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Changes in Biochemical Indices in Lead-Induced Toxicity Following Administration of Methanolic Extracts of C*urcuma longa Rhizomes* and C*ucumis sativus* Fruits

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Abstract:

Biochemical indices are important biomarkers of health and disease state in both humans and rodents. This study was designed to evaluate plausible changes in biochemical indices in lead-induced toxicity following administration of methanolic extracts of C*urcuma longa rhizomes* and C*ucumis sativus* fruits. Twenty five (25) male rats weighing between 145g-200g were purchased, acclimatized and subsequently randomly distributed into five (5) groups(5 rats each) and were treated for 28 days as follows: Group 1: Control group (received distilled water and normal feed only); Group 2: received a daily dose of 2.25mg/kg lead for 28 days; Group 3: rats received 2.25mg/kg lead + 200mg/kg C*urcuma longa rhizome* extract; Group 4: rats received 2.25mg/kg lead + 500mg/kg C*ucumis sativus* extract; Group 5: rats received 2.25mg/kg lead + 200mg/kg C*urcuma longa rhizome* + 500mg/kg body weight of C*ucumis sativus* extracts. Daily treatments with lead and extracts were orally. Testes were harvested and homogenized for determination of testicular cholesterol and nitric oxide levels on day 29, also blood was collected via direct cardiac puncture for serum liver enzymes and lipid parameters estimation. Expectedly, significantly higher values of testicular cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total cholesterol, triglyceride and low-density lipoprotein but decrease nitric oxide, total protein, albumin and high-density lipoprotein levels were observed amongst group 2 rats administered 2.25mg/kg lead compared to group 1 ratsp<0.05, suggesting a possible deleterious effect of lead. Predictably, individual doses of the extracts of C*urcuma longa rhizome* and C*ucumis sativus* administration to groups 3 and 4 rats significantly ameliorated the above parametersp<0.05, suggesting a likely beneficial effect of C*urcuma longa* and C*ucumis sativus* extracts with C*urcuma longa rhizome* showing greater potency compared to C*ucumis sativus*. Combined administration of both extracts to group 5 rats showed better therapeutic efficacy compared to both groups 3 and 4 rats. Results suggest that combined treatment with C*urcuma longa* and C*ucumis sativus* may present better outcome compared to individual administration. More studies are recommended to this end.

Keywords:

Changes, C*urcuma longa rhizome*, C*ucumis sativus*, lead, biochemical.

INTRODUCTION

The application of native medicinal plants in combating various ailments, including pollutants induced disease conditions has been on since time immemorial in virtually all countries of the world. The World Health Organization (WHO) reported that over 85% of African population, especially sub-Sahara Africa, including Nigeria still rely on native (folklore)medicine for basic healthcare needs (WHO, 2002). The concept of health promotion has become a legitimate part of health care and as such, there is a growing interest in natural plants-based products as against the synthetic medications because of concerns over features like their volatile nature, cost, high sensitivity to heat, availability and instability with possible side effects (Kurian *et al*., 2010; Ruiz Navajas *et al*., 2011). The intake of natural products has been linked to reduced risks of cardiovascular disease, cancer, diabetes and other ailments associated with ageing (Hamid *et al*., 2010). Plants have been known to be good and rich source of potential bioactive compounds which are of high benefit to health; These bioactive chemical compounds are often referred to as phytochemicals (Ezebuiro *et al*., 2020: Saronee *et al*., 2023).

With an exponential increase in civilization, man is apparently constantly and increasingly being exposed to the pollution of his natural environment (Balali *et al*., 2021). All these poses serious risk to man's health. The inability to detoxify inherent reactive intermediates, is largely responsible for many diseases (Saronee *et al.*, 2024).Heavy metals elicit harmful effects, as such exposure to these metals including lead acetate is on the increase, occasioned by industrial and anthropogenic engagements cum industrialization(Balali *et al*., 2021). Toxicity associated with heavy metals exposure affects virtually all the systems of the body (Balali *et al*., 2021).

C*urcuma longa* is an upright herb with orange roots capable of growing to approximately 2 feet (Sawant and Godghate, 2013). It is a member of the Zingiberaceae family with a pungent and sore taste rhizome, it is used widely in local traditional medicine and as cooking spice (Gopinathan *et al.*, 2011). Literature reports enormous medicinal applications in menstrual disorders, diabetes, inflammation, high cholesterol, reproductive abnormalities, wounds etc. (Sawant and Godghate, 2013).

Cucumber (*Cucumissativus* L.) is a member of the *Cucurbitaceae* family. The *Cucumis sativus* has the greatest economic significance of all the thirty known C*ucumis* species. It has dicotyledonous seeds which comes from the flower, and therefore classified as a fruit (McLean*et al*., 2013). Cucumbers are predominantly water-rich, with approximately 4% dry mass. It is commonly explored as a skin moisturizer, and in the treatment of diabetes, pellagra, jaundice, bone fragility, lipid peroxidation, anemia and urinary tract infections in our environment. Indigenous reports on the application of both plants individually and in combination as possible remedy for common illnesses have become popular in our locality. This study, therefore, presents a preliminary report on potential changes in biochemical indices in lead-induced toxicity following administration of methanolic extracts of C*urcuma longa rhizomes* and C*ucumis sativus* fruits.

MATERIALS AND METHODS

Plants Identification and extracts preparation

C*urcuma longa rhizomes* and C*ucumis sativus* fruits were purchased from Bori main market in Khana Local Government Area, Nigeria. Plants specimens were identified by Dr. E. Chimezie of Plant Science and Biotechnology Department, University of Port Harcourt, Rivers State. Ethical approval for our study was sought and obtained from the Ethics Committee of our University. This study was

conducted in line with the code of the United States Institute for Laboratory and Animal Research (1996).

Fresh rhizomes of C*urcuma longa* were bought, properly washed and air dried at room temperature for about 14 days to a stable weight. The rhizomes were subsequently macerated with the aid of a household blender. Dissolution of approximately 4.6g of obtained powder was done in 98% methanol for 48 hours with 90% yield. The rhizomes were extracted using percolation method.

Fresh cucumber fruits were thoroughly washed, oven dried and grinded using a blender. 3.1kg realized was soaked in 98% methanol for 60 hours with a yield of 63%. The mixture was filtered and concentrated with the aid of an evaporator at 40°C. Successfully obtained extracts were stored in airtight containers in the refrigerator prior use.

Procurement of Experimental Animals and Lead acetate

Twenty-five (25) adults male rats weighing between 145g and 200g were purchased from the Department of Physiology Animal House, University of Port Harcourt, Nigeria. The rats were kept in wiregauzed cages, acclimatized and treated under standard laboratory conditions. Lead acetate was purchased from Sigma-Aldrich Co., United States of America.

Induction of Lead toxicity

Lead toxicity was induced using a single daily oral dose of 2.25mg/kg bw of lead acetateas previously reported by Saronee *et al.,* 2024.

Acute toxicity study (LD_{50})

The LD_{50} of C*urcuma longa rhizomes* extract was estimated as previously reported by Yuandani, (2017) which was above 3000mg/kg bw.Likewise, the LD_{50} value of the extract of C*ucumis sativus* was as reported by Vivek *et al., (*2016)to be above 1000mg/kg bw.

Study design

Procured Wistar rats were randomly distributed into 5 groups (5 rats each). Lead toxicity was induced in rat groups 2, 3, 4 and 5by administering 2.25mg/kg bw of lead. The rat groups were treated thus for 28 days: Group 1: Negative control; rats in this group had free access to normal rat chow and tap water. Group 2: Positive control; rats in this group received no treatment after lead toxicityinduction. Group 3: lead + C*urcuma longa rhizome*; rats in this group were treated with 200mg/kg bw of C*urcuma longa rhizome* extract. Group 4: lead + C*ucumis sativus*; rats in this group were treated with 500mg/kg bw C*ucumis sativus*. Group 5: lead + combined doses of C*urcuma longa rhizome*and C*ucumis sativus*; rats in this group were treated with 200mg/kg bw of C*urcuma longa rhizome*+ 500mg/kg bw of C*ucumis sativus* extracts. Lead and extracts of C*urcuma longa rhizome* and C*ucumis sativu s*were orally administered once a day using oral cannula for 28 days. On the 29thday, testes and blood samples were obtained by direct cardiac puncture for testicular and serum biochemical indices estimation.

Estimation of Testicular cholesterol, Nitric oxide, Liver enzymes and Lipid Profile levels

Testicular cholesterol was determined using Randox Biosciences kits. Nitric oxide level of experimental animals was assessed using Griess reagent as previously described by Singh *et al*., (2015**).**Serum liver enzymes were determined as described by Wallach, (2007).Blood total

cholesterol, triglycerides, low density lipoprotein and high-density lipoprotein cholesterol levels were assessed using randox kits as described by Stein (1986), Chawla (2003), Ijeoma *et al*., (2020) and Saronee *et al*., (2020).

Statistical Analysis

Generated data were analyzed using ANOVA (SPSS). Values were reported as Mean ± standard error of mean. Ap-value < 0.05 was considered significant at 95% confidence interval.

RESULTS

Table 1: Values of testicular cholesterol and nitric oxide in methanolic extracts of C*urcuma longa* and C*ucumis sativus*treated rats.

Groups	Testicular Cholesterol (mg/dl)	Testicular Nitric Oxide (µmol/l)
GROUP 1: Negative Control	3.70±0.01[b]	85.09±0.10[b]
GROUP 2: Positive Control (2.25mg/kg lead)	5.43±0.08[a]	64.83±0.08[a]
GROUP 3: Lead + C*urcumalonga*(200mg/kg)	3.96±0.08[ab]	76.99±0.01[ab]
GROUP 4: Lead + C*ucumissativus*(500mg/kg)	4.56±0.05[ab]	74.04±0.29[ab]
GROUP 5: Lead + C*urcumalonga*(200mg/kg)+ C*ucumissativus*(500mg/kg)	3.40±0.03[ab]	89.90±0.09[ab]

All values are expressed as mean ± SEM. [a]= p<0.05 compared to Negative control.[b]= p<0.05 compared to Positive control.

Table 1 shows significantly higher values of testicular cholesterol with lower values of testicular nitric oxide amongst group 2 rats (Positive Control) administered 2.25mg/kg bw of lead p<0.05 compared to Group 1 (Negative control) rats, suggesting a potential harmful effect of lead acetate. Individual administration of extracts of C*urcumalonga* rhizome and C*ucumissativus* to Groups 3 and 4 (Lead + C*urcuma longa*)and (Lead + C*ucumis sativus*) rats significantly reversed the initial harmful effects p<0.05 caused by lead compared to Group 2 (Positive control) rats, indicating a potential reversibility and ameliorative effects of C*urcuma longa* and C*ucumis* sativus in lead induced toxicity. The effects of C*urcuma longa* for instance, observed amongst group 3 (Lead + C*urcuma longa*) rats were significant compared to group 4 (Lead + C*ucumis sativus*) rats p<0.05, demonstrating a possible greater potency of effect. Co-administration of both C*urcuma longa* and C*ucumis sativus* extracts to group 5 (Lead + C*urcumalonga*+ C*ucumis sativus*) animals significantly reduced testicular cholesterol level but increased testicular nitric oxideconcentration compared to Groups 1, 2, 3 and 4 rats p<0.05, showing a likely synergism of effects.

Table 2: Liver enzymes level in methanolic extracts of C*urcuma longa* and C*ucumis sativus* treated rats.

Groups	Aspartate aminotransferase (Iu/l)	Alanine aminotransferase (Iu/l)	Alkaline Phosphatase (Iu/l)	Total Protein (g/l)	Albumin (g/l)
GROUP 1: Negative Control	191.79±0.05[b]	149.57±0.01 [b]	258.19±0.08 [b]	7.13±0.00 [b]	3.21±0.08 [b]
GROUP 2: Positive Control (2.25mg/kg lead)	297.74±0.03[a]	220.50±0.08 [a]	310.22±0.01 [a]	5.27±0.01 [a]	3.01±0.05 [a]
GROUP 3: Lead + C*urcumalonga*(200mg/kg)	194.93±0.33[ab]	185.20±0.01 [ab]	263.52±0.01 [ab]	6.03±0.08 [ab]	3.17±0.05 [b]
GROUP 4: Lead + C*ucumissativus* (500mg/kg)	198.72±0.01 [ab]	194.20±0.05 [ab]	279.01±0.00 [ab]	5.96±0.08 [ab]	3.14±0.05 [ab]
GROUP 5: Lead + C*urcumalonga*(200mg/kg) + C*ucumissativus*(500mg/kg)	182.23±0.02 [ab]	146.53±0.01 [ab]	245.87±0.23 [ab]	7.44±0.01 [ab]	3.27±0.01 [ab]

All values are expressed as mean ± SEM. [a]= $p<0.05$ compared to Negative control.[b]= $p<0.05$ compared to Positive control.

Compared to Group 1(Negative control) rats in table 2, daily administration of a single dose of 2.25mg/kg body weight of lead acetate to Group 2 (Positive control) rats, caused a significant increase in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase but decreased total protein and albumin levels $p<0.05$, suggesting a probable hepatotoxic effect of lead at the administered dose in rats. Daily administration of a single dose of 200mg/kg and 500mg/kg bw of C*urcuma longa*and C*ucumis sativus* to animals in Groups 3 and 4 (Lead + C*urcuma longa)*and (Lead + C*ucumis sativus*) reversed the effects of lead acetate, with C*urcumalonga* showing a better effect compared to C*ucumis sativus*. Combined administration of both C*urcuma longa* and C*ucumissativus*to Group 5 (Lead + C*urcuma longa* + C*ucumis sativus*) rats caused a significant reduction in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase but increased total protein and albumin levels compared to Groups 3 and 4 ratsp<0.05, indicating a likely higher efficacy of the combined administration of both extracts in lead toxicity in male Wistar rats.

Table 3: Lipid profile level in methanolic extracts of C*urcuma longa* and C*ucumis sativus* treated rats.

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	High Density Lipoprotein (mg/dl)	Low Density Lipoprotein (mg/dl)
GROUP 1: Negative Control	2.30±0.05[b]	0.66±0.00 [b]	2.03±0.01[b]	1.63±0.08 [b]
GROUP 2: Positive Control (2.25mg/kg lead)	3.22±0.00[a]	1.03±0.02 [a]	1.00±0.03 [a]	2.55±0.03 [a]
GROUP 3: Lead + C*urcumalonga*(200mg/kg)	2.46±0.03[ab]	0.71±0.01 [b]	1.96±0.08 [ab]	2.03±0.08 [ab]
GROUP 4: Lead + C*ucumissativus* (500mg/kg)	2.80±0.01 [ab]	0.77±0.03 [ab]	1.86±0.05 [ab]	2.27±0.01 [ab]
GROUP 5: Lead +	2.23±0.02 [ab]	0.52±002 [ab]	2.20±0.05 [ab]	1.53±0.08 [ab]

C*urcumalonga*(200mg/kg) **+** **C*ucumissativus*(500mg/kg)**

All values are expressed as mean ± SEM. [a]= p<0.05 compared to Negative control.[b]= p<0.05 compared to Positive control.

In table 3, 2.25mg/kg bw of lead administration demonstrated a significant elevation in Total cholesterol, Triglyceride and Low-density lipoprotein levels but reduced High density lipoprotein concentration amongst Group 2 (Positive control) rats compared to Group 1 (Negative control) rats; indicating a possible hyperlipidemic effect of lead acetate. However, single daily administration of 200mg/kg and 500mg/kg body weight respectively of C*urcuma longa* and C*ucumis sativus* to Groups 3 and 4 rats caused a significant decrease in Total cholesterol, Triglyceride and Low density lipoprotein levels but elevated High density lipoprotein concentration compared to Group 2 rats p<0.05, suggesting a possible hypolipidemic effect of the extracts in lead toxicity, noteworthy that, C*urcuma longa* administration showed better hypolipidemic effect amongst Group 3 rats compared to C*ucumis sativus*treated rats (Group 4). By corollary, co-administration of both extracts to animals in Group 5 brought about significant changes in Total cholesterol, Triglyceride, Low density lipoprotein and High-density lipoprotein levels compared to Groups 3 and 4 rats p<0.05: our findings are suggestive of a potential synergistic and greater effects of the extracts when combined.

DISCUSION

The present study assessed changes in biochemical indices in lead-induced toxicity following administration of methanolic extracts of C*urcuma longa rhizomes* and C*ucumis sativus*fruits. Despite difference in study design, our findings validate recent reports on the modulatory functions of C*urcuma longa* on some biochemical parameters (Fortune *etal*., 2019; Saronee *et al.*, 2020; Ijeoma *et al.*, 2020). The significant reduction in testicular cholesterol with increased nitric oxide levels upon administration of individual extract copiouslypoint to possible hypotensive properties of the extracts, implying potential beneficial effects in lead toxicity. However, the precise mechanism of interaction of the two extracts in the changes made to testicular cholesterol and nitric oxide levels in lead toxicity is presently unclear. Comparatively, the effects of C*urcuma longa rhizomes* on testicular cholesterol and nitric oxide were significantly better than those administered C*ucumis sativus*only;implying perhaps a better hypotensive effect of C*urcuma longa* compared to C*ucumis sativus*. Our results suggest that apparently, co-administration of both extracts at 200mg/kg and 500mg/kg body weight exhibit some noticeable synergistic effects.

An elevation in serum liver enzymes levels: AST, ALT and ALP reflect increased permeability and cell rupture (Benjamin, 1978). Aspartate aminotransferase and alanine aminotransferase are markers of active liver injury and hepatocellular necroses [Davem and Scharschmidt, 2002]. AST activity spreads beyond the liver to the heart, kidney, brain and skeletal muscles, however, ALT function is confined to the liver [Benjamin 1978; Ikechukwu and Saronee, 2019]. Serum ALT and AST levels increases in hepatitis [Benjamin 1978; Ikechukwu and Saronee, 2019]. As a result, the significant reduction in AST, ALT and ALP with increasing total protein and albumin levels in the present study,suggest a beneficial outcome of the extract on lead acetate exposure.

Apparently, observed significant decrease in total cholesterol, triglyceride and low-density lipoprotein with elevated high density lipoprotein levels due to administration of both extracts, indicates that the extracts demonstrated a possible hypolipidemic efficacy with the combined administration showing a

better effect, this is suggestive of a potential beneficial effect in lead toxicity. These findings are consistent with Saronee *et al.,* 2020 and Ijeoma *et al.,* 2020.

Phytochemical examination of individual plant extracts has showed the presence of beneficial compounds like flavonoids, tannins, sterols, curcumin and glycosides, which could have been responsible for their synergistic effects in ameliorating lead toxicity associated complications (Okwu, 2001; Saronee *et al.,* 2019; Saronee *et al.,* 2023; Saronee *et al.,* 2024).

In conclusion, it can be deduced based on obtained results that individual doses of C*urcuma longa rhizome* and C*ucumis sativus* extractsameliorated lead induced toxicity with C*urcuma longa rhizome* showing greater potency compared to C*ucumis sativus.* Apparently,group 5 rats co-administered 200mg/kg and 500mg/kg bw of methanolic extracts of C*urcuma longa rhizome* and C*ucumis sativus* showed better ameliorative effects in the management of lead induced toxicity than the single administration of either extract, demonstrating possible synergism of effects.

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