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RESEARCH ARTICLE

Gastroprotective Effect of Aqueous *Achatina achatina* L. (Snail) Slime Extract on Indomethacin- and Acidified Ethanol-Induced Ulceration in Wistar Albino Rats

Florence N Nworah, Ifeoma F Chukwuma, Valentine O Nwanelo, Dionysus O Osuji, Sobenna S Onyeso, Golden O Iwuji, Linus N Ngwu, Chigozie P Odo, Charles C Okoroafor, Augustine C Nkwocha and Emmanuel C Ezeako*

Pharmacology and Pharm-biology Unit, Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria
*Corresponding author:emmanuel.ezeako.188647@unn.edu.ng

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A B S T R A C T

Snail slime is used widely in African traditional medicine, specifically in Nsukka Enugu, Nigeria, for wound management; however, this claim lacks scientific validation. Herein, we investigated the gastroprotective effect of aqueous *Achatina achatina* L. (snail) slime extract (ASSE) on indomethacin- and ethanol-induced ulceration in Wistar albino rat models. Biochemical analysis of ASSE showed appreciable levels of manganese, iron, zinc, copper, molybdenum, selenium, Vitamin C, B1, B2, and B3 and a basic pH. The experimental design consists of two Phases (five groups of five rats each). In both Phases, group I (positive control) received 3ml of distilled water, while groups II-V received 20 mg/kg body weight omeprazole (standard drug), 50, 100, and 200 mg/kg bw ASSE, respectively, via oral intubation. The various treatments lasted for 21-days. Following 24-hours fasting with access to only water (which spanned between the 22nd -23rd day), ulceration was induced separately on the experimental animals in Phase I with a single oral dose of indomethacin (30 mg/kg bw) and in Phase II with a single oral dose of acidified ethanol (1.5 ml/ kg bw), via the gastric gavage. The LD_{50} study showed safety up to 5000 mg/kg bw ASSE. After induction, a significant ($P<0.05$) increase in the number of ulcers and mean ulcer index were recorded in group 1 of both Phases; however, rats administered various concentrations of ASSE showed significant ($P<0.05$) amelioration of the ulceration in both Phases, and these were on par with the standard control. These results suggest that aqueous snail slime extract possesses gastroprotective potential.

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INTRODUCTION

Peptic ulcer disease (PUD) is a well pronounced and ubiquitous ailment across the globe. The recent logarithmic increase in the proportion of the human population with ulceration in Africa, especially in Nigeria (Madueke and Anosike, 2017), coupled with the fact that the numerous synthetic anti-ulcerative drugs that inundate the markets are not without several complications, informs the need for screening natural sources for possible drug candidates (Sabiu *et al*., 2015). It will be most appropriate to treat gastric ulcers with the use of natural products which comply with several economic and safety considerations and are nontoxic and efficacious. Although medical research has achieved many feats using plants in disease management, the use of animals' parts and secretions in disease management remains underexploited (Alade *et al*., 2018). The several ethical considerations associated with using animals and the fear of extinction of some vulnerable species seem to pose the greatest constrain on animal medicine. However, the recent development and upscale in animal husbandry and breeding settle these concerns (Göncü and Güngör, 2018).

Across the globe, Zootherapies have been in practice in places like East Asia, Latin America, and Europe for several centuries. Skin, feathers, blood, bones, meat, feces, and body slimes of wild and domestic animals are deployed in the folkloric management of several disease conditions that defiles orthodox medical interventions (Jugli *et al*., 2020). Hitherto, animal-based medicines remain an integral part of the systems of traditional medicine as well as most modern pharmaceuticals

worldwide (Alade *et al*., 2018). Two succinct examples include angiotensin-converting enzyme (ACE) inhibitors extracted from the venom of pit viper snake and Omega-3 PUFAs (polyunsaturated fatty acids) and dietary supplements extracted from certain fish oils (Costa-Neto, 2005). Several instances have been documented where extracts from easily accessible invertebrates such as snails, silkworms, stinkbugs, scorpions, and triton were deployed in treating hemorrhoids and other internal diseases (Lev, 2005). The sustained use of zootherapy from ancient times to the present day suggests its medical efficacy (Alade *et al*., 2018). Therefore, a substantial investigation of animal extracts in the forms used in folk medicines will be rewarding and might provide sufficient insight for efficient drug formulations and better management of chronic diseases such as peptic ulcers.

Snail slime has found wide application in several fields, such as cosmetics, as it contains collagen, allantoin, elastin and hyaluronic acid needed for skincare (Trapella *et al*., 2018). Studies have also demonstrated its antibacterial, antioxidant, anticancer, and wound healing potentials (Etim *et al*., 2016; Harti *et al*., 2016). Hitherto, snail mucus conveyed using milk or honey is used in treating inflammatory diseases in African folk medicine, precisely in Nsukka, Enugu, Nigeria, but this is void of scientific validation. This premise informs the present study. Hence, this study investigated the effectiveness of snail slime in preventing and protecting against NSAIDs- and ethanol-induced ulceration in Wistar albino rats.

MATERIALS AND METHODS

Materials: Indomethacin, omeprazole, ethanol (98%) and Hydrochloric acid were purchased from Kim-zee Pharmacy, Nsukka, Enugu, Nigeria. Distilled water, Shaking Incubator (Kottermann, Germany) and pH meter (Japan) were gotten from Biochemistry Laboratory, University of Nigeria, Nsukka. All reagents used in the study were of analytical grade and were obtained from well reputable companies.

Snail collection and identification: Fifty (50) adult African snails (*Achatina achatina* L.) were obtained from Ibagwa-Aka Market in Nsukka LGA in Enugu, Nigeria. The snails were subjected to morphological identification according to Bouchet *et al.* (2015). The snail was identified and authenticated at the Zoology garden of the Department of Zoology and Environmental Biotechnology, University of Nigeria, Nsukka by a zoologist.

Slime collection and extraction: Adult snails were euthanized by electric shocks of 5–10 volts, in 30–60 s. After washing in clean water to remove debris, the snails were mildly scrubbed to squeeze out the slime from their body. Graded amount (100 ml) of snail slime was introduced into a 2000 ml sterile conical flask containing 1000 ml of distilled water. The flask and its content was stopped with cotton and allowed to stay for 48 hours at 40°C in a Shaking Incubator (Kottermann, Germany) set at 200 × g. Afterward, the mixture was centrifuged at 3000 × g for 10 min. The resulting supernatant of the snail slime was lyophilized as aqueous extract (with yield of 8.3 mg extract/ml slime) and stored in a well-labeled sterile screw-capped bottle kept in a refrigerator at 4°C. The aqueous snail slime extract (ASSE) was used in the study and was vortex every time prior to administration.

Experimental animals: Fifty (50) adult albino rats (*Rattus norvegicus*) weighing 100-150 g and eighteen (18) albino mice weighing 55-85 g were used for the study. The experimental animals were procured from the animal house of the Department of Zoology and Environmental Science, University of Nigeria Nsukka. They were kept in standard cages and maintained in a well hygiene environmental condition (temperature of 23±1°C, relative humidity of 55±5% and a 12-hour/12-hour light/dark cycles), given standard feeds (Vital) and allowed to acclimatize for 14 days, before the commencement of the study.

Experimental design: The study adopted the completely randomized design (CRD) method. The study deployed fifty (50) Wistar albino rats and eighteen (18) Swiss albino mice. The experimental design consisted of two Phases made up of five groups of five rats each. In each Phase; group I (positive control) received 3 ml of distilled water, while groups II-V received 20 mg/kg bw omeprazole (standard drug), 50, 100, and 200 mg/kg body weight of aqueous snail slime extract (ASSE) via oral intubation, respectively. The various treatments regimens lasted for 21-days, after which ulcer was induced on animals in Phase I and II with indomethacin and acidified ethanol (EtOH/HCl), respectively, via oral intubation.

Ethical clearance: Permission and ethical clearance document for the safe conduction of the experimental processes and the prudent use of laboratory animals were obtained from the university ethics commission of the Department of Biochemistry University of Nigeria Nsukka. All the experimental procedures adhered to International protocols for the Care and Use of Laboratory Animals in Biomedical Research.

Acute Toxicity (LD_{50}) studies: Acute toxicity (LD_{50}) studies of ASSE were performed following the method described by Lorke (1983).

Induction of gastric ulcer: The study employed Sayanti *et al*. (2007) and Shin *et al*. (2020) methods in the ulcer induction process. Twenty-one days post-treatments, the animals were deprived of food for 24 h but allowed access to only water (spanning between the 22nd - 23rd day). Subsequently, induction of ulcers was performed on animals in Phase I with a single oral dose of indomethacin (30 mg/kg bw) and in Phase II with a single oral dosage of acidified ethanol (1.5 mL/ kg bw of 150 mM HCl in 60 % ethanol), via oral intubation. The animals were then euthanatized humanely by overdosing with an anesthetic agent (halothane) and then sacrificed.

Biochemical analysis of snail slime: Screening of the mineral and vitamin composition of ASSE followed the method described by AOAC (2005). The snail slime pH across several concentrations following serial dilution was determined using a pH meter.

Quantification of ulcerations: Quantification of ulcerations in the animal models followed the method of Szabo and Hollander (1985). Briefly, after 30 min for indomethacin- and 6 hours for absolute ethanol-induction, each stomach was excised along the greater curvature and subjected to antiulcer studies. Animal stomachs were washed clean with normal saline and fastened on a white corkboard. The scoring of the ulcer was on a 0–5 grading scale based on the severity of the lesion using a dissecting microscope having a square-grid eyepiece. Quantification of the damaged mucosal area as a percentage of the total surface area of the glandular stomach and the mean ulcer index and percentage ulcer inhibition of the extract for each group followed the method of Vogel (2002). The formula thus used is as follows:

$$\text{Mean Ulcer Index} = 1 - \frac{\text{Ulcer treated}}{\text{Ulcer control}}$$

$$\text{Percentage Ulcer inhibition} = 1 - \frac{\text{Ulcer treated}}{\text{Ulcer control}} \times 100$$

Statistical analysis: The data obtained were analyzed statistically with one-way ANOVA in SPSS (statistical product and service solutions) version 23, using Descriptive and Duncan tables. Values thus obtained were expressed as the Mean ± standard error of mean SEM (n=3). The subsets of a column with different alphabets as superscripts differ significantly (P<0.05).

RESULTS

Vitamin and Mineral Composition and pH of Aqueous Snail Slime Extract: Determination of some biochemical constituents of the aqueous snail slime extract (ASSE) revealed the presence of essential vitamins such as Vit. C, E, B1, and B3 in appreciable levels (Fig. 1). Similarly, high levels of essential minerals such as iron, manganese, zinc, selenium, and molybdenum were also present in moderate levels (Fig. 2). In addition, the snail slime also recorded basic pH at various dilutions (Fig. 3).

Results of acute toxicity studies: Acute toxicity studies of ASSE demonstrated safety of the extract for oral administration at all studied doses and as such neither death nor changes in behavior were recorded, as presented in Table 1.

Effect of ASSE on Indomethacin-Induced Gastric Ulcer in Rats: It is well-documented that pre-prandial administration of an overdose of NSAIDs on experimental animals results in ulcer progression. From the result, as presented in Table 2, untreated rats inflicted with a single dose of 30 mg/kg bw indomethacin (positive control) after a 24-hours fast showed a significant (P<0.05) higher degree of ulceration as apparent in the high ulcer number score and mean ulcer index value. Interestingly, the result recorded a significant (P<0.05) decrease in ulcer number and mean ulcer index in rats pretreated with varying dosages (50, 100 and 200 mg/kg) of ASSE when compared with the positive control rats; and these were at par with that obtained in the group administered standard drug (20 mg/kg bw Omeprazole) before induction. In addition, the % ulcer inhibition (58.57, 47.80 and 59.91) observed in the groups pretreated with varying concentrations (50, 100 and 200 mg/kg) of ASSE respectively, was not significantly (P>0.05) different compared with the % ulcer inhibition (55.0) observed in the standard control.

Table 1: Acute toxicity test (LD_{50}) of ASSE on albino mice

Groups	Dose (mg/kg)	Mortality
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Group 4	1600	0/3
Group 5	2900	0/3
Group 6	5000	0/3

Table 2: Anti-ulcerative effect of ASSE on Indomethacin-induced gastric ulcer

Groups	Treatments	Ulcer number	Mean Ulcer Index	% Ulcer Inhibition
Group1	Positive control	1.67±0.58^{b}	35.00±9.557^{c}	---
Group2	20 mg/kg bw omeprazole	0.45±0.52^{a}	15.75±6.850^{ab}	55.00^{a}
Group3	50 mg/kg ASSE	0.70±0.36^{a}	14.50±8.021^{a}	58.57^{a}
Group4	100 mg/kg bw ASSE	0.62±0.35^{a}	18.25±6.652^{b}	47.86^{a}
Group5	200 mg/kg bw ASSE	0.47±0.21^{a}	14.03±7.188^{a}	59.91^{a}

Results are presented as mean ± SD; (n=5). Values in the same column having different superscripts differ significantly (P<0.05).

Table 3: Anti-ulcerative effect of Snail slime on ethanol-induced gastric ulcer

Groups	Treatments	Ulcer number	Mean Ulcer Index	% Ulcer Inhibition
Group1	Positive control	1.47±0.47^{c}	8.25±1.500^{c}	---
Group2	20 mg/kg bw omeprazole	0.33±0.34^{a}	3.82±2.160^{a}	53.70^{a}
Group3	50 mg/kg ASSE	0.50±0.22^{b}	5.00±3.559^{b}	39.39^{a}
Group4	100 mg/kg bw ASSE	0.35±0.24^{a}	4.73±2.363^{ab}	42.67^{a}
Group5	200 mg/kg bw ASSE	0.20±0.08^{a}	3.75±0.957^{a}	54.54^{a}

Values are presented as mean ± SD, (n=5). Values in the same column having different superscripts differ significantly (P<0.05).

Effect of ASSE on Ethanol-Induced Gastric ulcer in Rats: The effect of ASSE on ethanol-induced ulceration in rats is shown in Table 3. The result showed that pre-prandial administration of absolute ethanol on rats; resulted in ulceration, as apparent in the high ulcer number score and mean ulcer index recorded in the untreated group (positive control). Contrarily, groups treated with varying concentrations (50, 100, and 200 mg/kg) of ASSE showed significant (P<0.05) decrease in ulcer number and mean ulcer index compared with the positive control, and these were at par with that obtained in the group pretreated with standard drug (20 mg/kg bw Omeprazole). The result recorded a significant (p <0.05) decrease in ulcer number and mean ulcer index in rats administered varying dosages (50, 100, and 200 mg/kg) of ASSE when compared with the positive control rats. In addition, the % ulcer inhibition (58.57, 47.80 and 59.91) observed in the groups pretreated with varying concentrations (50, 100, and 200 mg/kg) of ASSE, respectively, was not significantly (P>0.05) different compared with the % ulcer inhibition (55.0) observed in the standard control. Moreover, the observed % ulcer inhibition (39.39, 42.67, and 54.54) exhibited by the groups administered varying concentrations (50, 100, and 200 mg/kg) of ASSE before ethanol intoxication was close to the % ulcer inhibition (53.70) observed in the standard control group; and this was not significantly (P>0.05) different.

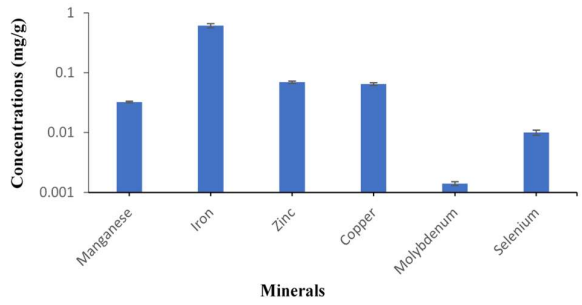

Fig. 1: Mineral composition of the ASSE

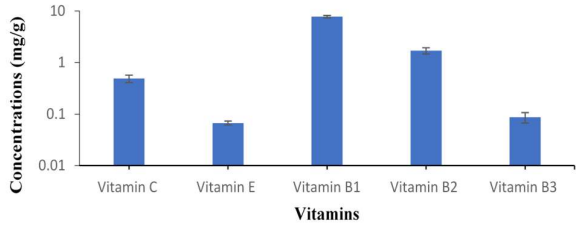

Fig. 2: Vitamin composition of the Snail Slime

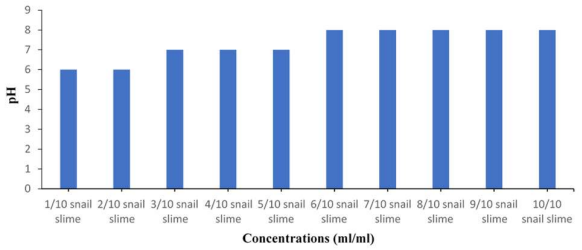

Fig. 3: pH of ASSE at different concentrations.

DISCUSSION

The result of this study revealed that aqueous snail slime extract (ASSE) is rich in some vital minerals such as manganese, iron, zinc, copper, selenium, and molybdenum and vitamins such as vitamin C, B1, B2, B3, and E, and has a basic pH. These agree with previous studies that stated that minerals are inorganic elements found in animal processes necessary to sustain life (Tardy *et al*., 2020). Animal-sourced minerals are less chelated by antinutrients and are readily available; and are better off than plant-sourced minerals (Samtiya *et al*., 2020). In the same vein, Olorunnisola *et al*. (2019) proposed that vitamins are critical for wound healing, cell growth and development, antioxidant activities and proper cell-cell communication in animals.

The basic pH exhibited by ASSE suggests its potency in neutralizing acidity in the stomach. Investigation of the effect of pharmacological agents on stomach physiology and the modulation of ulceration usually deploy some biochemical parameters such as gastric volume and pH, to ascertain the mucosal integrity upon exposure to toxicants (Saheed *et al*., 2015; Shin *et al*., 2020). Ghosh *et al*. (2011) proposed that pH measurements provide an insight into the degree of acidity and gastric secretions. A low pH value indicates elevated concentrations of hydrogen ions in the stomach and is linked to the etiology of gastric damage and ulceration.

The acute toxicity study showed that the ASSE did not demonstrate any toxicity in mice up to 5000 mg/kg bw. These align with a previous study by Adikwu and Nnamani (2006), which reported that snail mucus was well-tolerated in mice, and they did not exhibit any symptom of overt toxicity.

Results in Tables 2 and 3 showed that pre-prandial administration of an overdose of indomethacin and acidified ethanol, respectively, effectively induced ulceration in rats, manifesting in the high number of ulcers and mean ulcer index in untreated animals (positive control). However, induction with ethanol (Table 3) showed a lesser number of ulcers and mean ulcer indices when compared with Indomethacin induction (Table 2). This could be because ethanol has a slower mechanism of ulcer pathogenesis than NSAIDs. A previous study by Karampour *et al*. (2018) reported a similar result. The toxicants may have exerted the lethal effects via the initiation of free radical production or the inhibition of prostaglandin synthesis necessary to maintaining the mucosal integrity. These agree with previous studies that showed that both NSAIDs (indomethacin) and ethanol exert similar effects on the pathogenesis of peptic ulcers (Sabiu *et al*., 2015); by suppressing the expression of the enzyme COX1 thereby inhibiting the conversion of arachidonic acid to prostaglandin (Cadirci *et al*., 2007). These further result in increased activities of neutrophils and their local release of ROS and other chemokines such as PAF, histamine, and TNF-α from the peritoneal mucosal mast cell (Juhn *et al*., 2008). Subsequently, COX1 inhibition may result in the release of endothelin-1, a potential vasoconstrictor that causes intensified mucosal damage (Spinella, 2004). The Inhibitory actions of NSAIDs (indomethacin) on the synthesis of prostaglandin results in the distortion in mucosa integrity and stems from the increased production of gastric acid (Ajani *et al*., 2015). This is also at par with the works of Ajani *et al*. (2015) and Karampour *et al*. (2018), which deployed indomethacin and ethanol, respectively, to alter gastric secretions and induce ulceration in rats.

Conversely, rats pretreated with various concentrations of ASSE before induction with either of the toxicants (Indomethacin and EtOH/HCl) exhibited a significant ($P<0.05$) decrease in the number of ulcers and mean ulcer indices when compared with the positive control. The ulcer inhibitory effect of the extract in each case was similar to that obtained with the standard drug (omeprazole). Additionally, the % ulcer inhibition exerted by the extract followed a dose-dependent trend and was highest at the highest concentration in both Phases. These suggest that the extract possesses gastroprotective potentials akin to the reference drug. Perhaps, it may be that the aqueous snail slime extract exerts its effect via pH-lowering; neutralizing gastric acid secretion. Studies have implicated gastrointestinal damage to be a consequence of the erosion of mucin contents and the mucosa layers by drugs or *Helicobacter pylori* activities (Madueke and Anosike, 2017; Shin *et al*., 2020). These erosions may be exacerbated by assaults from both internal (e.g., pepsin) and external (e.g., drugs) ulcer-causative agents (Sabiu *et al*., 2015). In the same vein, Newar and Ghatak (2015) stated that mucus plays several functions in animals, such as emollient, lubricating,

moisturizing, adhesive, and protective functions. Moreover, Trapella *et al*. (2018) proposed that snail mucus may be composed of active ingredients necessary to treat skin disorders.

Conclusions: The result suggests that aqueous snail slime extract possesses anti-ulcer potential against NSAIDs- and alcohol-induced ulceration. These thus shed light on its use in folkloric medicine and give insight into its potential as a possible candidate for drug formulation for peptic ulcer prevention and management.

Recommendation: Further studies to elucidate the biochemical mechanism of snail slime in exerting the observed gastroprotective effect; to give further credence to the result of the present study are underway.

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Authors contribution: We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. In addition, FNN envisioned and proposed the research design; FNN, IFC, ECE, ODO, CCO and ACN carried out the study; VON, SSO, GOI, CPO, LNN and ECE analyzed and interpreted the obtained data; ECE wrote the paper. All the authors proofread and approved the final document.

REFERENCES

Adikwu M and Nnamani P, 2006. Some physiological and toxicological properties of snail mucin extracted from *Archachatina marginate.* Biol Res 3:1-6.

Ajagun EJ, Anyaku CA and Afolayan MP, 2017. A survey of the traditional, medical and non-medical uses of animal species and parts of the indigenous people of Ogbomoso, Oyo State. IntJ Herbal Med 5:26-32. www.floraljournal.com

Ajani E, Sabiu S, Bamisaye F, *et al*., 2015. Ethanolic leaf extract of *Langenaria breviflora* (bitter gourd) inhibits gastric onslaught inindomethacin-induced ulcerated rats. J Appl Biosci 85:7871.

Alade GO, Arueniobebh F and Ajibesin KK, 2018. Animals and animal products as medicines: A survey of Epie-Atissa and Ogbia people of Bayelsa State, Nigeria. J Pharm Pharmacognosy Res **6**:408-502. https//jppres.com/jppres

Association of official Analytical Chemists (AOAC), 2005. Official methods of Analysis. 15th Ed. Washington, DC. Associated of Analytical Chemistry.

Bouchet P and Rocroi JP, 2005. Classification and nomenclator of gastropod families. Malacologia 47:1-368.

Cadirci E, Suleyman H, Aksoy H, *et al*., 2007. Effects of *Onosma armeniacum* root extract on ethanol-induced oxidative stress in stomach tissue of rats. Chem Biol Interact 170:40–8.

Costa-Neto EM, 2005. Animal-based medicines: biological prospection and the sustainable use of zootherapeutic resources. An Acad Bras Ciênc 77:33-43.

Etim LB, Aleruchi C, Obande GA, 2016. Antibacterial properties of snail mucus on bacteria isolated from patients with wound Infection. Br Microbiol Res J 11:1-9.

Ghosh T, Lewis D I, Axon ATR, *et al*., 2011. Methods of measuring gastric acid secretion. review article. Aliment Pharmacol Ther 33:768–81.

Göncü S and Güngör C, 2018. The innovative techniques in animal husbandry, Anim Hus Nutr pp:3-22.

Harti AS, Sulisetyawati SW, Murharyati A, *et al*., 2016. The effectiveness of snail slime and chitosan in wound healing. Int J Pharma Bio Sci 5:76-80.

Jugli S, Chakravorty J and Meyer-Rochow VB, 2020. Zootherapeutic uses of animals and their parts: an important elements of the traditional knowledge of the Tangsa and Wancho of Eastern Arunachal Pradesh, North-east India. Environ Dev Sustain 22: 4699-734. https://doi.org/10.1007/s10668-019-00404-6

Juhn SK, Jung MK, Hoffman MD, *et al*., 2008. The role of inflammatory mediators in the pathogenesis of otitis media and sequelae. Clin Exp Otorhinolaryngol 1:117-38.

Karampour NS, Arzi A, Rezaie A, *et al*., 2019. Gastroprotective effect of zingerone on ethanol-induced gastric ulcers in rats. Medicina 55:64-53.

Lev E, 2003. Traditional healing with animals (zootherapy): medieval to present-day Levantine practice. J Ethnopharmacol 85:107–18.

Lorke D, 1983. Determination of acute toxicity. Arch Toxicol 53:275-9.

Madueke AC and Anosike CA, 2017. Anti-ulcerogenic effect of the chloroform extract of *Chasmanthera dependens* on indomethacin and aspirin-induced ulcer in rats. J Nutr Ecol 4:1–7.

Newar J and Ghatak A,2015. Studies on the adhesive property of snail adhesive mucus. Langmuir 31:12155–60.

Olorunnisola OS, Ajayi AF, Okeleji OL, Oladipo AA, *et al*., 2019.Vitamins as antioxidants. J Food Nutr Res 2:214-35.

Sabiu S, Garuba T, Sunmonu TO, *et al*., 2015. Indomethacin-induced gastric ulceration in rats: Ameliorative roles of *Spondias mombin* and *Ficus exasperate.* Pharm Biol 54:180–6.

Saheed S, Olarewaju S, Taofeeq G, *et al*., 2015. Combined administration of *Spondiasmombin* and *Ficusexasperata* leaf extracts stall Indomethacin-mediated gastric mucosal onslaught in rats. Afr J Trad Complement Altern Med 12:45.

Samtiya M, Aluko RE and Dhewa T, 2020. Plant food anti-nutritional factors and their reduction strategies: an overview. Food Prod Process Nutr 2:6-20.

Sayanti B, Susri RC, Subrata C, *et al*., 2007. Healing properties of some Indian medicinal plants against indomethacin-induced gastric ulceration of rats. J Clin Biochem Nutr 41:106–14.

Shin J, Park JH, Kim KS, *et al*., 2020. Antiulcer Activity of Steamed Ginger Extract against Ethanol/HCl-Induced Gastric Mucosal Injury in Rats. Molecules 25: 4663-4678; doi:10.3390/molecules 25204663.

Spinella F, 2004. Inhibition of cyclooxygenase-1 and -2 expression by targeting the endothelin a receptor in human ovarian carcinoma cells. Clin Cancer Res 10:4670-9.

Szabo S and Hollander D, 1985. Pathways of gastrointestinal protection and repair: mechanisms of action of sucralfate. Am J Med 86:23-31.

Tardy A, Pouteau E, Marquez D, *et al*., 2020. Vitamins and minerals for energy, fatigue and cognition: a narrative review of the biochemical and clinical evidence. Nutrients 12:228.

Trapella C, Rizzo R, Gallo S, *et al*., 2018. Helix complex snail mucus exhibits pro-survival, proliferative and pro-migration effects on mammalian fibroblasts. Sci Rep 8:17665-75.

Vogel HG, 2002. Drug discovery and evaluation, pharmacological assay. 2nd Ed, Springer-verlag Berlin Heidelberg, Germany. pp:759-67.