Antioxidant activity of aqueous extract of *Piliostigma thonningii* following Indomethacin induced gastric mucosa onslaught in male *wistar* Albino Rats.

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**Running Title:** Antioxidant Activity of Aqueous Extract of *Piliostigma thonningii*

**Abstract**

Antioxidant activity of *P. thonningii* extract following indomethacin induced gastric mucosa onslaught in male Wistar albino rats was carried out on 36 male rats that were divided into six (6) groups of 6 rats each. Group one (1) served as control and was given 0.5ml of normal saline (vehicle). Group two (2) was treated with 100mg/kg body weight of the drug (Cimetidine). Group three (3), five (5) and six (6) were given 100, 100 and 200mg/kg body weight of the extract respectively. The vehicle and extract were administered orally while the drug was administered intra-muscularly for 12days. After 12days of administration, all rats were fasted for 24 hours, gastric ulceration was then induced using 40mg/kg body weight of indomethacin orally only to group 2, 4, 5 and 6 respectively. Twelve (12) hours after indomethacin administration all rats were sacrificed after been anaesthetised with chloroform, the abdomen of each rats was opened to remove the stomach, Liver, Kidney and Testes respectively for the determination of SOD, Catalase, lipid peroxidation (MDA) activity and tissue protein concentration. The result shows significant (P<0.05) increase in tissue protein, SOD, Catalase but significant (P<0.05) decrease in MDA in groups treated with the extract compared with the control .Similar pattern was also exhibited with Cimetidine treated group. The untreated group a significant (P<0.05) decrease in tissue protein, SOD, Catalase with a significant increase (P<0.05) in MDA when compared with the control. The biochemical and physiological alterations are indications that the extract of *P.thonningii* leaf has a dose dependent protective effect in indomethacin-mediated gastric mucosa onslaught, which can be attributed to its antioxidant potential or activity.

**Keywords:** Antioxidant activity, Indomethacin, *Piliostigma thonningii*, Ulcer, Mortality, Morbidity

**Introduction**

An ulcer is basically an inflamed break in the skin or mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance [1]. About 19 out of 20 peptic ulcers are duodenal while gastric ulcers found in the stomach wall are less common [2]. The gastric mucosa is continuously exposed to potentially injurious agents such as acids, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs [3]. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility.
Symptoms of ulcer include epigastric pain of a burning or gnawing nature (postprandial pain and pain relieved by food or antacids), nausea, vomiting, belching and bloating.

Reactive oxygen species (ROS) are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) control their accumulation [4]. Any imbalance in the activity of these enzymes normally leads to faulty disposal of free radical and its accumulation. These ROS are responsible for oxidation of tissues leading to lipid peroxidation and tissue damage. They are also responsible for oxidation of bases in cellular DNA making them mutagenic, cytotoxic and cross linking agents, which in turn causes uncontrolled expression of certain genes causing increased multiplication of cells leading to cancer [5]. Antioxidants seemed to have protective role in gastric ulcers [6]. Antioxidant agents are compounds that have the potentials to scavenge reactive oxygen species of free radicals. These free radicals play important roles in energy production, synthesis of some biomolecules, phagocytosis and cell growth. It is well known that antioxidant activity in higher plants has often been associated with phenolic compounds, which have been demonstrated to be present in both Piliostigma species [7]. Generation of free radicals in the body beyond its antioxidant capacity leads to oxidative stress which has been implicated in diseases like cancer, diabetes, hypertension, inflammation and AIDS [8, 9].

Piliostigma thonningii is an underexplored leguminous plant that belongs to family of Caesalpiniacea is commonly known in African and across other sub-saharan countries as follows: Carmel’s foot (English) Kameel spoor (Africans), Mukolokote (Venda); Mokogoropo (North Sotho .In Nigeria it is known locally as abefe in Yoruba, kalgo in Hausa, okpoatu in Igbo, nyihar in Tiv, omepa in Igede, ejei-jei in Igalal, obepe in Yala and Kidakpam in Obudu languages [8-10].

Ulcers are deep lesions penetrating through the entire thickness of the gastrointestinal tract (GIT) mucosa and muscularis mucosa. H. Pylori is the main cause of stomach ulcers. H. Pylori is a gram negative bacillus, motile, microaerophilic flagellated and spiral shaped bacteria [11]. Type 1 strian of H. Pyloripossess a pathogenic activity, which encodes, gene A (CagA). Gastric acid is established as one of the major ulcerogenic factor for the induction of gastric ulcer disease. It has been reported that about 50% of gastric ulcer patients are pepsin and acid hypersecretors. But on the other hand, gastric acid plays a stringent role in gastric defenses to prevent bacterial colonization and reduced the ability to entrance in the mucosal layer [12]. Acid secretion is suggested to be stimulated by three principle secretion secretagogues: histamine, acetylcholine and gastin receptor on the surface of parietal cells, receptor that are sensitive to muscarinic effect of acetylcholine released from the vagus nerve and probably receptor responsive endogenous circulating gastrin [13]. Gastrin stimulates acid secretion either by direct stimulation of parietal cells or by the release of histamine from Extracellular cells.

Helicobacter pylori (H. Pylori) is etiologically linked to several major gastro duodenal diseases, the mechanism of its action has not been fully explained. However, it has been suggested that free radicals are closely related with gastric ulcer and gastritis [14]. Oxygen free radicals are detrimental to the integrity of biological tissues and mediate their injury. The mechanism of damage involves lipid peroxidation, which destroys cell membranes with the release of intracellular components, such as lysosome enzymes, leading to further tissue damage. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage [15]. The generation of the superoxide anion as a mechanism of damage is well established in different models of acute and chronic injury, but it has not been clarified whether this radical is involved in gastric mucosal damage [16].

Reactive oxygen species (ROS) are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) control their accumulation [6]. Any imbalance in the activity of these enzymes normally leads to faulty disposal of free radicals and its accumulation. These ROS are responsible for oxidation of tissues leading to lipid peroxidation and tissue damage. They are also responsible for oxidation of bases in cellular DNA making them mutagenic, cytotoxic and cross linking agents, which in turn causes uncontrolled expression of certain genes causing increased multiplication of cells leading to cancer [16]. Antioxidants seemed to have protective role in gastric ulcers. Therefore, this study entails the effect of aqueous leaf extract of Piliostigma thonningii on some antioxidant enzymes following indomethacin induced gastric ulcer in albino wistar rats.
Plant material

Fresh leaves of *P. thonningii* were collected from Igoli Road, Cross River University of Technology, Cross River State, Nigeria. The leaves were taken to Federal College of Forestry (FCOFJ) Jos in Plateau State, Department Herbarium for identification and authentication. The Voucher number #25 has been deposited for future reference at the department’s (FCOF J) Herbarium.

Preparation of plant material

Fresh leaves of *P. thonningii* were air-dried at room temperature for twenty (20) days, macerated and pulverized into powdery form using the blender and then sieved.

Aqueous extraction

Three hundred (300) g of powdered *P. thonningii*, leaves were dissolved with 1200mls of distilled water for 24 hours in a refrigerator. Thereafter, it was filtered with muslin cloth and filtered using Whatman filter No1. The filtrate was evaporated to dryness and the percentage yield was calculated reconstituted into dosage and administered into rats.

Experimental animal

Thirty-Six (36) wistar albino rats (120-200) g were obtained from the Animal Holding Unit of the Department of Medical Biochemistry, Cross River University of Technology Cross River State, Nigeria. The animals were allowed to undergo acclimatization period for seven (7) days before the commencement of the research. Each rat was housed in a plastic cage. The animal room was ventilated and kept at room temperature and relative humidity 29±2°C and 70% with 12 hours natural light dark cycle and were allowed free access to standard feed and water. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily.

Anti-ulcer activity

The experiment was carried out on 36 male rats that were divided into six (6) groups of 6 rats each. Group one (1) served as control and was given 0.5ml of normal saline (vehicle). Group three (3), five (5) and six (6) were given 100, 100 and 200mg/kg body weight of the extract respectively while group two (2) was treated with 100mg/kg body weight of the drug (cimetidine). The vehicle and extract were administered orally while the drug was administered intra-muscularly for 12days. After 12days of administration ,all rats were fasted for 24 hours,

gastric ulceration was then induced by the administration of 40mg/kg body weight of indomethacin orally only to group 2, 4, 5 and 6 respectively. Twelve(12) hours after indomethacin administration all rats were sacrificed after been anaesthetised with chloroform, the abdomen of each rats was opened to remove the stomach, Liver, Kidney and Testes for the determination of their superoxide dismutase (SOD), catalase, lipid peroxidation (MDA) activity and tissue protein concentration.

Protein determination by folin-ciocalteau (lowry) method

Protein was determined according to the method of [17] using Standard protein solution: 0.2 mg per ml of bovine serum albumin (BSA).

PRINCIPLE:
The Folin-Ciocalteau reagent was used in the quantification of proteins by [17]. In its simplest form the reagent detects tyrosine residues due to their phenolic nature. The reaction of a protein in solution with the Folin reagent occurs in two stages:

1. Reaction with 
   \[ \text{Cu}^{++} + \text{protein} \rightarrow \text{Cu}^{++} \cdot \text{protein} \]

2. Reduction of the phosphomolybdic-phosphotungstic reagent by the Cu++ - protein complex.

The reduced complex gives a blue solution with an absorption in the red portion of the visible spectrum (600-800 nm).

Preparation of reagents for protein determination

Reagent A: 2% Sodium Carbonate (Na2Co3), in 0.1N Sodium hydroxide NaOH

Reagent B: 0.5 % Copper Sulphate (CuSO4. 5H2O) in 1% Potassium Sodium tartate

Reagent C: Prepare freshly, alkaline Copper Solution: Mix 50ml of A and 1ml of B, prior to use.

Reagent D: Folin- Ciocalteau reagent.

Procedure

Nine (9) ml of distilled water was pipetted into 1 ml of sample (serum) to make 10 times dilution. Then 2 ml of reagent C (working solution) was added to each tube and were mixed thoroughly and allowed the mixture to stand for about 10 minutes. 0.2 ml of Folin-Ciocalteau reagent was thereafter mixed with it and the mixture was mixed thoroughly and the tubes were then kept in a dark
cupboard for about 30 minutes. Absorbance was read at 660nm for all the tubes.

**Determination of standard protein curve**

Take another 6 clean test tubes. Pipette 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of the standard protein solution, (100ug/ml to tubes 1-6 respectively). Appropriate amount of distilled water was added to make a volume of 1ml. 2ml of reagent c was added to each tube mixed thoroughly and allowed to stand for 10 mins. Then 0.2ml of Folin-Ciocalteau reagent was added and mixed thoroughly and then the tubes were kept in a dark cupboard for about 30 min. The absorbance at 660nm of all the tubes against the blank was determined.

**Determination of superoxide dismutase (SOD) activity**

The levels of total SOD activity in the tissues were determined by the method of [18]

**Principle**

The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O$_2^-$) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

**Reagents**

- **0.3 mM Epinephrine**
  - 0.01 of epinephrine (Sigma Chemical) was dissolved in 17ml of distilled water.
- **0.05 M Carbonate buffer (pH 10.2)**
  - 14.32g of Na$_2$Co$_3$.10H$_2$O and 4.20 g of NaHCO$_3$ were dissolved in distilled water and made up to 1000 ml with distilled water and the pH adjusted to 10.2.

**Procedure**

An aliquot of 0.2 ml of each of the tissue homogenates was added to 2.5 ml of 0.05 carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3 ml of freshly prepared 0.3 mM epinephrine to the mixture. The absorbance of the sample was measured at 450 nm using spectrophotometer.

Change in ab/ min = A5-A1/2.5

% Inhibition = \( \frac{\text{Increase in abs of sample}}{\text{Increase in abs of blank}} \times 100\% \)

I unit SOD == amount that cause 50% inhibition.

**Determination of catalase activity**

Catalase activity was determined according to the method of [19].

**Principle**

This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H$_2$O$_2$ with the formation of perchromic acid as an unstable intermediate. The chromate acetate then produced is measured colorimetrically at 570-610nm. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H$_2$O$_2$ for different periods of time. The reaction was stopped at a particular time by the addition of dichromate acetate mixture and the remaining H$_2$O$_2$ is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

**Reagents**

- (a) 5% K$_2$Cr$_2$O$_7$
  - 5g of potassium heptaoxodichromate (VI) was dissolved in some distilled Water and the solution were made up to 100ml with the same.
- (b) 0.2M Hydrogen Peroxide (H$_2$O$_2$)
  - 11.50ml of 30% (w/w) H$_2$O$_2$ was diluted with distilled water in a volumetric flask and the solution was made up to 500ml.
- (c) Dichromate/acetate acid solution
  - This reagent was prepared by mixing 5% solution of K$_2$Cr$_2$O$_7$ with glacial acetic (1:3 by volume)
- (d) 0.01M phosphate buffer, pH 7.0
  - 3.58g of Na$_2$HPO$_4$ 12H$_2$O and 1.19g of NaH$_2$PO$_4$2H$_2$O were dissolved in 900ml of distilled water. The pH was adjusted to 7.0 and distilled water was then added to make up to 1 litre.

**Colorimetric determination of H$_2$O$_2$ standard curve**

Different amounts of H$_2$O$_2$ ranging from 10 to 100µmoles were pipette into test tubes and 2ml of dichromate/acetate
was added to each. Addition of the reagent instantaneously produced an unstable blue precipitate of perchromic acid. Subsequent heating for 10mins in a boiling water-bath changed the colour of the solution to stable green due to formation of chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made up to 3ml and the optical density measured with a spectrophotometer at 570nm. The concentrations of the standard were plotted against absorbance.

**Determination of catalase activity in samples**

1ml of the supernatant fraction of the tissue homogenate was mixed with 19ml distilled water to give a 1:20 dilution. The assay mixture contained 4ml of solution H$_2$O$_2$ (800 μmoles) and 5ml of phosphate buffer, pH 7.0 in a 10ml flat bottom flask. 1ml of properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1ml portion of the reaction mixture was withdrawn and blown into 2ml dichromate/acetic acid reagent at 60 seconds interval. The hydrogen peroxide contents of the withdrawn sample were determined by the method described above.

The monomolecular velocity constant $K$ for the decomposition of H$_2$O$_2$ by catalase was determined by using the equation for a first-order reaction.

$$K = \frac{1}{t} \log \frac{S_0}{S}$$

Where $S_0$=initial concentration of H$_2$O$_2$ and $S$ = concentration of H$_2$O$_2$at 1min interval. The values of $K$ were plotted against time in minutes and the velocity constant of catalase $K(O)$ at 0 minute was determined by extrapolation.

The catalase content of enzyme preparation was expressed in terms of catalase feihahigkeit or “Kat f” (which is equivalent to micromole H$_2$O$_2$ consumed per min mg protein) according to Von Euler and Josephson (1927):

$$\text{Kat f} = \frac{K \cdot \text{O}}{\text{Mg protein/ml}}$$

**Assessment of lipid peroxidation**

A breakdown product of lipid peroxidation thiobarbituric acid reactive substances (TBARS) was measured by the method of [19]

**Principle**

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535nm.

**Reagents**

Hydrochloric acid (0.25N)

250ml of 1N HCl was diluted with distilled water and made up to 100ml.

TCA-TBA-HCl-Stock

The stock solution contained equal volumes of trichloroacetic acid (TCA) 15% (w/v) (Sigma chemical Co, London) in 0.25N hydrochloric acid and 2-thiobarbituric acid (TBA) 0.375% (w/v) (Sigma Chemical Co, London) in 0.25N hydrochloric acid. Dissolution of TBA was aided by shaking in a boiling water bath.

**Procedure**

One volume of the test sample and two volume of stock reagent were mixed in a cooked test tube and heated for 15 minutes on a boiling water bath. After cooling at room temperature, the precipitate was removed by centrifugation at 1000 x g for 10 minutes and the absorbance of the supernatant was measured at 532nm against blank containing all the reagents except test sample.

**Calculation**

The malondialdehyde (MDA) concentration of the sample was calculated from the absorbance using an extinction coefficient of 1.56 x 105 M$^{-1}$ cm$^{-1}$ according to the method of [20]

$$\text{MDA (moles / g tissue)} = \frac{\text{absorbance} \times \text{T.V}}{E_{532} \times \text{Vs} \times \text{g tissue}}$$

Where $E_{532} = \text{Molar extinction coefficient for MDA}$

$\text{T.V} = \text{total volume of reaction mixture}$

$\text{Vs} = \text{Volume of Sample}$

$\text{g} = \text{gram}$

**Results**

The effect of the extract of P. thonningii leaf on kidney lipid peroxidation (MDA) produce a significant reduction ($p<0.05$) in all the treated groups when compared with the untreated group (Fig.1). Furthermore, the effect of extract of P. thonningii leaf on liver lipid peroxidation following indomethacin mediated gastric mucosa onslaught showed a significant increase ($P<0.05$) in groups treated with cimetidine (standard drug), indomethacin without
treatment group (negative control) and the treated with 100mg/kg body weight of the extract while other treated groups shows no significant difference when compared with the control (Fig. 2). The effect of extract of *P. thonningii* leaf on stomach lipid peroxidation following indomethacin induced gastric ulceration shows a significant increase (P<0.05) in the indomethacin without treatment group whereas other treated groups shows no significant difference when compared with the control (Fig. 3). Similar pattern were shown in the testes lipid peroxidation (Fig. 4).

The effect of *P. thonningii* leaf on kidney SOD following indomethacin induced ulceration shows a significant increase (P<0.05) when compared with the control while the groups treated with 100 and 200mg/kg body weight of the extract produced a significant decrease (P<0.05) when compared with the control, likewise more significant decrease were observed in the ulcerated group without treatment when compared with the control (Fig 5). The effect of extract of *P. thonningii* leaf in liver SOD following indomethacin induced ulceration shows significant decrease (P<0.05) in all extract treated group and Cimetidine group (standard drug) with an exception of extract only group which shows a significant increase (P<0.05) (Fig 6). The effect of extract of *P. thonningii* leaf on stomach SOD following indomethacin mediated gastric mucosa onslaught shows a significant increase (P<0.05) in extract only and cimetidine treated groups while there was a significant decrease (P<0.05) in the groups treated with 100 and 200mg/kg body weight of extract and indomethacin without treatment group (negative control) compared to the control (Fig 7). Similar pattern was observed in the testes SOD (Fig 8).

The effect of extract of *P. thonningii* on kidney protein following indomethacin induced gastric ulceration shows a significant decrease (P<0.05) in all treated groups with compared to the control group (Fig. 9). The effect of extract of *P. thonningii* on liver protein following indomethacin induced gastric ulceration shows a significant decrease (P<0.05) in all treated groups with an exception in the extract only treated group which shows a significant increase (P<0.05) when compared with the control group (Fig. 10). The effect of extract of *P. thonningii* on stomach protein following indomethacin induced gastric ulceration show a significant decrease (P<0.05) in the cimetidine (standard drug) and indomethacin treated group (negative control) while other treated groups shows a significant increase (P<0.05) compared to the control group (Fig. 11). The effect of the extract of *P. thonningii* on testes protein following indomethacin induced gastric ulcer shows no significant different in group treated with extract only compared with control group while other treated groups shows significant decrease (P<0.05) when compared with control group (Fig. 12).

The effect of extract of *P. thonningii* leaf on kidney catalase activity following indomethacin mediated gastric mucosa onslaught reveals a significant decrease (P<0.05) in indomethacin induced untreated group, low dose and high dose compared with the control whereas groups treated with extract only and cimetidine had no significant difference when compared with the control group (Fig. 13). Likewise, Fig 14 reveals the effect of extract of *P. thonningii* leaf on liver catalase activity following indomethacin mediated gastric mucosa onslaught. All treated groups produced a significant decrease (P<0.05) when compared with the control group. The effect of *P. thonningii* leaf on stomach catalase activity following indomethacin mediated gastric mucosa onslaught shows a significant decrease (P<0.05) in all treated group with exception of the group treated with extract only which shows a significant increase (P<0.05) compared to the control (Fig. 15). The effect of extract of *P. thonningii* leaf on testes catalase following indomethacin mediated gastric mucosa onslaught shows no significant difference different (P<0.05) in all the treated group with an exception in the indomethacin induced untreated group (negative control) when compared with the control group (Fig. 16).
Antioxidant Activity of Aqueous Extract of *Piliostigma thonningii*

**Figure 1**: Effect of aqueous extract of *P. thonningii* leaves on kidney lipid peroxidation following indomethacin mediated gastric mucosa onslaught.

**Figure 2**: Effect of aqueous extract of *P. thonningii* leaves on liver lipid peroxidation following indomethacin mediated gastric mucosa onslaught.

**Figure 3**: Effect of extract of *Piliostigma thonningii* leaves on stomach lipid peroxidation following indomethacin mediated gastric mucosa onslaught.

**Figure 4**: Effect of aqueous extract of *P. thonningii* leaves on testis lipid peroxidation following indomethacin mediated gastric mucosa onslaught.
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Figure 5: Effect of aqueous extract of *P. thonningii* leaves on kidney SOD following indomethacin mediated gastric mucosa onslaught.

Figure 6: Effect of aqueous extract of *P. thonningii* leaves on liver SOD following indomethacin mediated gastric mucosa onslaught.

Figure 7: Effect of aqueous extract of *P. thonningii* leaves on stomach SOD following indomethacin mediated gastric mucosa onslaught.

Figure 8: Effect of aqueous extract of *P. thonningii* leaves on testis SOD following indomethacin mediated gastric mucosa onslaught.
Figure 9: Effect of aqueous extract of *P. thonningii* leaves on kidney protein following indomethacin mediated gastric mucosa onslaught.

Figure 10: Effect of aqueous extract of *P. thonningii* leaves on liver protein following indomethacin mediated gastric mucosa onslaught.

Figure 11: Effect of aqueous extract of *P. thonningii* leaves on stomach protein following indomethacin mediated gastric mucosa onslaught.

Figure 12: Effect of aqueous extract of *P. thonningii* leaves on testis protein following indomethacin mediated gastric mucosa onslaught.
Figure 13: Effect of aqueous extract of P. thonningii leaves on kidney catalase following indomethacin mediated gastric mucosa onslaught.

Figure 14: Effect of aqueous extract of P. thonningii leaves on liver catalase following indomethacin mediated gastric mucosa onslaught.

Figure 15: Effect of aqueous extract of P. thonningii leaves on stomach catalase following indomethacin mediated gastric mucosa onslaught.

Figure 16: Effect of extract of Piliostigma thonningii leaves on testis catalase following indomethacin mediated gastric mucosa onslaught.
Discussion

Gastric ulcer is one of the diseases responsible for high mortality and morbidity among the less privileged in Africa and beyond, possibly due to wide-spread or usage of NSAIDs recently or due to poor understanding of the pathophysiology of this disease [21]. Studies investigating new active compounds are needed. As well, various pharmaceutical products currently used for treatment of gastric ulcers are not completely efficient and cause many adverse side effects. Consequently, it is necessary to develop more effective agents that are also less toxic, with medicinal plants being an attractive source for the development of new drugs because of their wide array of active ingredients [22].

In this study we used indomethacin to induce gastric mucosa ulcer. Indomethacin is known to induce the reactive oxygen metabolites in animal models, which may contribute to mucosal injury. These free radicals also damage the cellular antioxidant enzymes such as CAT, SOD among others, acting as the first line of cellular defense against oxidative injury, this might lead to aggravated tissue damage during stomach ulceration. Indomethacin-induced stomach ulceration can triggers severe oxidative stress in gastric tissue causing damage to key bio molecules such as lipids. This was apparent from the stimulated lipid oxidation leading to increased accumulation of MDA as well as reduction in the gastric activity of CAT. As a matter of fact indomethacin being a NSAID is widely used in clinical practices due to its efficacy and various therapeutic effects, on the other hand acute gastrointestinal lesions are the most serious and frequent side effects of NSAIDs, making them the most common cause of gastro-duodenal ulcers in Western countries [21,23]. Ulcer formation induced by Indomethacin, is known to be co-related with inhibition of cyclooxygenase (COX₁ and COX₂), that prevents prostaglandin biosynthesis [24] which in turn inhibits the release of mucus a defensive factor against gastrointestinal damage.

Recently, much attention has been focused on oxygen derived free radicals which play an important role in the pathogenesis of gastric ulcer apart from the interactive processes like many other tissue degeneration situations. Oxygen derived free radicals cause tissue injury through lipid peroxidation. Oxygen handling cells have different systems, e.g. superoxide dismutase (SOD), peroxidases and catalases which are able to protect them against the toxic effects of oxygen derived free radicals. As shown in this present results, treatment with extract of *P.thonningii* leaf significantly reverted the indomethacin-induced changes or alterations in MDA and CAT. This significant reduction in MDA levels along with significant increase in SOD and CAT level suggest decreased lipid peroxidation and cytoprotective or antioxidant activity of extract of *P.thonningii*. Cimetidine also provided a marked suppression of oxidative damage due to its excellent radical scavenging capacity; it brought MDA level closer to normal levels, but less than observed in the extract with concomitant increase in CAT level and possibly with the release of Nitric oxide. Nitric oxide (NO) is an endogenous defensive factor for gastric cells and exhibits gastro protective properties against different types of aggressive agents [25]. It is also involved in the maintenance of mucosal integrity through the regulation of mucus and alkaline secretion, gastric motility and microcirculation [26]. NO is known to modulate acid levels, gastric mucus secretion, and blood flow in gastric tissues, prevention membrane lipid peroxidation, protection against NSAID damage by promotion of prostaglandin synthesis [27].

In the present study, it appears that *P.thonningii* has bioactive compounds which have synergistic relationship with NO synthesis. Indomethacin also significantly reduced total protein for negative control group but significantly increased in groups treated with high and low doses of *P.thonningii* suggest that the Plant might contain a bioactive compound which might aid in protein synthesis or antibody or free radical scavenging property hence aiding its antioxidant activity by preventing the formation of free radicals or by scavenging superoxide anions.

Our results show that treatment with extract of *P.thonningii* at the dose of 100 and 200mg/kg body weight significantly decreased the level of lipid peroxidation product (MDA) when compared to untreated ulcerated rats. The activities of both SOD and Catalase were decreased in ulcerated untreated groups and maintained to near normalcy in treated group with the exception of the extract treated group which shows a significant increase compared to the control group.

Conclusion

The present results of biochemical and physiological alterations are indications that the extract of *P.thonningii* leaf has a dose dependent cytoprotective effect against indomethacin-mediated gastric mucosa onslaught, which can be attributed to its antioxidant activity.

Conflict of interest

The authors declare that there is no conflict of interest to reveal.
References