 0.04, 1.29 ± 0.03 and 1.11 ± 0.06, respectively, compared to the ulcer control group (3.46 ± 0.02). These findings were comparable to the reduction produced by the reference drug, Omeprazole (0.56 ± 0.08) (Table 4).

Histopathological studies

Figures 1 to 5 show the histological investigations of normal control, ulcer control (negative control) rats and rats pre-treated with Omeprazole, and methanol extract at 200, 400 and 600 mg/kg body weight.

The results showed that injuries were not observed in the gastric mucosa for the normal control group (Figure 1). Rats that received ethanol without pre-treatment (ulcer control group) exhibited severe lesions around the gastric portion of the stomach (as presented in Figure 2). Disruptions to the surface mucosal epithelium, leucocytes infiltration and chronic oedema in the submucosal layer were also seen. The rats that received Omeprazole (25 mg/kg body weight) displayed normal epithelium (Figure 3) just as seen in control rats (Figure 1).

There was moderate disruption of epithelial mucosa of rats given 200 mg/kg of methanol extract (Figure 4), but there were minor haemorrhages in some rats given 400 m/kg of methanol extract (Figure 5). Gastric lesions were not observed in rats pre-treated with 600 mg/kg of methanol extract (Figure 6).

Antimicrobial activity of methanol extract of *N. lotus* leaves

The sensitivity of the microorganisms to the methanol extract is presented in Table 5. *Pseudomonas aeruginosa* exhibited the highest sensitivity to methanol extract. The methanol extract of *N. lotus* inhibited the growth of all microorganisms tested. *Salmonella typhi,* and *Aspergillus niger* displayed the least sensitivity to the effect of the extract used.

Discussion

Traditional healers have employed *N. lotus* leaves to treat many conditions, including ulcer (Rtibi *et al.,* 2015). The result of acute toxicity test revealed that all the rats used in each experimental group survived, demonstrating that the LD_{50} is

greater than 6000 mg/kg (OECD, 2001). Therefore, the absence of mortality in different doses of these extracts administered in the experimental procedures ensured the safety of the doses (Fernandes *et al.,* 2010; Haule *et al.,* 2012; Rtibi *et al.,* 2015).

The gastro-protective effect of methanol extract of *N. lotus* was evaluated in ulcer model induced by ethanol. It is well-established that ethanol causes gastric sore by directly damaging gastric mucosal cells, leading to increased lipid peroxidation and the development of free radicals in the system (Krishna *et al.,* 2014; Yau *et al*., 2017). The stomach is sensitive to ethanol. Oral administration of 95% ethanol disrupts the cyto-protective mechanism in *vivo* and induces gastric mucosal damage by making the mucus membrane vulnerable to hydrochloric acid attack (Rao *et al.,* 2004). The extract at different doses reduced the mean ulcer indices to various degrees. The reduced ulcer index value compared to the control group indicated that the methanol extract of *N. lotus* has significantly protected the gastric mucosa against ethanol challenge, pointing to the extract's strong free radical scavenging and cytoprotective effect. The antioxidant potential of *N. lotus* was reported by Aliyu *et al.* (2018). The effect of methanol *N. lotus* extracts followed a dose-dependent manner and the gastro-protective effect recorded in rats treated with extracts was comparable to that of omeprazole (standard drug), a drug popularly used for gastric ulcer treatment. A further increase in the concentration of extracts might have an even greater effect than that of Omeprozole.

All the doses of the methanol extract of *N. lotus* leaves significantly reduced mucosal damage in the indomethacin-induced ulcer model suggesting the possible mobilization and involvement of prostaglandin (Jude *et al*., 2011). A genotoxic drug such as Indomethacin and other related non-steriodal anti-inflammatory drugs (NSAIDs) induce gastric lesion by halting endogenous prostaglandins biosynthesis (Akinbo and Eze, 2017; Okpalaeke *et al*., 2019). Prostaglandins have been reported to possess gastro-protective activity of stabilizing gastric microcirculation (Vane, 1971), enhancing bicarbonate and mucous secretions, and inhibiting the secretion of gastric acid (Akah *et al*., 2007). Furthermore, prostaglandin inhibition results in successive synthesis of leukotrienes, which could bring about vasoconstriction of mucous and decrease the flow of blood (Dajani and Agrawal, 1995). As a result, the ability of the extract to significantly confer protection on the rats against indomethacin-induced ulcers suggests a possible cyto-protective effect, probably mediated by increasing the production of prostaglandin (Jalilzadeh, 2015).

The hypothermic restraint stress model has been linked with increased secretion of gastric acid and a reduction in pH (Murakami *et al*., 1985). Cold restraint induces physical and psychological stress in rats, causing the release of histamine in the stomach; this leads to increased secretion of gastric acid and decreased production of mucus which consequently leads to the development of ulcers (Bhajoni *et al*., 2016). Several studies have also reported the possible involvement of leukotriene-C4 (LTC4) in ulcers induced by stress. Thus, histamine is believed to be a key player in the pathogenesis of ulcers induced by stress since it is a potent activator of gastric acid secretion (Akah, 2007). The methanol extract of *N. lotus* displayed a significant dose-dependent reduction in the ulcer index induced by hypothermic restraint stress, suggesting the role of histamine in its mechanism (José *et al*., 2017). Further studies are needed to elucidate the detailed mechanism. The pathogenesis of ulcers is caused by numerous aetiologic factors. However, the ability of the extracts to protect against ulcers caused by ethanol may suggest that they could prevent the development of ulcers by inhibiting one or more different inciting stimuli (Rao *et al*., 2004).

Several peptic ulcer disorders have been linked to the formation of colonies in the gastrointestinal system by microorganisms (Akah *et al*., 2007). Even though *H. pylori* could not be used in this study, the methanol extract had inhibitory effect on the growth of other Gram-negative enteric organisms that are in the same class as *H. pylori*, which can be attributed to the plant extract's antiulcer properties.

Many phyto-constituents, including alkaloids, flavonoids, terpenes, reducing sugars and tannins were identified via phytochemical assay. These phytochemicals are reported to have antiulcer activity (Aktay *et al.,* 2004). Flavonoids have gastroprotective and antiulcer effects. The biological effects of flavonoids have been explained by numerous gastroprotective mechanisms which include increases in mucosal PGE_2, increases in mucosal blood flow, free radical scavenging during hyperoxidation of the lipid membrane, decreases secretion of histamine from mast cells, mediated through inhibition of histidine carboxylase, inhibition of H^+/K^+-ATPase, inhibition of *H. pylori* growth and acts as free radical scavengers (Sharath *et al*., 2015). However, because of their effects on protein precipitation and vasoconstriction, tannins may inhibit the onset of ulcers. Their astringent effect assists to precipitate microproteins at the ulcer site, creating an impenetrable layer on the mucosa lining that prevents gut secretions and protection from toxins and other irritants and promotes PGE_2 production (Bhajoni *et al*., 2016). Terpenes are known to have antiulcer potential, and it has been revealed that this activity is brought about by activation of cellular protection, decreasing gastric vascular permeability and a reduction of mucosal prostaglandins metabolic-cyto-protective action. While tannins transform the mucosal outermost layer irresponsive to chemical stimulation, saponins may activate factors involved in mucous membrane protection (Borelli and Izzo, 2000).

In addition, the extracts contained alkaloids, which are known to have an impact on mucous membrane integrity. According to Neelam *et al.* (2012), alkaloids like hyoscine-N-methyl bromide have been reported to decrease the secretion of acids (Borelli and Izzo, 2000).

Conclusion

Our findings have revealed that the methanol extract of *N. lotus* leaves possesses gastro-protective activity. This result could justify the traditional use of this plant in the treatment of ulcers.

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