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Evaluation of antioxidant activity of lime juice extract of cashew bark in normal and wistar rats with indomethacin toxicity

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Abstract

This study evaluated the effect of lime juice extract of cashew bark (LJECB) on serum antioxidant status in normal and Wistar rats with indomethacin toxicity. Fresh *Anacardium occidentale* bark was pulverised and extracted using fresh juice of *Citrus aurantifolia*. Forty two female Wistar rats (150-200 g) were purchased and randomised into seven groups (n=6): Group 1 (normal control); Group 2 (Indomethacin 30 mg/kg only); Group 3 (Indomethacin 30 mg/kg + Gecrol antacid); Group 4 (Indomethacin 30 mg/kg + LJECB 400 mg/kg); Group 5 (Indomethacin 30 mg/kg + LJECB 800mg/kg); Group 6 (LJECB 400 mg/kg only), Group 7 (LJECB 800 mg/kg only). Post-treatment with LJECB or Gecrol antacid was commenced 4 hours for 14 days. On the 15th day, the animals were sacrificed, and the serum was collected aand used for antioxidant, lipid peroxidation (malonialdehyde MDA), and total protein assays based on standard protocols. Results showed that treatment with 400 mg/kg LJECB elevated the nitric oxide (NO), superoxide dismutase (SOD), and MDA levels, but lowered the activities of glutathione peroxidase (GPx) and catalase (CAT). Also, 800 mg/kg of LJECB elevated the NO, CAT, SOD, and MDA levels, but lowered the level of GSH, GPx, and total protein. The study concludes that LJECB provoked a significant increase in antioxidant status (NO, SOD, and CAT) but lowered the activity of GPx and reduced glutathione (GSH). However, the elevation of lipid peroxidation biomarker (MDA) suggests a possible damage to the stomach endothelial membranes. Therefore, caution should be exercised when consuming the LJECB for therapy.

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Introduction

Oxidative stress has been described as a component of many disease conditions (Forman and Zhang, 2021). Oxidative stress is a physiological condition characterized by an imbalance between oxidant production and the body's antioxidant defense mechanisms in favour of oxidants, leading to damage in biological systems (Aminjan *et al.*, 2019). Free radicals including reactive oxygen species (ROS) and peroxides are known to mediate cellular damage via oxidation of biomolecules including lipids, proteins, and DNA (Ayala *et al.*, 2014). Oxidative stress has been linked to the development of several chronic conditions including cardiovascular diseases, neurodegenerative disorders, premature aging, DNA disruption, lipid peroxidation, and gastric mucosal injuries (Valko *et al.*, 2007). The extent to which oxidants promote human diseases varies in biological systems. Therefore, raising the antioxidant status may not be effective in all disease conditions (Forman and Zhang, 2021). The context of oxidants or reactive species is comprised of oxygen and nitrogen radicals, each with unique reactive properties and interactions (Murphy *et al.*, 2022). Therefore, there is a need to study the species of the oxidants that promote cellular damage to provide a holistic approach in rational antioxidant drug design.

Several studies have evaluated oxidative stress in living systems and observed that production of ROS is dependent on enzymatic and non-enzymatic reactions (Pizzino *et al.*, 2017). The human body produces the oxidants or free radicals via the normal biological processes, including breathing, food digestion, alcohol or drug metabolism, and energy production. The natural antioxidant mechanism normally helps to eliminate the free radicals. A controlled production of free radicals were reported to have beneficial roles in cellular defense against invasive pathogens, cell signaling, etc (Saleem *et al.,* 2023). However, if the body fails to control the production or scavenge them, they can cause a negative chain reaction in the body (Vona *et al.*, 2021). This reaction has the potential to destroy cell membranes, inhibit the activity of important enzymes, stop vital cellular processes, stop normal cell division, destroy deoxyribonucleic acid (DNA), and prevent the production of energy (Sharifi-Rad *et al.*, 2020).

Increased production of ROS is a hallmark that characterises the toxicity of many xenobiotics, including chemotherapeutic drugs (Jomova *et al.,* 2023). The impact of oxidative stress on a biological system is dependent on several factors including the nature of oxidant, the site, and intensity of production, a functional repair system, and endogenous antioxidant system (Pizzino *et al.*, 2017). Since the organism may not be able to control these factors on its own, there is a need to fortify the body with abundant antioxidant compounds to enable it protect the body from free radical insult; irrespective of the source, type, and abundance (Fountoucidou *et al.*, 2019).

Antioxidants, whether in form of diets or supplements, play a crucial role in maintaining cellular health by neutralizing ROS and preventing oxidative damage (Vona *et al.,* 2021). Antioxidants can be obtained from various sources including fruits, seeds, vegetables, and herbs (Saleem *et al.*, 2023). Citrus fruits are particularly known for their rich antioxidant content due to the presence of bioactive compounds like ascorbic acid, phenolic compounds, and flavonoids (Saleem *et al.,* 2023). Lime (*Citrus aurantifolia*) is one such citrus fruit that has gained attention for its potential health benefits (Rahaman *et al.*, 2023). Synthetic antioxidants are known to protect against oxidative damages. However, research has shown that some synthetic antioxidants are not as effective as natural antioxidants as they produce toxic effects when consumed as supplements or remedies (Sen, 2014).

The genus *Anarcadium* has received great recognition in recent years due to their nutritional, biological, and pharmacological benefits (Salehi *et al.*, 2020). The leaves, fruits, and stem parts contain beneficial secondary metabolites. A plant with several medicinal benefits, however those that have drawn the most interest are their antioxidant, antibacterial, and anticancer properties (Salehi *et al.*, 2020).

In Nigeria, the bark is used for relief of hemorrhoids, diarrhea, pains, skin infection, inflammatory diseases, type 2 diabetes, and fever (Barbosa-Filho *et al.*, 2014). The leaves are used for malaria, yellow fever, diarrhea, ulcer, blisters, itching, warts, rheumatism, hypertension, and sore throat (Salehi *et al.*, 2020). In Ghana, the leaves are used for toothache and sore gums, dysentery, diarrhea, and piles. In Benin, the root is used for cough, stomach pain, tooth decay, hypertension, and malaria (Salehi *et al.*, 2020).

Fruit cashews is delicious and a good source of vitamins, minerals, and other important nutrients. It contains significant amounts of vitamin C and minerals, nearly five times higher than that of oranges. The fruit contains a few volatile substances, such as carboxylic acids, terpenes, and esters (Bicalho and Rezende, 2001). The bark, leaves, and nuts are rich in tannins and flavonoids, representing an economic source of antioxidants for both nutraceutical and pharmaceutical benefits (Salehi *et al.*, 2020). Anarcadic acid is abundant in the cashew nutshell. While cardanol and cardol are the principal components of cashew nutshell liquid (Kumar *et al.*, 2002).

Citrus aurantifolia (lime; family: Rutaceae) also known as miracle fruit, is a globally cultivated plant (Jaina *et al.*, 2020). Mexico and Argentina are the world's largest producers of lime averaging 2,270 and 1,450 metric tons per year (Statista, 2023). Lime fruit juice is a widely consumed cuisine in many cultures due to its acclaimed medicinal purposes such as antibacterial, anti-inflammatory, anticancer, antidiabetic, anti-hypertensive, antioxidant properties (Liu *et al.*, 2014).

Materials and methods

Collection of plant samples

Fresh *Anacardium occidentale* (Cashew) stem bark was collected at the Adeleke University campus, Ede, Osun State. Fresh and healthy *Citrus aurantifolia* (Lime) fruits were purchased at Ede market, Osun State, Nigeria. The two plants were authenticated (Voucher Number: IFE-18169, Voucher Number: IFE-18170) respectively at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of Citrus aurantifolia juice extract of Anacardium occidentale

Preparation of C. aurantifolia juice

Fresh *C. aurantifolia* fruits (ripe) were rinsed with tap water and sliced with knife. The juice was squeezed into a clean basin and filtered severally with sieve cloth until a clear filtrate solution was obtained. The residues were discarded and the filtrate was kept in refrigerator until use.

Preparation of A. occidentale

Fresh stem bark of *A. occidentale* was rinsed in tap water and dried. The sample was cut into small pieces and pulverized mechanically using mortar and pestle to expose the cells that contain the phytochemicals.

Extraction of A. occidentale stem bark

The Pulverized stem bark (1.0 kg) of *A. occidentale* was soaked in 3 L of *C. aurantifolia* (Lime) for 48 h inside a refrigerator. Thereafter, the solution was filtered severally and the filtrate was concentrated *in vacuo* using a rotary evaporator at the Department of Biochemistry, Adeleke University, Ede. This produced the crude extract labeled as LJECB.

Ethical clearance

An ethical clearance (AUERC/2024/66IR/01) was collected from the Ethical Committee of Adeleke University, Ede, Osun State, Nigeria.

Experimental animals

Forty two (42) non-parous female Wistar rats (150-200 g) were purchased from an animal breeder in Ogbomoso, Oyo State, Nigeria. The animals were acclimatized for seven (7) days in a well-ventilated animal house at the Animal Facility Unit, Department of Biochemistry, Adeleke University, Ede. The animals were fed with standard feed and water *ad libitum*.

Experimental design

The study adopted curative treatment model. The animals were randomized into seven groups (n = 6) as follows: Group 1 (normal control; distilled water); Group 2 (negative control; Indomethacin 30 mg/kg only); Group 3 (positive control; Indomethacin 30 mg/kg + Gecrol antacid); Group 4 (Indomethacin 30 mg/kg + LJECB 400 mg/kg); Group 5 (Indomethacin 30 mg/kg + LJECB 800mg/kg); Group 6 (LJECB 400 mg/kg only), Group 7 (LJECB 800 mg/kg only).

Induction of gastric ulcer

Prior to the induction of gastric ulcer, the experimental animals were fasted for 24 h but allowed free access to water *ad libitum*. Thereafter, 1 ml of 30 mg/kg indomethacin stock was orally administered to the rats in Group 2-5 (Sabiu *et al*., 2015). After spiking ulcer with indomethacin, the animals were fasted for 4 h with free access to water to allow for ulcer development.

Treatment/ administration

After 4 hours of indomethacin induction, treatment commenced with 1 ml of Gecrol antacid (5 ml/kg) administered to the ulcerated animals in Group 3. Also, 1 ml of LJECB (400 or 800 mg/kg) was administered orally to the ulcerated animals in Group 4 and 5. Also, 1 ml of LJECB (400 or 800 mg/kg) was administered orally to the normal rats in Group 6 and 7 to evaluate the extract's effect only. Treatment with the reference drug or the extract lasted for 14 days between 9:00 - 10:00 a.m. in the morning. Animals were given food and water *ad libitum* throughout the 14 days of treatment.

Animal Sacrifice, Blood collection, and Blood Serum

Twenty four (24) hours after the last administration, the animals were anesthetized with mild inhalation of diethyl ether and sacrificed via cardiac puncture. The blood sample was collected into plain sample vials. After blood collection, the abdomen was opened and the stomach was excised and washed in isosaline to remove the gastric content. The stomach was also examined for stomach mucosal lesions.

Preparation of blood serum

The clotted blood samples were centrifuged at 3000 rpm (704 x g) for 10 min and their supernatants (serum) were collected and stored in sterile vials. This was kept in the refrigerator for biochemical analyses.

Antioxidant assays

Determination of reduced glutathione (GSH)

The GSH concentration in the serum was determined based on standard method (Ellman, 1959; Adegbola *et al*., 2022). The serum (1 ml) was treated with 1 ml of sulphosalicyclic acid (4%) for deproteination. Then, the mixture was centrifuged at 4000 rpm for five minutes and the supernatant was collected. The supernatant (0.5 ml) was added to 4.5 ml of Ellman's reagent (5,5' -Dithiobis-2-nitrobenzoate, DTNB). For blank preparation, 0.5 ml of sulphosalicyclic acid and 4.5 ml of Ellman's reagent were mixed. The absorbance of the sample was measured at 412 nm. The concentration of GSH was extrapolated from the standard curve and presented as µg/GSH/g sample.

Glutathione peroxidase (gpx) assay

The analysis of Gpx activity was carried out according to the method of Özyürek *et al*. (2012). The analysis was carried out in duplicate by mixing 0.1 ml of the homogenates with 0.5 ml phosphate buffer (pH 8.0), 0.1 ml sodium azide, 0.2 ml GSH, 0.1 ml H_2O_2, and 1 ml of distilled water. The mixture was incubated at 37°C for 3 min, then 0.5 ml TCA was added and centrifuged at 3000 rpm for 10 min. Then 0.1 ml of the supernatant was collected and mixed with 0.9 ml disodium hydrogen phosphate and 1 ml of DTNB. The absorbance was read in spectrophotometer at 412 nm against the blank solution which contained other components except the homogenate. The activity of the GPx was calculated using the expression:

GPx (µmol/ml)= (Absorbance of sample × Concentration of standard)/ (Absorbance of standard × Volume of serum (ml))

Superoxide dismutase assay

Superoxide dismutase assay was carried out according to the method of McCord and Fridovich (1969) based on the inhibition on pyrogallol

autooxidation. The serum (100 µl) was pipetted into clean test tubes in duplicate followed by addition of 2.5 ml Tris buffer and 100 µl of EDTA. The enzyme reaction was initiated by addition of 300 µl of pyrogallol. The increase in absorbance was measured at 420 nm, every 30 seconds interval for 150 secs against the blank containing 100 µl of distilled water in place of homogenate. The differences in absorbance per minute were calculated using the expression:

Change in Absorbance per minute (ΔAbs /min) = $B_5 - B_0/2.5$

Where, B_5 = Absorbance at 150 secs

B_0 = Absorbance at 30 secs

The activity of SOD was expressed as unit/mg of protein, where 1 unit of SOD is expressed as the amount of SOD required to cause 50% inhibition of oxidation of pyrogallol.

Catalase assay

The activity of catalase was assayed according to the modified method of Sinha (1972) as reported by Adegbola *et al.* (2022). The assay mixture consisted of 0.2 ml of serum mixed with 2 ml of (30 mM H_2O_2) in 50 mM potassium phosphate buffer pH 7.0 made up to 3 ml with distilled water. Decrease in the absorbance was monitored at 240 nm in 30 sec intervals for 3 min against the blank which consisted of distilled water and other reagents except the serum. The activity of the enzyme was expressed as µmoles of H_2O_2 decomposed /min/mg of protein.

Catalase activity (units/ml) = ΔA/min/ SV ×0.0436 ×df ×TV

ΔA= slope of the graph of absorbance against min; df = dilution factor; TV = total reaction volume (3 ml); 0.0436= Extinction coefficient hydrogen peroxide; Sv = sample volume.

Catalase activity (U/mg protein)= Units/ml/mg protein/ml

Malondialdehyde (mda) assay

The lipid peroxidation assay was determined via the thiobarbituric acid (TBA) method that measures the amount of malondialdehyde (MDA) products (Schmedes *et al.*, 1989). To 0.5 ml of stomach homogenate supernatant, 0.5 ml of phosphate buffer (0.1 M, pH 8.0) was added and 0.5 ml of 24% trichloroacetic acid (TCA). The resulting mixture was incubated at room temperature for 10 min, followed by centrifugation at 2000 rpm for 20 min. To 1 ml of supernatant was added 0.25 ml of 0.33% thiobarbituric acid (TBA) in 20% acetic acid and the resulting mixture was boil at 95°C for 1 hr. The resulting pink colour product was cooled and absorbance was read at 532 nm. (Extinction coefficient of MDA, $\varepsilon 532 = 1.53 \times 10^5\ M^{-1}\ cm^{-1}$).

Total protein assay

The total protein concentration was measured based on Lowry *et al.* (1951) method. The serum (20 µl) or the gastric juice (20 µl) was added to alkaline copper reagent (200 µl) composed of 2% (w/v) Na_2CO_3 in 0.1 M NaOH, 0.5 % (w/v) $CuSO_4.5H_2O$ and 1 % Na-K tartrate.$4H_2O$ (98:1:1v/v/v). The mixture was vortexed and allowed to stand for 10 min followed by addition of Folin-Ciocalteau reagent (20 µl). The reaction mixture was vortexed and allowed to stand at room temperature in the dark for 60 mins after which absorbance was read at 660 nm against the reagent blank prepared to contain 20 µl distilled water in place of the sample. The protein concentration of the test samples was estimated from BSA standard curve and expressed as µg/ml.

Nitric oxide (no) radical scavenging assay

The nitric oxide assay was determined according to the modified method of Grisham and Bryan (2007) using Griess reagent method. Exactly 100 µl of Griess reagent, 300 µl of serum sample, and 2.6 ml of distilled water were added into a test tube, and incubated at room temperature for 30 min. The blank was prepared by mixing 100 µl of Griess reagent and 2.9 ml of distilled water in a test tube. The absorbance was measured at 548 nm against the reagent blank. The NO levels were determined with reference to standard curve and results were presented in µmol/L.

Data analysis

Data were expressed as Mean ± SEM. Differences between the Mean values of the control and treated

groups were determined by One-way analysis of variance (ANOVA) with Dunnett post-hoc test using Graph pad prism 5.0. Significant difference was considered if $p < 0.05$.

Results

Effect of LJECB on serum reduced glutathione level in normal and wistar rats with indomethacin activity

The effects of LJECB on serum GSH is shown in Fig. 1. There was a significant ($p<0.05$) increase in serum GSH level of the negative control (Group 2) when compared with the normal control (Group 1). However, Group 3 (positive control) treated with Gecrol antacid showed higher level of GSH compared to the negative control and normal control groups. Interestingly, treatment with LJECB at 800 mg/kg significantly lowered the GSH level to normal. Normal Group 6 and 7 that received 400 and 800 mg/kg extract showed a significant increase in GSH level when compared with the normal control that received only the distilled water. The increase in GSH levels after *in vivo* administration with LJECB extract suggests enhanced antioxidant defense mechanisms and its potential to combat oxidative stress and protect the cells against oxidative damage.

Effect of LJECB on serum glutathione peroxidase activity in normal and wistar rats with indomethacin toxicity

The effects of LJECB on serum GSH is shown in Fig. 2. There was a non-significant increase in serum GPx activity of the negative control (Group 2) when compared with the normal control (Group 1). The normal Group 7 that received 800 mg/kg extract showed a non-significant increase in GPx activity when compared with the normal control. Following LJECB treatment, there was a non-significant rise in GPx activity, indicating that the plant extract may have a minor effect on the antioxidant defence system in female Wistar rats.

Effect of LJECB on serum superoxide dismutase (sod) activity in normal and wistar rats with indomethacin toxicity

The effects of LJECB on serum SOD is shown in Fig. 3. Indomethacin administration induced a significant increase in SOD activity in negative control (Group 2) compared to the normal control (Group 1). Treatment with LJECB at 400 and 800 mg/kg caused a significant two-fold increase in SOD when compared with the normal or negative control groups. The normal Group 6 and 7 that received only 400 or 800 mg/kg extract also showed a significant increase in SOD activity when compared with the normal control. Likewise the positive control (Group 3) treated with Gecrol antacid, high antioxidant SOD activity was also observed.

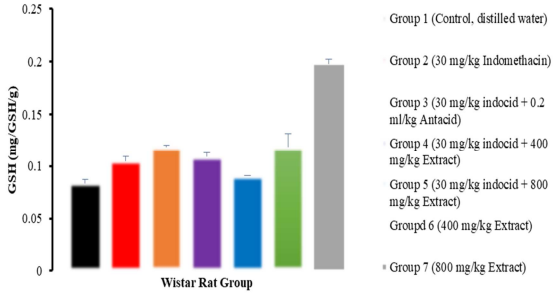

Fig. 1. Effect of LJECB on serum reduced glutathione level in normal and wistar rats with indomethacin activity

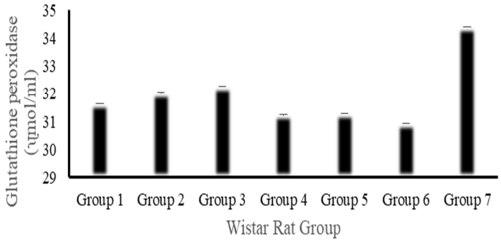

Fig. 2. Effect of LJECB on serum glutathione peroxidase level in normal and Wistar rats with indomethacin activity

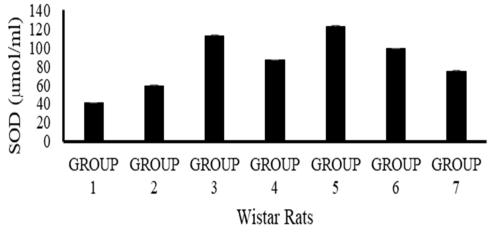

Fig. 3. Effect of LJECB on SOD activity in normal and Wistar rats with Indomethacin toxicity

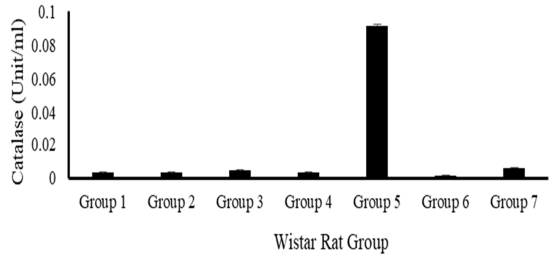

Fig. 4. Effect of LJECB on serum catalase activity in normal and Wistar rats with indomethacin toxicity

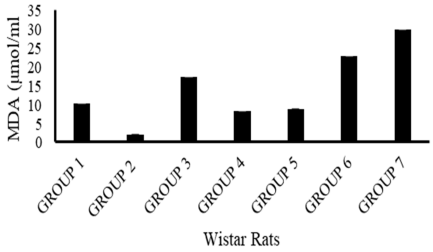

Fig. 5. Effect of LJECB on Serum MDA in normal and Wistar rats with indomethacin toxicity

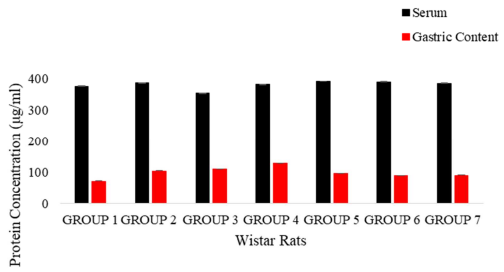

Fig. 6. Effect of LJECB on total protein concentration in normal and Wistar rats with indomethacin toxicity

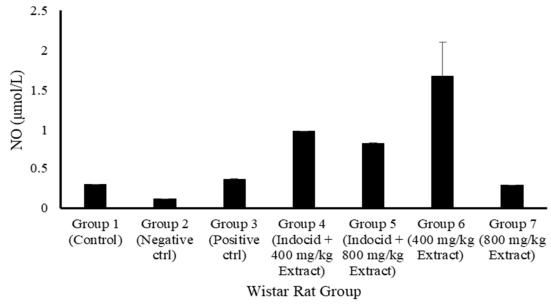

Fig. 7. Effect of LJECB on nitric oxide (NO) concentration in normal and Wistar rats with indomethacin toxicity.

Effect of LJECB on serum catalase activity in normal and wistar rats with indomethacin toxicity

The effect of LJECB on serum CAT is shown in Fig. 4. Indomethacin administration induced a non-significant increase in catalase activity in the negative control group compared to the normal control. Treatment with the LJECB at 800 mg/kg caused a significant increase in the catalase activity compared with the normal control group.

Effect of LJECB on serum malondialdehyde (mda) in normal and wistar rats with indomethacin toxicity

MDA, a product of lipid peroxidation, is used as a biomarker of oxidative stress. The effect of LJECB on MDA concentration is shown in Fig. 5. There was a significant decrease in MDA level in the negative control group when compared with the normal control (Group 1). However, treatment with the Gecrol antacid caused a significant increase in MDA level when compared with the normal or negative control group. When treated with the LJECB, the MDA level was restored to normal as in the normal control group. The normal groups in 6 and 7 however, showed a significant increase in MDA when compared with the normal or negative control groups. Suggesting that consumption of LJECB without underlying disease condition may trigger undue lipid peroxidation.

Effect of LJECB on total protein in normal and wistar rats with indomethacin toxicity

The effects of LJECB on protein concentration in the serum and gastric content is shown in Fig. 6. Indomethacin administration induced a significant increase in serum protein concentration in negative control (Group 2) compared to the normal control (Group 1). Treatment with gecrol (Group 3) caused a further spike in serum total protein concentration. However, administration of LJECB at 800 mg/kg caused a significant reduction in the serum total protein concentration. Administration of 400 or 800 mg/kg of LJECB to the animals in Group 6 and 7 produced no significant effect when compared with the normal control. The total protein concentration was found to be significantly higher in serum when compared with the gastric juice.

Effect of LJECB on nitric oxide (NO) concentration in normal and Wistar rats with indomethacin toxicity

The NO is a gastric defensive or protective factor against indomethacin-mediated ulcer. The effect of LJECB on NO concentration in normal and Wistar rats with indomethacin toxicity is shown in Fig. 7. There was a significant decrease in the NO level in the negative control (Group 2) compared to the normal control (Group 1). Conversely, the positive control group (Group 3) treated with Gecrol antacid restored the NO level to the normal level as compared with the normal control. Group 4 and 5 treated with 400 and 800 mg/kg of LJECB showed a significant increase in NO level compared to the normal control. As a defensive factor, the increase in nitric oxide level suggests some protective effects on the gastric vascular system. The normal rats in Group 6 that was administered with 400 mg/kg of LJECB showed a significant increase in NO level. While administration of 800 mg/kg of LJECB to normal rats in Group 7 showed a nonsignificant difference in NO concentration when compared with the normal or negative control.

Discussion

This present study investigated the effects of lime juice extract of cashew bark (LJECB) on serum antioxidant profile in female Wistar rats with indomethacin toxicity. NSAIDs including indomethacin produce damage to the gastro-intestinal mucosa in both humans and animals. This explains why their use is associated with high risk of hemorrhage, perforation of mucosal lining in ulcerative conditions (Yuji and Toshikazu, 2006). The molecular mechanism that allows NSAIDs like indomethacin to propagate gastro-intestinal toxicity is through the non-selective inhibition of cyclooxygenase 1 and cyclooxygenase 2. As a result, the COX-I is not able to produce sufficient prostaglandins required for housekeeping function (Botting, 2007), including maintenance of the stomach endothelial walls. Thus, leading to the pathogenesis of gastric ulcer and hemorrhage (Enas *et al.*, 2024). Ulceration may likely provoke rapid release of reactive oxygen species (ROS) which may lead to oxidative stress (Danisman *et al.,* 2023).

Studies have shown that, a common denominator for pathogenesis of gastric ulcer is free radical perturbation (El-Ashmawy, 2016). The most important and viable first line of defense in combating oxidative stress is through the use of endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ighodaro *et al.*, 2018). Basically, these enzymatic antioxidants help in the conversion of peroxides and hydroperoxides to water and oxygen molecules which are subsequently removed from the cells without the risk of causing molecular damage (Ighodaro *et al.*, 2018). The nonenzymatic antioxidants, including reduced glutathione (GSH) also protect the cells against oxidative damage by serving as electron donors to the radicals (Cadet *et al.*, 2017). Some antioxidant mechanisms, including elevated level of nitric oxide have been shown to increase the mucosal content of prostaglandin and mucus in the gastric mucosa which suggests cyto-protective effects. Indomethacin may mediate gastric ulceration by creating an imbalance between offensive factors (pepsin, gastric acid, ROS) and defensive factors (prostaglandins, bicarbonate ions, mucin, growth factors, and nitric oxide) (Serafim *et al.*, 2023).

This present study observed a significant increase in the activities of antioxidant proteins (GSH, CAT, SOD, and GPX) in indomethacin-induced group (negative control), suggesting possible accumulation of ROS. The study however, observed a decrease in NO level in the negative control group with indomethacin toxicity. The decrease in NO level is consistent with previous studies, showing that indomethacin inhibits NO production by inhibiting the activation of endothelial nitric oxide synthase (eNOS) (Arumugam *et al.*, 2014).

However, groups treated with LJECB only showed a considerably higher level of NO, GSH, CAT, SOD, GPX. The increase in NO level following the administration of LJECB suggests a possible protective effect on the gastric vascular system. Some studies reported increase in NO level of ulcerated rat (Raish *et al.,* 2021) which negates our study present

finding. Moreover, treatment with Gecrol antacid was associated with increase in NO level. The effects of antacid on nitric oxide level may vary depending on the specific antacid used and the mechanism involved. It is possible that the antacid treatment may have influenced nitric oxide mechanism through effects on gastric acid secretion.

Furthermore, MDA is used as a biomarker of lipid peroxidation-mediated oxidative stress. In this study, the negative control group was found to have a significantly lower level of MDA than the normal control group. Suggesting that intake of indomethacin may be associated with maintenance of cellular membrane health. However, treatment with Gecrol was associated with elevated MDA level; indicating that intake of gecrol may be associated with peroxidative stress on the gastric mucosal wall. When treated with the LJECB, the MDA level was restored to normal as in the normal control group. Administration of 400 or 800 mg/kg of LJECB to normal rats in groups 6 and 7 was associated with a significant increase in MDA when compared with the normal or negative control groups. Suggesting that consumption of LJECB without an underlying condition may trigger undue lipid peroxidation.

The observation that indomethacin-induced stress in Wistar rats significantly elevated the SOD levels is in agreement with previous studies (Danisman *et al.*, 2023). SOD has been shown to functionally transform superoxide anion into hydrogen peroxides (H_2O_2) and molecular oxygen (O_2). An increase in SOD activity suggests an increase in the intra-cellular concentration of H_2O_2 resulting from superoxide dismutation (Asma *et al.*, 2014).

In contrast, indomethacin-induced stress caused a non-significant difference in catalase activity in the negative control group compared to the normal control. Treatment with the LJECB at 800 mg/kg was associated with a significant increase in the CAT activity. CAT is known to convert H_2O_2 to H_2O; likewise, the GPx. considering the possible abundance of H_2O_2 from superoxide dismutase activity (Ray and Husain, 20002). One expected the CAT and GPx activities to be significantly high in the negative control compared with the normal. Instead, a non-significant difference was observed in the activities of both enzymes; suggesting that the indomethacin treatment may have affected the CAT and GPx via different mechanisms.

Considering the level of GSH, it was observed that the indomethacin-stressed negative control had significantly higher level of GSH than the normal control group. Reduced glutathione is an antioxidant which confers bio-protection on cells. It has effective protection against toxic effects of substances on tissues. It has been reported that roughly 90% of all glutathione pool are stored in human system in reduced form (GSH) and mobilized during oxidative stress. The observed increase in the indomethacin-stressed negative control could possibly be to combat the toxic effect of indomethacin. Treatment with 800 mg/kg of LJECB showed a significant decrease in GSH compared to the negative and normal control groups.

Moreover, the total protein concentrations in both serum and gastric juice were also compared. It was observed that more proteins were present in the serum than in the gastric juice. Indomethacin administration induced a significant increase in serum protein concentration in negative control (Group 2) compared to the normal control (Group 1). Treatment with gecrol (Group 3) even caused a further spike in serum total protein concentration. However, administration of 800 mg/kg of LJECB was associated with a significant reduction in the serum total protein concentration. Administration of 400 or 800 mg/kg of LJECB to the animals in Group 6 and 7 produced no significant effect when compared with the normal control. The total protein concentration was found to be significantly higher in gastric content juice when compared with the serum.

Indomethacin-induced stress was found to cause a significant reduction in lipid peroxidation (MDA), and NO levels; as well as an increase in the antioxidant status. Administration of LJECB in female Wistar rats with indomethacin stress for 14

days, caused an increase in non-enzyme (NO and GSH) and enzymatic antioxidants (SOD, catalase) as well as a non-significant decrease in GPX activity. While the plants' extract elicited potent antioxidant property, it was however, found to be associated with significant lipid peroxidative power in high doses. Therefore, prolong or frequent consumption of the plants' extract is strongly discouraged.

Conclusion

In conclusion, the LJECB elicited antioxidant property in the experimental rats. The increase in nitric oxide levels suggested that there might be some protective effects on the gastric vascular system, while the reductions in glutathione levels suggests that the extract may also have some potential as a detoxifying agent. However, the significant increase in malondialdehyde levels in ulcer-treated groups could be a cause of concern, as this may indicate increased oxidative stress on the gastric endothelial cells. Therefore, it may be wise to exercise caution in using this extract, particularly at high doses or frequent dosage.

References

Adegbola MV, Godwin A, Ntwasa M, Ayeleso AO, Oyedepo TA. 2022. Potential Effect of *Syzygium aromaticum* (Cloves) Extract on Serum Antioxidant Status and Lipid Profiles in Wistar Rats with Artesunate Toxicity. Appl. Sci. **12**, 8216.

Aminjan HH, Abtahi SR, Hazrati, E, Chamanara M, Jalili M, Paknejad B. 2019. Targeting of oxidative stress and inflammation through ROS/NF-kappaB pathway in phosphine-induced hepatotoxicity mitigation. Life Sci. **232**, 116607.

Arumugam SN, Varghese J, Gautham TP, Visalakshi J, Molly J. 2014. Indomethacin inhibits activation of endothelial nitric oxide synthase in the rat kidney: Possible role of this effect in the pathogenesis of indomethacin-induced renal damage. Chemico-Biological Interactions **221,** 77-87.

Asma B, Ranajoy C, Shankar M. 2014. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal disease. Physiol. Rev. **94**, 329–354.

Ayala A, Munoz MF, Arguelles S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. 360438.

Barbosa-Filho VM, Waczuk EP, Kamdem JP, Abolaji AO, Lacerda SR, Da Costa JGM. 2014. Phytochemical constituents, antioxidant activity, cytotoxicity and osmotic fragility effects of Caju (*Anacardium microcarpum*). Indus Crops Prod. **55**, 280–8.

Bicalho B, Rezende CM. 2001. Volatile compounds of cashew apple (*Anacardium occidentale* L.). Zeitschr Nat Sect C. **56**, 35–9.

Botting, R. 2007. Cyclooxygenases in Biology and Disease. In: Schmidt, R., Willis, W. (eds) Encyclopedia of Pain. Springer, Berlin, Heidelberg. **978**, 32.

Bryan NS, Grisham MB. 2007. Methods to detect nitric oxide and its metabolites in biological samples. Free. Radic. Biol. Med. **43**, 645–657.

Cadet J, Davies KJA, Medeiros MHG, Di Mascio P, Wagner JR. 2016. Formation and repair of oxidatively generated damage in cellular DNA. Free Radic. Biol. Med. **107**, 13–34.

Danisman B, Cicek B, Yildirim S, Bolat I, Kantar D, Golokhvast KS, Nikitovic D, Tsatsakis A, Taghizadehghalehjoughi A. 2023. Carnosic Acid Ameliorates Indomethacin-Induced Gastric Ulceration in Rats by Alleviating Oxidative Stress and Inflammation. Biomedicines **11(3)**, 829.

Ellman GL. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. **82**, 70–77.

El-Ashmawy NE, Khedr E, El-Bahrawy HA, Selim HM. 2016. Gastroprotective effect of garlic in indomethacin induced gastric ulcer in rats. Nutrition **32**, 849–854.

Enas RA, Miada FA, Nada MA, Dalia HA, Azza MAZ, Faisal HA, Naseh AA. 2024. Apple extract protects against indomethacin-induced gastric ulcers in rats by suppressing oxidative stress- The implication of Nrf-2/HO-1 signaling pathway: In silico and in vivo studies, Journal of Functional Foods **112**, 105926.

Forman H J, Zhang H. 2021. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nature Reviews Drug Discovery, **20,** 689–709.

Fountoucidou P, Veskoukis AS, Kerasioti E, Docea AO, Taitzoglou IA, Liesivuori J. 2019. A mixture of routinely encountered xenobiotics induces both redox adaptations and perturbations in blood and tissues of rats after a long-term low-dose exposure regimen: The time and dose issue. Toxicol. Lett. **317**, 24–44.

Ighodaro OM, Akinloye OA. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine **54**, 287–293.

Jaina S, Arora P, Popli H. 2020. A comprehensive review on Citrus aurantifolia essential oil: its phytochemistry and pharmacological aspects. Brazilian Journal of Natural Sciences **3**, 354.

Jomova K, Raptova R, Alomar SY. 2023. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol **97**, 2499–2574.

Kumar P., Paramashivappa R, Vithayathil PJ, Subba Rao PV, Srinivasa Rao A. 2002. Process for isolation of cardanol from technical cashew (*Anacardium occidentale* L.) nut shell liquid. J Agric Food Chem. **50**, 4705–8.

Liu S, Lou Y, Li Y, Zhang J, Li P, Yang B, Gu Q. 2022. Review of phytochemical and nutritional characteristics and food applications of Citrus L. fruits. Frontiers in Nutrition **9**, 968604.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with Folin-Phenol reagent. Journal of Biological Chemistry 193, 265-275.

McCord JM, Fridovich I. 1969. Superoxide dismutase. An enzymic function for erythrocuprein (Hemocuprein). Journal of Biological Chemistry **244**, 6049–6055.

Murphy MP, Bayir H, Belousov V. 2022. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. Nat Metab **4**, 651–662.

Özyürek M, Baki S, Güngör N, Çelik SE, Güçlü K, Apak R. 2012. Determination of biothiols by a novel on-line HPLC-DTNB assay with post-column detection. Anal. Chim. Acta **750**, 173–181.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. 2017. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. **2017**, 8416763.

Rahaman MM, Hossain R, Herrera-Bravo J, Islam MT, Atolani O, Adeyemi OS, Owolodun OA, Kambizi L, Daştan SD, Calina D, Sharifi-Rad J. 2023. Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. Food Sci Nutr. **11(4)**, 1657-1670.

Raish M, Shahid M, Bin Jardan YA, Ansari MA, Alkharfy KM, Ahad A, Abdelrahman IA, Ahmad A, Al-Jenoobi FI. 2021. Gastroprotective Effect of Sinapic Acid on Ethanol-Induced Gastric Ulcers in Rats: Involvement of Nrf2/HO-1 and NF-κB Signaling and Antiapoptotic Role. Front Pharmacol. **25**(12), 622815.

Ray G, Husain SA. 2002. Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol. **40**(11), 1213-32.

Sabiu S, Garuba T, Sunmonu T, Ajani E, Sulyman A, Nurain I, Balogun A. 2015. Indomethacin-induced gastric ulceration in rats: Protective roles of *Spondias mombin* and *Ficus exasperata*. Toxicol Rep. **8**(2), 261-267.

Saleem M, Durani AI, Asari A, Ahmed M, Ahmad M, Yousaf N, Muddassar M. 2023 Investigation of antioxidant and antibacterial effects of citrus fruits peels extracts using different extracting agents: Phytochemical analysis with in silico studies. Heliyon. **9(4)**, e15433.

Salehi B, Gültekin-Özgüven M, Kirkin C, Özçelik B, Morais-Braga MFB. 2020. Antioxidant, Antimicrobial and Anticancer Effects of Anacardium Plants: An Ethnopharmacological Perspective. Frontiers in Endocrinology. Front. Endocrinol., **11**.

Schmedes A, Holmer GA. 1989. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. Journal of Oil & Fat Industries **66**, 813-817.

Sen Li, Guowei Chen, Chao Zhang, Man Wu, Shuyan Wu, Qing Liu, 2014. Research progress of natural antioxidants in foods for the treatment of diseases, Food Science and Human Wellness **3**(3-4), 110-116.

Serafim C, Araruna ME, Júnior EA, Diniz M, Hiruma-Lima C, Batista L. 2020. A Review of the Role of Flavonoids in Peptic Ulcer (2010-2020). Molecules **25**(22), 431.

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho WC, Sharifi-Rad J. 2020. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front. Physiol. **11**, 694.

Sinha .K. 1972. Colorimetric assay of catalase. Anal. Bioche., **47**, 389–394.

Statista 2023. Global leading lemon & lime producers 2022/23. https://www.statista.com/statistics/1045016/world-lemons-and-limes-major-producers.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. **39**(1), 44-84.

Vona R, Pallotta L, Cappelletti M, Severi C, Matarrese P. 2021. The Impact of Oxidative Stress in Human Pathology: *Focus* on Gastrointestinal Disorders. Antioxidants **10**, 201.

Yuji N, Toshikazu Y. 2006. Oxidative stress involvement and gene expression in indomethacin-induced gastropathy. Redox Report **11**(6), 243-253.