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Antioxidant and anti-inflammatory functions of turmeric and cucumber juice following lead-induced toxicity in male Wistar rats

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Abstract

Reactive oxygen species and free radicals generated by oxidative stress and inflammation have been implicated in various clinical conditions. This study aimed to evaluate the antioxidant and anti-inflammatory effects of turmeric and cucumber juice following lead-induced toxicity in male Wistar rats. Twenty-five male rats (170–250 g) were randomly divided into five groups (n = 5 per group). Lead toxicity was induced in all rats by a single daily oral dose of 2.25 mg/kg, except in Group 1, which served as the control. The treatment was administered for 28 days as follows: Group 1 (control) received no treatment; Group 2 (Pb only) received 2.25 mg/kg of lead; Group 3 (Pb + TURM) received 2.25 mg/kg of lead with 1 ml of turmeric juice; Group 4 (Pb + CUM) received 2.25 mg/kg of lead with 1 ml of cucumber juice; and Group 5 (Pb + TURM + CUM) received 2.25 mg/kg of lead with 1 ml of both turmeric and cucumber juice. On day 29, blood samples were collected for serum analysis of antioxidants (malondialdehyde [MDA], superoxide dismutase [SOD], catalase [CAT]) and inflammatory markers (interleukin-6 [IL-6] and tumor necrosis factor-alpha [TNF-α]). Rats in Group 2 (Pb only) exhibited significantly higher levels of MDA, IL-6, and TNF-α, and lower levels of SOD and CAT compared to the control group ($p < 0.05$), indicating oxidative damage and inflammation. Treatment with turmeric and cucumber juice in Groups 3 and 4 significantly reduced MDA, IL-6, and TNF-α, and increased SOD and CAT levels ($p < 0.05$). Co-administration of both juices in Group 5 resulted in even more pronounced effects, suggesting a synergistic benefit. The mechanism of action remains under investigation.

Keywords

Turmeric, Cucumber, Lead, Antioxidant, Anti-inflammatory and Rats.

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INTRODUCTION

From time immemorial, a good number of plants variety have been used for purposes of good health. Most ethnobotanical divisions and parts of plants have been deployed as extracts orjuice and may possess antioxidant and anti-inflammatory functions related to diseases, including diabetes, heavy metal toxicity, atherosclerosis and cancer(Sharma *et al.*, 2010). Toxicants and xenobiotics induced oxidative stress causes free radicals generation, which are causatives of most complicatedailments associated with disease states (Sharma *et al.*, 2010).Oxidative stress induced by toxicants causes membrane lipid peroxidation and mitochondria abnormality which are responsible for cell death (Adil *et al.*, 2016: Mcgill *et al.*, 2012). Anti-inflammatory interventions can modulate the gut microbiota composition, this way, prevalent ailments whose etiology is associated with immune dysfunction or repeated inflammation can be prevented by plants consumption through downregulation of pro-inflammatory cytokineslike interleukins and tumor necrosis factors. Additionally, bioactive ingredients of plants can regulate possible oxidative stress caused because of imbalance in the synthesis of reactive oxygen species coupled with the antioxidant potential of cellular enzymes (Saronee *et al.*, 2023).

Turmeric is a rhizomatous medicinal perennial herbaceous plant of the Zingiberaceae family, it is cultivated widely in many countries of the world like India, China, Nigeria etc. (Akter *et al*., 2019). It grows between 3 to 5 feet tall, with oblong leaves with funnel-shaped, yellowish rhizome growing below the soil (Iweala *et al.*, 2023). Turmeric is being used in most of the Asian countries as a medicinal herb, it is a well sought after phyto-medicinal plant in Nigeria and has been in constant use in folklore medicine in most African countries (Okwu, 2001). The rhizome is mostly used and has been confirmed therapeutic in the management of various ailments like oxidative stress, gonorrhea, inflammation, syphilis, stomach aches, blood cell disorder, bloody diarrhea, diabetes, reproductive abnormalities, toothaches and poisoning(Okwu, 2001;Fortune *et al*., 2019 and Saronee *et al*., 2019)

Cucumber (*Cucumis sativus*)is a member of the *Cucurbitaceae* family and is a widely distributed vine herb with cylindrical fruits used as culinary vegetables, it is a widely consumed salad vegetable worldwide (Hegazy*et al.*,2018). It is a known cultivated crop in major countries of the world. Cucumber has been reported to possess in-vitro anti-inflammatory, antioxidant, antifungal, antibacterial,and anti-diabetic activities while the juice has been reported to be rich in several phytochemical compounds including flavonoids, cardiac glycosides, alkaloids, terpenoids, tannins, saponins, coumarins, sterols and terpenes (McLean *et al*., 2013: Dan-Jumbo *et al.*,2024). Anecdotal applications of turmeric and cucumber juice in traditional medicine are enormous. Though, scientific reports validating this anecdotal use of the juice of turmeric and cucumber are relatively scarce in our environment. The present study, therefore aimed to investigate the antioxidant and anti-inflammatory functions of turmeric and cucumber juice following lead induced toxicity using male Wistar rats as models.

MATERIALS AND METHODS

Procurement of Experimental Rats, Leadand Induction of Lead Toxicity

Twenty-five (25) male Wistar rats (170 to 250g) were purchased from PAMO University of Medical Sciences animal house. They were housed in transparent polycarbonate cages with wired top covers, with 12 hours light/dark cycle and were fed with normal rat chow with unhindered access to clean water. The animals were acclimatized for two weeks and were subsequently grouped for the experiment. Lead acetate was obtained from Eddy Chemicals and Safety Supply Co. Port Harcourt, Rivers State, Nigeria. Lead toxicity was induced with single oral daily administration of 2.25mg/kg bw of lead in the morning hours (between 8-9AM daily)in line with Saronee *et al.,* 2024.

Purchase and Preparation of Turmeric and Cucumber Juice

Turmeric and cucumber used for this study were bought from a local fruit market in Port Harcourt, Rivers state, and were duly identified and authenticated. Turmeric juice was prepared according to the method of Gul and Bakht (2015). After collection, a known quantity of turmeric rhizomes was washed thoroughly and grinded using an electronic blender with 400ml of distilled water. The solution was later filtered using a sieve and a mesh cloth, and the juice obtained was put in a universal bottle and stored in the refrigerator for preservation prior administration. The process was done in the Department of Physiology Laboratory, PAMO University of Medical Sciences.

Cucumber juice was prepared according to the method of Aderinolaand Abaire, 2019. Fresh cucumbers werewashed thoroughly and grinded without water, using a blender. The solution was later filtered using a sieve and a mesh cloth, and the juice obtained was also put into a universal bottle and stored in the refrigerator for preservation. This study was conducted in line with the guidelines set by the United States Institute for Laboratory and Animal Research (1996).

Ethical Approval

Ethical approval for the study was sort and obtained from the Research Ethics Committee of PAMO University of Medical Sciences and ethical approval code of PUMS/REC/2024022 was given.

Acute Toxicity Study

The acute toxicity study (LD_{50}) of turmeric was estimated as reported by Shafira *et al.,*2022 and Yuandani, 2017, ≥3000mg/kg bw. Similarly, the LD_{50} value of Cucumber was as previously reported by Vivek *et al*., (2016)to be above 1000mg/kg bw.

Experimental Design

Experimental animals were randomly distributed into 5 groups of 5 rats per group. Induction oflead toxicity was done with a single daily oral dose of 2.25mg/kg bw of lead in all the rats except group1 rats, as previously described by Saronee *et al.*,(2024).

Group 1:Control; animals in this group had free access to tap water and normal rat chow. Group 2: Pb only; animals in this group received noadditional treatment after induction of lead toxicity. Group 3: Pb + TURM; rats in this group got 1ml of turmeric juice only. Group 4: Pb + CUM; animals in this group were given 1ml of Cucumber juice only. Group 5: Pb + TURM + CUM; animals in this group received 1ml of turmeric and 1ml of Cucumber juice following induction of lead toxicity. All administrations were orally done once a day in the morning hours (between 8-9AM daily)using oral cannula for 28 days. 24 hours after the last administration, the rats were anesthetized using diethyl ether and blood collected by cardiac puncture for serum antioxidants (MDA, SOD and CAT) and inflammatory markers (IL-6 and TNF-Alpha) determination.

Measurement of Oxidative Stress and Inflammatory Markers

Malondialdehyde (MDA) level was assessed using the method described by Wasowicz ,1993. Under acidic condition, MDA produced from the peroxidation of fatty acid membranes react with chromogenic reagent-2- thiobabituric acid to yield a pink colored complex which is measured at 532nm. As previously described by Sun*et al.*,1988, superoxide enzyme activity measurement was carried out with the aid of a spectrophotometer. Superoxide anion formation of blue formazan was estimated at 560nm. Determination of catalase activity was based on the splitting of hydrogen peroxide which was subsequently measured using a spectrophotometer at 240nm (Aebi, 1974). Inflammatory markers were determined using standard laboratory procedures.

Statistical Analysis

Obtained data were analyzed with one-way ANOVA followed by a post hoc (LSD test) with SPSS Version 23.0. Results were presented in figures (figures 1-2). A P value $< 0.05$was considered statistically significant.

RESULTS AND DISCUSION

Effects of turmeric and cucumber juice on Malondialdehyde (MDA), Superoxide dismutase (SOD) and Catalase (CAT) levels.

Figure 1 showed significant higher values of MDA and lower SOD and CAT levels amongst group 2 (Pbonly) rats when compared to group 1 (Control) rats ($p<0.05$), indicating a possible lipid peroxidative effect of lead acetate at the administered dose. This finding is consistent with Saronee *et al.,* (2024), in which lead acetate disrupted reproductive functions and some biochemical indices in experimental animals. However, administration of 1ml each of turmeric and cucumber juice to animals in groups 3 (Pb + TURM) and 4 (Pb + CUM) rats

caused a significant reduction in MDA level and increased SOD and CAT concentration when compared to group 2 (Pbonly) rats (*p<0.05*), indicating a potential free radical scavenging and reversibility potentials of the administered juice in lead toxicity. These findings collaborate earlier reports from our center in which extracts and other products of plants reversed the deleterious biochemical and glycemic effects of lead acetate and phenyl hydrazine in laboratory rats(Gbaranor *et al.*,(2024): Dan-Jumbo *et al.*,(2024):Saronee *et al.*,(2019): Saronee *et al.*,(2020) and Ijeoma *et al.*,(2020) with turmeric showing a better effect as observed amongst group 3 rats compared to group 4 rats: highlighting a potential greater potency of turmeric juice compared to cucumber juice. By corollary, co-administration of both juice to group 5 (Pb + TURM + CUM) rats elicited a significant reduction in MDA level and increased SOD and CAT levels when compared to group 2 (Pb only) rats (*p<0.05).* Comparatively, co-administration of both juice to group 5 rats lowered serum MDA level and increased blood SOD and CAT levels more than the single administration of either juice, suggesting a potential greater effect of the combined administration of both juices compared to individual juice administration. Antioxidants are molecules that can delay or inhibit oxidative reactions usually catalyzed by free oxygen radicals, antioxidants prevent chain reactions leading to lipid peroxidation through the removal of free radicals and preventing further oxidation reactions (Vilioglu *et al.*, (1998), Oloyede and Afolabi (2012). The antioxidant efficacies of many products of plants have been associated with the presence of bio-active phenolic compounds like flavonoids, diterpenes, tannins etc. (Johnson*etal*., (2022), Pietta, (1998), Saronee *et al*., (2023) and Fortune *et al*., (2019). Flavonoids, Phenols, and tannins are useful antioxidants in the management of oxidative stress and other associated disorders. Phytochemical screening and characterization of plants products including turmeric and cucumber juice have revealed the presence of antioxidant compounds which may account for the observed antioxidant efficacies (Johnson*etal*., (2022).

Effects of turmeric and cucumber juice on interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) levels.

Compared to group1 (Control) rats, administration of 2.25mg/kg body weight of lead acetate to rats in group 2 induced a significant increase in IL-6 and TNF-alpha levels observed amongst group 2 (Pbonly) rats*(p<0.05*) as shown in figure 2; indicating a potential pro-inflammatory effect of lead acetate in experimental animals. This is in consonance with Patra *et al.,* (2011). Upon administration of 1ml each of turmeric and cucumber juice respectively to groups 3 (Pb + TURM) and 4 (Pb + CUM) rats, a significant decrease in the above inflammatory indices was observed, compared to group 2 rats (*p<0.05*), indicating a potential anti-inflammatory property of both juice, with a more decrease in IL-6 and TNF-alpha observed amongst turmeric treated animals compared to cucumber treated rats, demonstrating a likely better anti-inflammatory effect of turmeric compared to cucumber. For group 5 rats, co-administration of turmeric and cucumber juice significantly lower IL-6 and TNF-alpha levels when compared to group 2 rats (*p<0.05*). By comparison, group 5 rats co-treated with both juices relatively lowered IL-6 and TNF-alpha levels more, compared to the other single

treatment groups, suggesting a possible greater potency of the combined treatment with both juice relative to groups 3 and 4 rats treated with individual doses of the juice. Abnormality occurs when inflammatory response is not enough to eliminate antigenic stimuli like foreign bodies or antigenic materials and/or other inflammatory triggers including lead, urate crystals and excessreactive oxygen species (ROS) synthesis (Rose-John, 2018).Cytokines, including chemokines, interleukins, interferon, tumor necrosis factor, transforming growth factor, and cell adhesion molecules are known markers of inflammation (Rose-John, 2018). Persistent inflammation because of prolonged stimulation by chemical, biologicalor physical agents can prove problematic, as it can cause chronic inflammation (Germolec *et al*., 2018).

Conclusively, this study reports that co-administration of turmeric and cucumber juice caused significant and synergistic increase in superoxide dismutase, catalase, but decrease malondialdehyde, interleukin 6 and tumour necrosis factor alpha levels following lead induced toxicity in male Wistar rats. Our findings suggest a possible beneficial effects of the juice of turmeric and cucumber on oxidative stress and inflammation following lead administration in Wistar rats, with turmeric demonstrating a better ameliorative effect than cucumber and the co-administration of both juices showing greater potency compared to the single administration of either juice.The exact mechanism of action of the juices requires further investigation.

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LEGEND TO FIGURES

Fig. 1: Shows the effects of turmeric and cucumber juice on malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) levels.

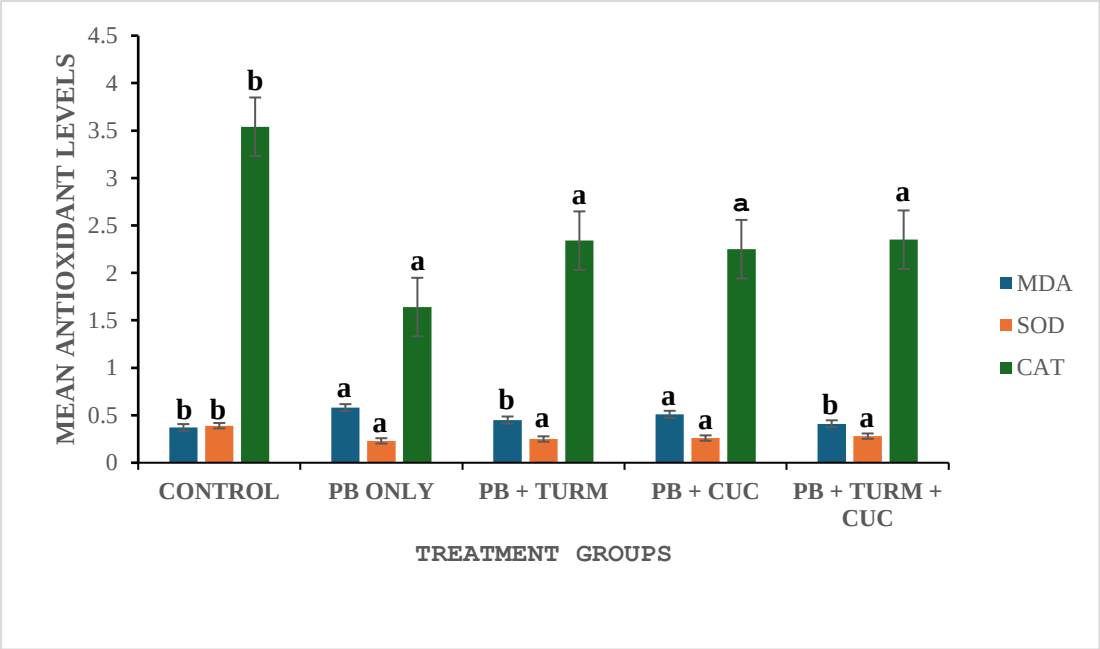

Figure 1:

All values are expressed as mean ± SEM.[a]= $p<0.05$ compared to Control.[b]= $p<0.05$ compared to PB Only

Fig. 2: Shows the effects of turmeric and cucumber juice on interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) levels

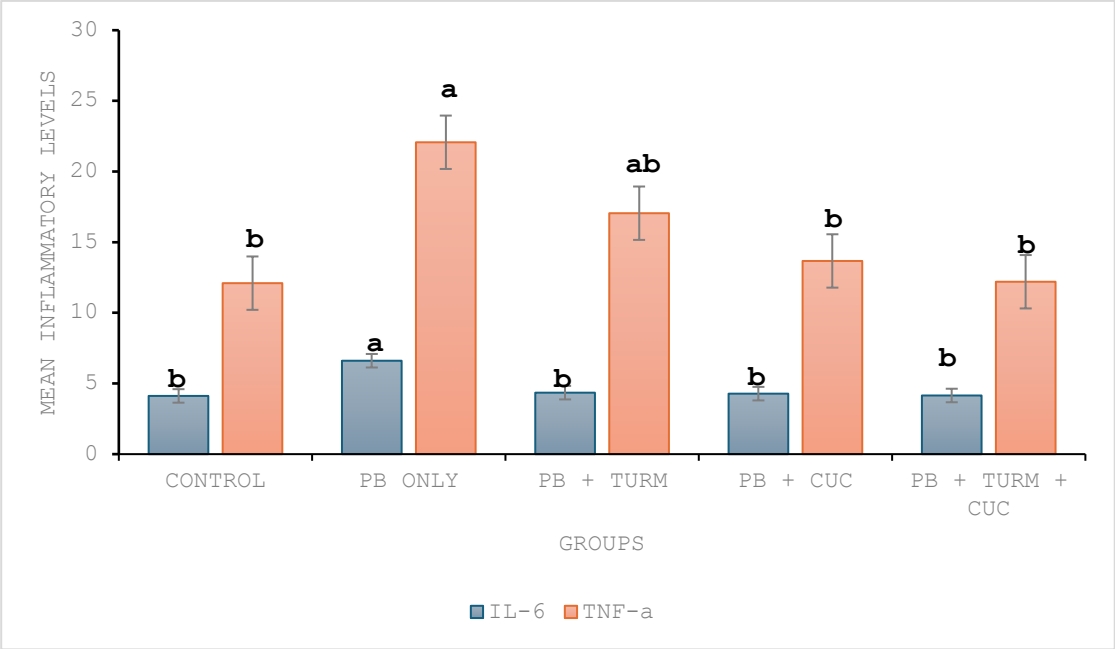

Figure 2:

All values are expressed as mean ± SEM. [a]= $p<0.05$ compared to Control.[b]= $p<0.05$ compared to PB Only