American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS American Journal of Biochemistry and Molecular Biology

ISSN 2150-4210
DOI: 10.3923/ajbmb.2020.35.44

Research Article
Regulation of Connective Components in Indomethacin-induced Gastric Ulcer Healing in Wistar Rats

[1]S.Tijani Abiola, [1,2]Fasawe M. Oluwaseun, [1]Mrakpor O. Ono-jefe-eroro and [3]Ebenezer O. Farombi

[1]Department of Biochemistry, Bowen University, Iwo, Osun State, P. O. Box 284, Nigeria
[1,2]College of Health Science, Bowen University, Iwo, Osun State, P. O. Box 284, Nigeria
[3]Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria

Abstract

Background and Objective: There are growing evidences that non-steroidal anti-inflammatory drugs (NSAIDs)-induced depression of mitochondrial functions and activation of collagenase by lesions in the stomach. Therefore, this study sought to investigate possible mechanism of action of oleic acid rich fraction of n-hexane extract of Chasmanthera dependens (OHECD) in indomethacin-induced gastric ulcer. **Materials and Methods:** Gastric ulcer was induced using indomethacin orally. Gastric ulceration was assessed and gastric ulcer healing were analyzed by assessing the ulcer score, ulcer index, percentage healing, mucus content and levels of proline, hydroxyproline, nitric oxide and activities of pepsin and citrate synthase in the serum and gastric tissues. **Results:** Indomethacin (IND) caused severe gastric mucosa damage with ulcer score and ulcer index of 3.75±0.20 and 2.62±0.37 relative to control group at $p<0.05$, respectively. However, treatments with OHECD and standard drug cimetidine (CIM) significantly heal the ulcer with percentage ulcer healing of 61.60±0.42, 74.36±0.86 for different doses of OHECD and CIM, 68.15±0.20 respectively. Similarly, indomethacin administration decreased mucus content, proline, hydroxyproline, nitric oxide and citrate synthase activity with concomitant increase in pepsin activity, interestingly, OHECD or CIM significantly increased mucus content, proline, hydroxyproline, nitric oxide and citrate synthase activity with concomitant attenuation in pepsin activity. **Conclusion:** Overall, administration of OHECD stem promotes gastric ulcer healing by up regulating connective tissue components and recovery of mitochondrial function by increasing the mucus content, proline, hydroxyproline, nitric oxide and citrate synthase activity and decreased the peptic activity from drug-induced assault.

Key words: Chasmanthera dependens, gastric ulcer, mitochondrial, collagenase, drug induction, anti inflammatory, lesions

Citation: S. Tijani Abiola, Fasawe M. Oluwaseun, Mrakpor O. Ono-jefe-eroro and Ebenezer O. Farombi, 2020. Regulation of connective components in indomethacin-induced gastric ulcer healing in wistar rats. Am. J. Biochem. Mol. Biol., 10: 35-44.

Corresponding Author: Tijani S. Abiola, Department of Biochemistry, Bowen University, Iwo, Osun State, P.O. Box 284, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed drugs in the world and are extensively used to alleviate clinical cases of pain and inflammation[1], prevention and treatment of ischemic heart disease[2] and neoplasia[3]. However, these drugs are well known for stomach ulceration and delayed ulcer healing properties[4] by interfering with the action of growth factors, decreasing epithelial cell proliferation in the ulcer margin, decreasing angiogenesis in the ulcer bed and slowing the maturation of the granulation tissue[5]. Significant enhancement of gastric ulcer healing is promoted by a number of drugs including proton pump inhibitors, H_2 receptor antagonists, antisecretory drugs (e.g., omeprazole), prostaglandin analogs, mucosal defense agents (e.g., sucralfate) and various growth factors[6], but clinical evaluation of these drugs have shown incidence of relapses, side effects and drug interactions[7]. A drug with multiple mechanism of protective and healing action may be highly effective in minimizing tissue injury in human diseases. It has been observed that herbal drugs and formulations which possess potent antioxidant property are effective in healing experimentally induced gastric ulcer[8,9]. Furthermore, mechanisms underlying delayed healing need to be well understood so that new therapies can be developed[10] and the increasing interest in traditional medicines have been attributed to the economic advantage they provide, the accessibility and assumed safety they offer when compared to conventional medicines[11,12].

Chasmanthera dependens (Hochst) belongs to the family Menispermaceae is usually used in traditional medicine as a remedy for treating a variety of ailments. It is used medicinally for sprains and bruises[13], venereal diseases, topically as pain killers, as a general tonic for physical or nervous debilities[14] and has also been reported to possess anti-inflammatory and analgesic effects on laboratory animals[15,16], antimicrobial properties[17], antifungal activity[18] and fertility enhancement properties[19]. Its phytoconstituents include alkaloids such as; protoberberine, jatrorrhizine, palmatine, columbamine, lysicamine (oxonuceferine), O,O-dimethylcorytuberine, anonaine as well as the furanoid diterpene 8-hydroxycolumbine, flavonoids, tannins, essential oils like oleic acid etc.

Recently, different studies shown that the methanol extract of *Chasmanthera dependens* has significant gastro protective activity against indomethacin-induced gastric ulceration in rats[20] by its antioxidant action. Hence, objective of the present study was to observe the methanol extract of *Chasmanthera dependens* to prepare the oleic acid-enriched extract (designated as OHECD in this study) and test edits gastric ulcer healing activity in rats.

MATERIALS AND METHODS

Study area: *Chasmanther adependens* stems were collected in July, 2017 from Iwo, Osun state, Nigeria. Iwo is located on latitude 7.6292°N and longitude 4.1872°E.

Chemicals and drugs: Tris-HCl buffer, Triton X-100, Griess reagent, sodium nitrite, Bovine serum albumin, sodium hydroxide, Folin-Ciocalteu reagent, sodium hydroxide, copper sulphate, sodium carbonate, sodium potassium tartrate, chloramine-T, Ehrlich's reagent, sodium acetate trihydrate, hydroxyproline, ammonium hydroxide solution (NH_4OH), magnesium chloride ($MgCl_2$), ferric chloride ($FeCl_3$), ninhydrin, proline, tyrosine, alcian blue, sucrose, orcinol, n-Hexane, citric acid monohydrate and n-propanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA), indomethacin (Fabrique par: Yangzhou No.3 Pharmaceutical Company Limited, Jiangsu, China) and cimetidine (Glaxosmithkline) were obtained from Pharma Aid, a licensed pharmaceutical store in Nigeria. All other chemicals/reagents used were of analytical grade.

Plant collection, authentication and preparation: Fresh stems of *Chasmanthera dependens* stems were obtained from Iwo, Osun state, Nigeria, authenticated by Mr. Esimekhuai DPO at the Department of Botany of the University of Ibadan, Nigeria voucher specimen (UIH-22478) was deposited at the University herbarium.

The shade dried *Chasmanthera dependens* stems were pulverized into coarse powders using a blender (bravo 3 jars mixer grinder). The oleic acid rich fraction of *Chasmanthera dependens* was obtained using a modified method of Ogunlesi *et al.*[21], the essential oil from the pulverized stem was collected into hexane with the use of a Soxhlet extractor, yielding a pale-yellow liquid. The n-hexane was removed from the pale-yellow liquid using rotary evaporator at 40°C and the yellow-liquid was stored at -4°C until ready to use.

Animals: Forty adult male Wistar rats weighing (150±20 g) were purchased from the Central Animal House, Faculty of Basic Medical Science, College of Medicine, University of Ibadan, Ibadan, Nigeria. Animals were kept in a temperature-controlled room (25±2°C) with 12 h light and 12 h dark cycle. The rats were kept under standard laboratory conditions and were fed with standard rat's pellet (Ladokun Feeds, Nigeria)

with fresh water *ad libitum*. They were acclimatized for 7 days, after which they were randomly divided into five groups. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of animals[22] and as approved by the Research Ethical Committee, Bowen University, Nigeria. The "Principle of Laboratory Animal Care" (NIH publication No. 85-23) guidelines and procedures were considered in this study[23]. The rats were deprived of food for 24 h but had free access to clean water prior to the commencement of the experiment.

Experimental protocol for ulceration and assessment of healing: Forty male Wistar rats with average weight of 150.0±20.0 g were divided into five groups of eight rats per group. Rats in group 1 served as the control rats and received 1 mL kg^{-1} b.wt., corn oil. The rats in group 2, 3, 4 and 5 received 30 mg kg^{-1} b.wt., indomethacin to induce gastric ulcer according to the procedure described by Bhattacharya *et al*.[24]. Twenty four hours after gastric ulcer induction, rats in groups 3 and 4 were post-treated with 200 mg kg^{-1} and 400 mg kg^{-1} b.wt., oleic acid rich fraction of n-hexane extract of *Chasmanthera dependens* (OHECD) and rats in group 5 received 50 mg kg^{-1} b.wt., of cimetidine (CIM) a standard drug, respectively for 7 days. All administration was done orally (p.o) and corn oil was used as vehicle to dissolve all the drugs.

After 24 h of the last doses of OHECD or CIM, all the rats were anaesthetized with ether and stomach of each rat was removed and excised along the greater curvature and ulceration was scored. Gastric juice was used for determining ulcerogenic parameters, post-mitochondrial fraction of the stomach was used for all other biochemical assays and stomach sections were used for histological assessment.

Collection of the gastric juice: After the animals were sacrificed, their stomachs were removed following ligature of the oesophocardiac junction, washed with distilled water and dried between filter paper and opened along the greater curvature. The gastric juice was drained and centrifuged at 2000 rpm for 10 min and used for the determination of all ulcerogenic parameters.

Preparation of gastric mucosal tissue: Portions of each gastric tissue were homogenized with 200 mM potassium phosphate buffer, pH 7.4 with a homogenizer. Tissue homogenates were centrifuged for 15 min at 10,000 rpm and 4°C and then the supernatants were removed and used for the biochemical analysis.

Determination of ulcer score and ulcer index: Degrees of ulceration in the animals were quantified[25]. The damage scores were assessed by grading the gastric injury on a 0-4 scale based on the severity of hyperemia and hemorrhagic erosions, 0: Normal mucosa, 0.5: Hyperemia, 1: One or two lesions, 2: Severe lesions, 3: Very severe lesions and 4: Mucosa full of lesions (lesions-hemorrhagic erosions, hyperemia-vascular congestions). The sum of the total scores divided by the mean damage score is expressed as the damage score. The experiments were performed by two investigators blinded to the groups and the treatment of the rats. Percentage healing for each group was calculated using the formula:

$$\text{Healing (\%)} = \frac{\text{UIC} - \text{UIT}}{\text{UIC}} \times 100$$

Where:
UIC = Ulcer index for control
UIT = Ulcer index for test

Determination of gastric pH content, gastric juice volume, total acidity and acid output: The effect of indomethacin, oleic acid rich fraction of n-hexane extract of *Chasmanthera dependens* (OHECD) and cimetidine (CIM) on these parameters were determined using the method of Boeing *et al*.[26]. The total acidity acid and outputs were calculated using the following equations:

$$\text{Total acidity (mEq L}^{-1}) = \text{Volume of NaOH} \times \text{Normality of NaOH} \times 100/0.1$$

$$\text{Acid output } (\mu\text{Eq h}^{-1}) = \text{Acidity (mEq L}^{-1}) \times \text{Volume of gastric juice (mL 4 h}^{-1})$$

Determination of the mucin and mucus contents: Gastric mucosal mucus content was determined by the method of Martins *et al*.[27] and mucin content was determined according to the method of Winzler[28].

Determination of the levels of nitric oxide, hydroxyproline and proline: Gastric mucosal homogenate nitrite and nitrate were estimated as index of Nitric Oxide (NO) production and was quantified based on the Griess reaction as described by Cortas and Wakid[29]. Hydroxyproline level was determined according to the method of Reddy and Enwemeka[30] and proline level was estimated by the method of Troll and Lindsley[31].

Estimation of pepsin activity: The effect of indomethacin, oleic acid rich fraction of n-hexane extract of *Chasmanthera*

dependens (OHECD) and cimetidine (CIM) were assessed on the peptic activity was determined colorimetrically according to the method of Boeing *et al.*[26].

Determination of gastric citrate synthase activity: Gastric mucosal citrate synthase activity was determined by the method described by Lewis[32].

Histological assessment of the gastric tissue: The fundic portion of stomach was sectioned for histological studies. The tissue samples were fixed in 10% formalin and embedded in paraffin. The sections (5 μm) were cut using microtome, stained with hematoxylin and eosin and assessed under an Olympus microscope (BX41, Hamburg, Germany).

Statistical analysis: The results were expressed as mean±standard error of mean (mean±SE) (n = 8 in each group). Data were analyzed by one-way analysis of variance. Means values were compared using Duncan test. The SPSS statistical package by IBM was used and the value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Effect of OHECD on ulcer score, ulcer index, gastric content pH and gastric juice volume: Oral administration of indomethacin to rats caused ulceration in the gastric mucosal of the rats as revealed in the marked increase in ulcer score, ulcer index and gastric juice volume with concomitant decrease in the gastric content pH as shown in Fig. 1a-d when compared to control group. The ulcer score and ulcer index for the indomethacin untreated group were found to be 3.75 and 2.62, respectively for the 14 days of the study. Treatment with 200 and 400 mg kg^{-1} OHECD produced a dose dependent decrease in ulcer score and ulcer index. Cimetidine significantly ($p<0.01$) decreased the ulcer score and ulcer index. Furthermore, OHECD and CIM significantly ($p<0.01$) decreased the gastric juice volume almost the same value with increase in pH concomitantly.

Effects OHECD on total acidity and acid output: Oral administration of indomethacin increased the total acidity and acid output of the ulcerated untreated group when compared with the control group as shown in Fig. 2a, b, but treatment with different doses of oleic acid rich fraction of *C. dependens* and cimetidine decreased these parameters significantly ($p<0.01$) with the higher dose of oleic acid rich fraction from *C. dependens* (400 mg kg^{-1}) more effective than cimetidine.

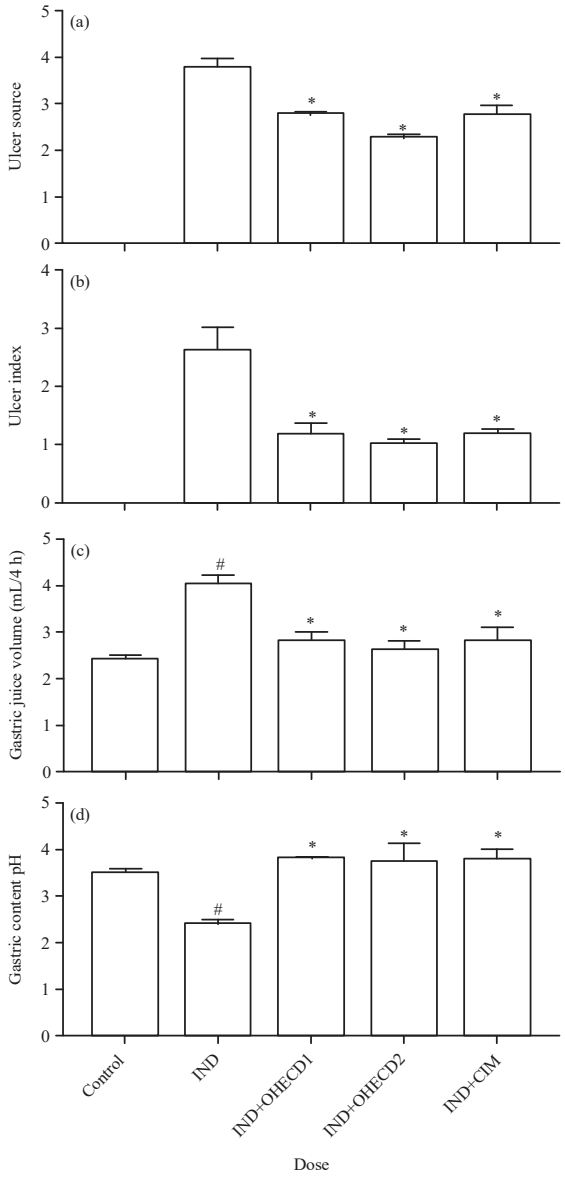

Fig. 1(a-d): Bar graphs showing effect of oleic acid rich fraction of n-hexane extract of Chasmanthera dependens on (a) Ulcer score, (b) Ulcer index, (c) Gastric juice volume and (d) Gastric content pH. Each bar represents mean values±S.E. of 8 rats/group

IND: 30 mg kg^{-1}, OHECD1: 200 mg kg^{-1}, OHECD2: 400 mg kg^{-1} and CIM: 50 mg kg^{-1}.*$p<0.01$ significantly different from the IND group, #$p<0.01$ significantly different from the control group

Effect of OHECD on mucin and mucus contents: Oral administration of indomethacin caused a significantly ($p<0.01$) decrease in the mucin and mucus contents in the stomach of the rats when compared with the control group (Fig. 3a, b). Groups treated with 200 and 400 mg kg^{-1} of OHECD showed

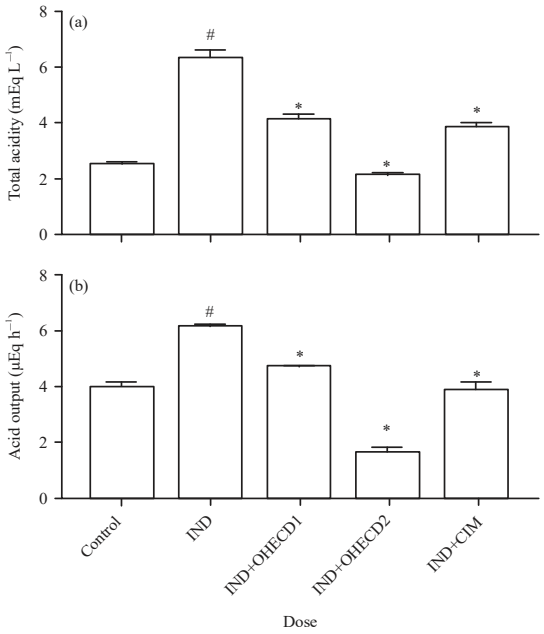

Fig. 2(a-b): Effect of oleic acid rich fraction of n-hexane extract of Chasmanthera dependens on (a) Total acidity and (b) Acid output
IND: 30 mg kg^{-1}, OHECD1: 200 mg kg^{-1}, OHECD2: 400 mg kg^{-1} and CIM: 50 mg kg^{-1}. *p<0.01 significantly different from the IND group, #p<0.01 significantly different from the control group

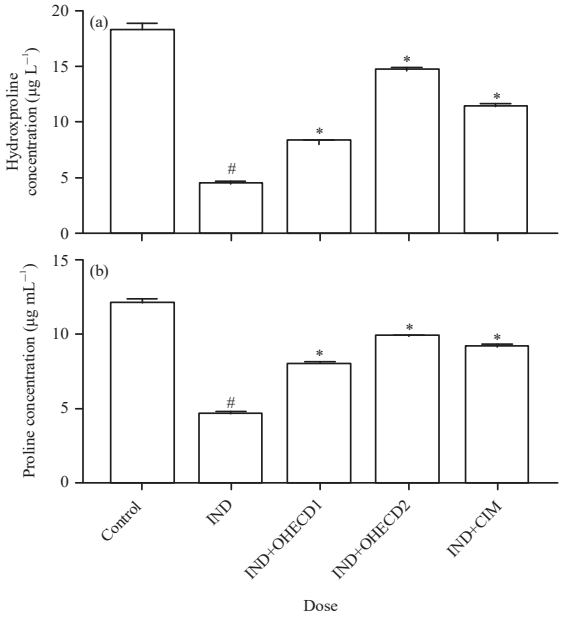

Fig. 4: Effect of oleic acid rich fraction of n-hexane extract of Chasmanthera dependens on (a) Hydroxyproline level and (b) Proline level
IND: 30 mg kg^{-1}, OHECD1: 200 mg kg^{-1}, OHECD2: 400 mg kg^{-1} and CIM: 50 mg kg^{-1}. *p<0.01 significantly different from the IND group, #p<0.01 significantly different from the control group

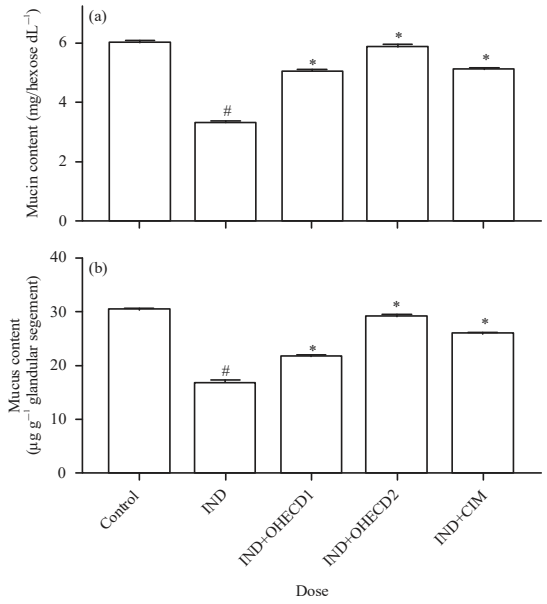

Fig. 3(a-b): Effect of oleic acid rich fraction of n-hexane extract of Chasmanthera dependens on (a) Mucin content and (B) Mucus content
IND: 30 mg kg^{-1}, OHECD1: 200 mg kg^{-1}, OHECD2: 400 mg kg^{-1} and CIM: 50 mg kg^{-1}. *p<0.01 significantly different from the IND group, #p<0.01 significantly different from the control group

a dose dependent increase in these parameters. Furthermore, group treated with the standard drug showed notable increase in the mucin and the mucus contents.

Effect of hydroxyproline and proline levels: Compared to the control group, tissue hydroxyproline and proline levels were decreased in the ulcerated untreated rats (Fig. 4a, b). But treatments with OHECD or cimetidine significantly increased hydroxyproline and proline. OHED at 400 mg kg^{-1} showed a marked increase than cimetidine.

Effect of OHECD on nitric oxide level, pepsin and citrate synthase activities: Administration of indomethacin reduced tissue NO level and citrate synthase activity in the ulcerated untreated rats when compared to the control group (Fig. 5a-c). Tissue level of NO and citrate synthase activity were markedly increased in the OHECD-treated groups, respectively. Also, group treated with CIM showed marked increase in NO level and citrate synthase activity with 400 mg kg^{-1} of OHECD showing a better up regulation of citrate synthase than cimetidine. Mucosal pepsin activity was markedly increased in the ulcerated untreated rats when compared to the normal control. But treatment with OHECD

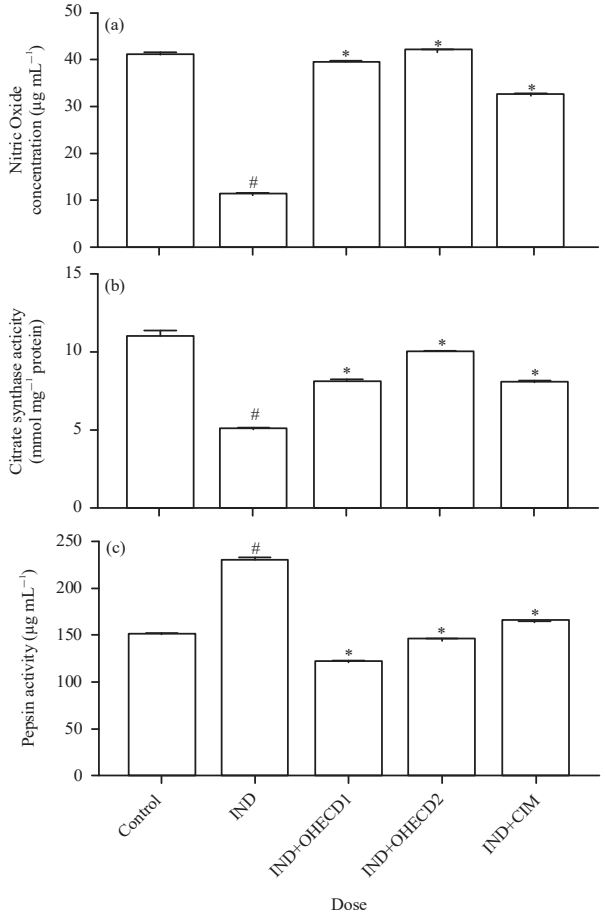

Fig. 5(a-c): Effect of oleic acid rich fraction of n-hexane extract of Chasmanthera dependens on (a) Nitric oxide level, (b) Citrate synthase activity and (c) Pepsin activity

IND: 30 mg kg^{-1}, OHECD1: 200 mg kg^{-1}, OHECD2: 400 mg kg^{-1} and CIM: 50 mg kg^{-1}. *p<0.01 significantly different from the IND group, #p<0.01 significantly different from the control group

and CIM significantly (p<0.01) reduced the activity of this enzyme with 400 mg kg^{-1} OHECD given a better reduction than CIM (Fig. 5c).

Assessment of ulcer healing: Administration of indomethacin (30 mg kg^{-1}) orally extensive ulceration in the glandular portion of the gastric mucosa in the stomach of the rats as evident in the histological assessment of indomethacin untreated group when compared with the control group as shown in Fig. 6a, b. Rats treated with OHECD (200 and 400 mg kg^{-1}) produced a better architectural structure (Fig. 6c, d) and rats that received 50 mg kg^{-1} CIM also show some level of healing as revealed in Fig. 6e.

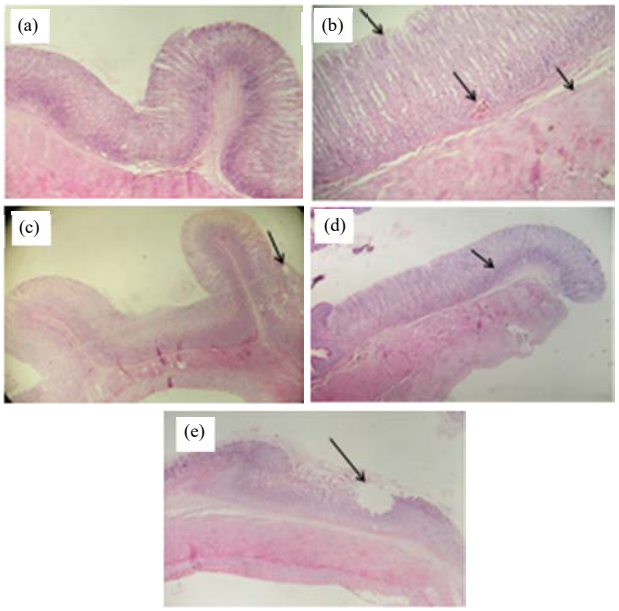

Fig. 6(a-e): Representative histology of gastric tissue sections are shown at 10x magnification, (a) Normal control showed normal architectural structure of the gastric mucosa and submucosal layers, (b) Ulcerated untreated showed gastric lesion ulceration with inflammatory cells infiltration (arrow) and oedema in submucosal layer, (c) Ulcerated+200 mg kg^{-1} OHECD showed reduced gastric lesions with normal mucosa and oedema in submucosal layer, (d) Ulcerated+ 400 mg kg^{-1} OHECD: showed normal mucosal and submucosal layers with very few infiltration and (e) Ulcerated +50 mg kg^{-1} CIM: showed markedly reduced gastric lesions with normal mucosa and some inflammatory cells infiltration (arrow)

DISCUSSION

The process of gastric ulcer healing is a tightly coordinated process which is regulated by transcription factors, growth factors and cytokines[33]. The balance between the aggressive and protective factors in the stomach is vital to obtain good quality of gastric ulcer healing[34]. Reports have shown that oleic acid has some notable gastroprotective and healing activities, as it reduces acid secretion, stimulates prostaglandins synthesis (especially prostaglandin E_2 known to increase cell proliferation and prostacyclin known to stimulate mucosal blood flow and mucus-bicarbonate secretion), thus increasing the pH and setting the conditions for faster healing[35,36]. The remarkable healing ability of the

methanol extract of *Chasmanthera dependens* stem in our previous study against the indomethacin-induced gastric ulcer encouraged us to delve into this present study of the probable modulatory effect of the OHECD on gastric ulcer healing.

Biochemical analysis of gastric secretions (for pH, gastric volume, bicarbonate and pepsin) and mucosal integrity for stomach is usually employed to ascertain its status following exposure to pharmacological agents[37]. The pH gives an idea of the level of acidity and volume of gastric secretions. Low pH value is a manifestation of decreased hydrogen ion concentration in gastric juice. This has been linked to pathogenesis of gastric ulceration and delayed healing in experimental animals[38]. Abdallah *et al*.[39] have also attributed gastrointestinal injury to eroded mucin content. This erosion is facilitated by onslaughts of both internal (pepsin and oxidants produced in the gastric lumen) and external (drugs and chemicals) aggressive agents on mucosal epithelia.

This present study revealed that indomethacin induced aggressive factors via increase gastric juice volume, free and total acidity as well as a decrease in gastric juice pH indicated altered hydrophobicity, this is in accordance with the earlier report of Sabiu *et al*.[40].

In the present study, the significant increase in ulcer score, ulcer index and gastric juice volume following oral administration of indomethacin in the ulcerated rats may be attributed to either free radicals formation or inhibition of prostaglandin synthesis. Decreased prostaglandin level has been attributed to impaired gastric ulcer healing and increased gastric acid secretion which are important events in the etiology of mucosal ulceration. This correlates with numerous reports[37,41,42] where indomethacin was reported to have caused alterations in gastric secretions of rats. Conversely, post-treatments with the OHECD significantly reduced these parameters. Furthermore, CIM also decreased these parameters suggested that both the extract and CIM may have similar mechanisms of action. These results agree with other studies[26,43].

Gastric mucus and mucin content are the first line of defense of the stomach, which prevents acid and pepsin from destroying the gastric wall[44,45]. Moreover, gastric mucus has been reported to play important role in healing of ulcer[46]. A combination of events including release of preformed mucus, wound retraction and re-epithelialization are involved in ulcer-healing process after toxicological injury[47,48]. Besides providing significant buffering capacity for the neutralization of luminal acid, the mucus also offers protection against both endogenous aggressors and exogenous gastro toxic agents such as indomethacin, thereby enhancing the rate of local healing process[49]. In this study, the increased pepsin activity coupled with decrease in mucin secretion in the indomethacin-ulcerated rats reduced protective ability of the mucosal membrane against hemorrhage, thus, resulting in tissue damage. This implied decreased ability of the gastric mucosa to withstand the offensive onslaught of indomethacin. Treatment with OHECD facilitated ulcer healing process, which is associated with decreased pepsin activity and elevated mucin level in the gastric mucosa. This in turn has encouraged speedy wound healing of the ulcerated areas of the mucosal epithelia and shielded the gastric membrane, thus abrogating the catastrophic influence of indomethacin in the ulcerated rats[47]. This is indicative of enhanced mucus secretory potential of the OHECD and suggestive of their significant role in ulcer healing process. CIM also increased the mucus and mucin contents in the gastric tissue but to a lesser extent.

Hydroxyproline, a modified amino acid is a major component of collagen[50], which forms part of the connective tissue and plays key roles for collagen stability. Induction of gastric ulcer leads to increase in collagenase activity which breaks the peptide bonds in collagen which in turn leads to decrease in collagen levels and apparently, decrease in hydroxyproline levels[51]. Reports of various workers have shown that glyprolines (gelatin short peptides) consist of the amino acids glycine and proline and they are natural peptides generated in the organism in the course of collagen synthesis or decomposition[50]. They protect the stomach mucous tunic from injuries. This study revealed decreased hydroxyproline and proline levels in the indomethacin ulcerated untreated rats which is an indication of reduced collagen levels thus implying decreased structural protein of connective tissues. These is consistence with previous literature[50,51].

Nitric Oxide (NO) is a mediator of gastrointestinal mucosal defense but inconsistently, it also contributes to mucosal damage. Although nitric oxide synthase is responsible for synthesis of nitric oxide it has many isoforms. Mainly, cytoprotective endothelial (eNOS) and cytotoxic inducible (iNOS)[52]. Nitric oxide from cNOS improves the mucosal blood flow, protects the integrity of epithelial tissue and inhibits activation, adhesion and migration of leucocytes in the inflammatory[53] resulting in increasing mucus synthesis and accelerating ulcer healing[54]. This study showed down regulation of NO with indomethacin treatment leading to a decrease in mucus synthesis and mucosal barrier content which confirmed by biochemical and histopathological analysis, this result agreed with the previous studies[55,56]. Meanwhile treatment with OHECD or CIM increased NO level leading to increasing in mucus synthesis and restoration of the depleted gastric mucus levels.

Pepsin has important proteolytic enzyme, activated at pH of 1-4[26]. In this present study indomethacin significantly increased the activity of this enzyme in the ulcerated untreated rats indicated reduced protective ability of the mucosal membrane, thus decreased ability of the gastric mucosa to with stand the onslaught of indomethacin. This is in accordance with other studies[37,57]. Post-treatment with OHECD decreased the activity of this enzyme indicated that oleic acid suppresses acid secretion and interferes with proteolytic digestion. This result agreed with the reported work of different studies[26,43]. Cimetidine, also decreased the activity of this important enzyme.

Citrate synthase is one of the mitochondrial enzymes that are used to assess the integrity of the mitochondrion. The decreased activity of citrate synthase in the indomethacin ulcerated rats indicated that there was impairment of the mitochondria integrity after ulceration. This agreed with the previous report of El-Abhar[58]. Post-treatment with the oleic acid rich fraction of n-hexane extract of *Chasmanthera dependens* markedly elevated citrate synthase activity thereby indicated enhancement of the mitochondria integrity. Also, cimetidine elevated citrate synthase activity which indicated the enhancement of the mitochondrial integrity. This agreed with the report of El-Abhar[58]. The implication of this study would be to utilize oleic acid rich fraction of n-hexane extract of *Chasmanthera dependens* stem as an effective and alternate therapy for gastric ulceration.

CONCLUSION

Overall, this study has shown that oleic acid fraction of *Chasmanthera dependens* up-regulated the suppression of connective tissue components induced by indomethacin in rats stomachs and healing potential of oleic acid in indomethacin-induced gastric ulcer. The probable mechanism of action is via the upregulation of mitochondrial enzyme, increase in blood flow and connective tissue components.

SIGNIFICANCE STATEMENT

To the best of our knowledge, this is the first study of this kind to describe the successful healing capacity of oleic acid rich fraction of n-hexane extract of *Chasmanthera dependens* stem in indomethacin-induced gastric ulcer. This study shown that oleic acid can be considered as a therapeutic agent in gastric ulceration. Further studies are however needed to affirm the molecular mechanisms of action of this fraction used in this study.

REFERENCES

1. Yin, H., X.C. Pan, S.K. Wang, L.G. Yang and G.J. Sun, 2014. Protective effect of wheat peptides against small intestinal damage induced by non-steroidal anti-inflammatory drugs in rats. J. Integr. Agric., 13: 2019-2027.
2. Gladding, P.A., M.W. Webster, H.B. Farrell, I.S. Zeng, R. Park and N. Ruijne, 2008. The antiplatelet effect of six non-steroidal anti-inflammatory drugs and their pharmacodynamic interaction with aspirin in healthy volunteers. Am. J. Cardiol., 101: 1060-1063.
3. Piazza, G.A., A.B. Keeton, H.N. Tinsley, J.D. Whitt and B.D. Gary *et al*., 2010. NSAIDs: Old drugs reveal new anticancer targets. Pharmaceuticals, 3: 1652-1667.
4. Lanas, A., M.A. Perez-Aisa, F. Feu, J. Ponce and E. Saperas *et al*., 2005. A nationwide study of mortality associated with hospital admission due to severe gastrointestinal events and those associated with nonsteroidal antiinflammatory drug use. Am. J. Gastroenterol., 100: 1685-1693.
5. Schmassmann, A., 1998. Mechanisms of ulcer healing and effects of nonsteroidal anti-inflammatory drugs. Am. J. Med., 104: 43S-51S.
6. Okabe, S. and K. Amagase, 2005. An overview of acetic acid ulcer models-The history and state of the art of peptic ulcer research. Biol. Pharmaceut. Bull., 28: 1321-1341.
7. Omar, M., N. Adila, N. Abdullah, U.R. Kuppusamy, M.A. Abdulla and V. Sabaratnam, 2011. Nutritional composition, antioxidant activities and antiulcer potential of *Lentinus squarrosulus* (Mont.) mycelia extract. Evidence-Based Complement. Altern. Med., Vol. 2011. 10.1155/2011/539356.
8. Chatterjee, A., S. Chatterjee, S. Das, A. Saha, S. Chattopadhyay and S.K. Bandyopadhyay, 2012. Ellagic acid facilitates indomethacin-induced gastric ulcer healing via COX-2 up-regulation. Acta Biochim. Biophys. Sin., 44: 565-576.
9. Chatterjee, A., S. Chatterjee, A. B iswas, S. Bhattacharya, S. Chattopadhyay and S.K. Bandyopadhyay, 2012. Gallic acid enriched fraction of *Phyllanthus emblica* potentiates indomethacin-induced gastric ulcer healing via e-NOS-dependent pathway. Evidence-Based Complement. Altern. Med., Vol. 2012. 10.1155/2012/487380.
10. Rasik, A.M. and A. Shukla, 2000. Antioxidant status in delayed healing type of wounds. Int. J. Exp. Pathol., 81: 257-263.
11. Osemene, K.P., A.A. Elujoba and M.O. Ilori, 2013. A comparative assessment of herbal and orthodox medicines in Nigeria. Niger. J. Nat. Prod. Med., 17: 77-81.
12. Kunle, O.F., H.O. Egharevba and P.O. Ahmadu, 2012. Standardization of herbal medicines-A review. Int. J. Biodivers. Conserv., 4: 101-112.
13. Odugbemi, T., 2008. A Textbook of Medicinal plants from Nigeria. University of Lagos Press, Nigeria, ISBN: 978-978-48712-9-7, pp: 588.

14. Iwu, M.M., A.R. Duncan and C.O. Okunji, 1999. New Antimicrobials of Plant Origin. In: Perspectives on New Crops and New Uses, Janick, J. (Ed.). ASHS Press, Alexandria, VA., USA., ISBN-13: 9780961502706, pp: 457-462.
15. Onabanjo, A.O., T.A. John, A.A. Sokale and O.T. Samuel, 1991. Analgesic and antiinflammatory effects of *Chasmanthera dependens*. Int. J. Pharmacogn., 29: 24-28.
16. Morebise, O., E.O. Awe, J.M. Makinde and O.A. Olajide, 2001. Evaluation of the anti-inflammatory and analgesic properties of *Chasmanthera dependens* leaf methanol extract. Fitoterapia, 72: 497-502.
17. Okiei, W., M. Ogunlesi and M.A. Ademoye, 2009. An assessment of the antimicrobial properties of extracts of various polarities from *Chasmanthera dependens*, *Emilia coccinea* and *Cuscuta australis*, herbal medications for eye diseases. J. Applied Sci., 9: 4076-4080.
18. Adekunle, A.A. and S.O. Okoli, 2002. Antifungal activity of the crude extracts of *Alafia barteri* Oliver (Apocynaceae) and *Chasmanthera dependens* Hochst. (Menispermaceae). Hamdard Medicus, 45: 52-56.
19. Quadri, A.L. and M.T. Yakubu, 2017. Fertility enhancing activity and toxicity profile of aqueous extract of *Chasmanthera dependens* roots in male rats. Andrologia, Vol. 49, No. 10. 10.1111/and.12775.
20. Tijani, S.A., S.B. Olaleye and E.O. Farombi, 2018. Anti-ulcerogenic effect of the methanol extract of *Chasmanthera dependens* (Hochst) stem on male Wistar rats. J. Basic Clin. Physiol. Pharmacol., 29: 377-383.
21. Ogunlesi, M., W. Okiei, M. Ademoye and E.A. Osibote, 2010. Analysis of essential oil from the stem of *Chansmanthera dependens*. J. Nat. Prod., 3: 47-53.
22. PHS., 1985. Public health service policy on humane care and use of laboratory animals. Public Law 99-158, November 20, 1985, U.S. Department of Health and Human Services, National Institutes of Health, Washington, DC., USA.
23. APS., 2002. Guiding principles for research involving animals and human beings. Am. J. Physiol.-Regul. Integr. Comp. Physiol., 283: R281-R283.
24. Bhattacharya, S., S.R. Chaudhuri, S. Chattopadhyay and S.K. Bandyopadhyay, 2007. Healing properties of some Indian medicinal plants against indomethacin-induced gastric ulceration of rats. J. Clin. Biochem. Nutr., 41: 106-114.
25. Dokmeci, D., M. Akpolat, N. Aydogdu, L . Doganay and F.N. Turan, 2005. L-carnitine inhibits ethanol-induced gastric mucosal injury in rats. Pharmacol. Rep., 57: 481-488.
26. Boeing, T., L.M. da Silva, L.B. Somensi, B.J. Cury and A.P.M. Costa *et al*., 2016. Antiulcer mechanisms of *Vernonia condensata* Baker: A medicinal plant used in the treatment of gastritis and gastric ulcer. J. Ethnopharmacol., 184: 196-207.
27. Martins, J.L.R., O.R.L. Rodrigues, D.M. da Silva, P.M. Galdino and J.R. de Paula *et al*., 2014. Mechanisms involved in the gastroprotective activity of *Celtis iguanaea* (Jacq.) Sargent on gastric lesions in mice. J. Ethnopharmacol., 155: 1616-1624.
28. Winzler, R.J., 1955. Determination of Serum Glycoproteins. In: Methods of Biochemical Analysis, Glick, D.P. (Ed.). Interscience Publishers, New York, USA., pp: 279-311.
29. Cortas, N.K. and N.W. Wakid, 1990. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin. Chem., 36: 1440-1443.
30. Reddy, K. and C.S. Enwemeka, 1996. A simplified method for the analysis of hydroxyproline in biological tissues. Clin. Biochem., 29: 225-229.
31. Troll, W. and J. Lindsley, 1955. A photometric method for the determination of proline. J. Biol. Chem., 215: 655-660.
32. Lewis, B.D., 1990. Determination of citrate in urine by simple direct photometry. Clin. Chem., 36: 578-578.
33. Tarnawski, A.S., 2005. Cellular and molecular mechanisms of gastrointestinal ulcer healing. Dig. Dis. Sci., 50: S24-S33.
34. Oh, T.Y., B.O. Ahn, E.J. Jang, J.S. Park, S.J. Park, H.W. Baik and K.B. Hahm, 2008. Accelerated ulcer healing and resistance to ulcer recurrence with gastroprotectants in rat model of acetic acid-induced gastric ulcer. J. Clin. Biochem. Nutr., 42: 204-214.
35. Xing, J., S. Jinyao, S. Hu, B. Wang, Y. Dong, B. Yang and H.P. Kallio, 2012. Effects of sea buckthorn (*Hippophaë rhamnoides* L.) pulp oils on the gastric secretion, gastric emptying and its analgesic activity. J. Med. Plants Res., 6: 3240-3245.
36. Toma, W., L.L. Guimaraes, A.R.M.S. Brito, A.R. Santos and F.S. Cortez *et al*., 2014. Safflower oil: An integrated assessment of phytochemistry, antiulcerogenic activity and rodent and environmental toxicity. Rev. Bras. Farmacogn., 24: 538-544.
37. Adhikary, B., S.K. Yadav, K. Roy, S .K. Bandyopadhyay and S. Chattopadhyay, 2010. Black tea and theaflavins assist healing of indomethacin-induced gastric ulceration in mice by antioxidative action. Evidence-Based Complementary Alter. Med., Vol. 2011. 10.1155/2011/546560.
38. Lullmann, H., K. Mohr, A. Ziegler and D. Bieger, 2000. Color Atlas of Pharmacology. 2nd Edn., Thieme, New York, ISBN: 9780865778436, Pages: 166.
39. Abdallah, I.Z.A., H.A.H. Khattab and G.H. Heeba, 2011. Gastroprotective effect of *Cordia Myxa* L. fruit extract against indomethacin-induced gastric ulceration in rats. Life Sci. J., 8: 433-445.
40. Sabiu, S., T. Garuba, T.O. Sunmonu, A.O. Sulyman and N.O. Ismail, 2016. Indomethacin-induced gastric ulceration in rats: Ameliorative roles of Spondias mombin and ficus exasperata. Pharmaceut. Biol., 54: 180-186.
41. Ajani, E.O., S. Sabiu, F.A. Bamisaye, B.V. Adenigba, D.D. Awomoyi and M.M. Adeyanju, 2014. Hepatoprotective and antioxidative effect of ethanolic leaf extract of *Langenaria breviflora* (bitter gourd) on indomethacin-ulcerated rats. IOSR J. Pharm. Biol. Sci., 9: 61-68.

42. Ashraf, M.V., G. Thamotharan, S. Sengottuvelu, H.S. Sherief and T. Sivakumar, 2012. Evaluation of anti ulcer activity of *Ficus pumila* L. leaf extract in albino rats. Global J. Res. Med. Plants Indigen. Med., 1: 340-351.
43. Ateufack, G., E.C.D. Mokam, M. Mbiantcha, R.B.D. Feudjio, N. David and A. Kamanyi, 2015. Gastroprotective and ulcer healing effects of *Piptadeniastrum africanum* on experimentally induced gastric ulcers in rats. BMC Complement. Altern. Med., Vol. 15, No. 1. 10.1186/s12906-015-0713-5.
44. Lichtenberger, L.M., 1999. Gastroduodenal mucosal defense. Curr. Opin. Gastroenterol., 15: 463-472.
45. Allen, A. and G. Flemstrom, 2005. Gastroduodenal mucus bicarbonate barrier: Protection against acid and pepsin. Am. J. Physiol.-Cell Physiol., 288: C1-C19.
46. Ma, L., W.P. Wang, J.Y. Chow, S.T. Yuen and C.H. Cho, 2000. Reduction of EGF is associated with the delay of ulcer healing by cigarette smoking. Am. J. Physiol.-Gastrointest. Liver Physiol., 278: G10-G17.
47. Naito, Y., T. Yoshikawa, K. Matsuyama, N. Yagi and M. Arai *et al*., 1995. Effects of oxygen radical scavengers on the quality of gastric ulcer healing in rats. J. Clin. Gastroenterol., 21: S82-S86.
48. Luo, X.J., B. Liu, Z. Dai, Z.C. Yang and J. Peng, 2013. Stimulation of calcitonin gene-related peptide release through targeting capsaicin receptor: A potential strategy for gastric mucosal protection. Dig. Dis. Sci., 58: 320-325.
49. Alanko, J., A. Riuffa, P. Holm, I. Mulda, H. Vapatalo and T. Metsa-Ketela, 1999. Modulation of arachidonic acid metabolism by phenols: Relation to their structure and antioxidant/prooxidant properties. Free Radical Biol. Med., 28: 193-201.
50. Szpak, P., 2011. Fish bone chemistry and ultrastructure: Implications for taphonomy and stable isotope analysis. J. Archaeol. Sci., 38: 3358-3372.
51. Takeuchi, K., M. Ohishi, S. Ota, K. Suzumura and H. Naraoka *et al*., 2013. Metabolic profiling to identify potential serum biomarkers for gastric ulceration induced by nonsteroid a nti-inflammatory drugs. J. Proteome Res., 12: 1399-1407.
52. Abdel-Raheem, I.T., 2010. Gastroprotective effect of rutin against indomethacin-induced ulcers in rats. Basic Clin. Pharmacol. Toxicol., 107: 742-750.
53. Khattab, M.M., M.Z. Gad and D. Abdallah, 2001. Protective role of nitric oxide in indomethacin-induced gastric ulceration by a mechanism independent of gastric acid secretion. Pharmacol. Res., 43: 463-467.
54. Nishida, K., Y. Ohta and I. Ishiguro, 1998. Relationship between constitutive nitric oxide synthase activity and mucus level in the gastric mucosa of rats with stress. Pharmacol. Res., 38: 393-397.
55. Guha, P., A. Dey, B. Sarkar, M.V. Dhyani, S. Chattopadhyay and S.K. Bandyopadhyay, 2009. Improved antiulcer and anticancer properties of a *trans*-resveratrol analog in mice. J. Pharmacol. Exp Ther., 328: 829-838.
56. Chakraborty, S., S.K. Yadav, M. Subramanian, M. Iwaoka and S. Chattopadhyay, 2014. DL-trans-3,4-dihydroxy-1-selenolane (DHS_{red}) heals indomethacin-mediated gastric ulcer in mice by modulating arginine metabolism. Biochim. Biophys. Acta (BBA)-Gen. Subj., 1840: 3385-3392.
57. Bech, P.L., R. Xavier, N. Lu, N.N. Nanda, M. Dinauer, D.K. Podolsky and B. Seed, 2000. Mechanisms of NSAID-induced gastrointestinal injury defined using mutant mice. Gastroenterology, 119: 699-705.
58. El-Abhar, H.S., 2010. Coenzyme Q_{10}: A novel gastroprotective effect via modulation of vascular permeability, prostaglandin E_2, nitric oxide and redox status in indomethacin-induced gastric ulcer model. Eur. J. Pharmacol., 649: 314-319.