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The ameliorative effects of glutathione on biochemical indices of goats exposed to lead

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Abstract

Poisoning with lead is rampant in goats and it is a crucial threat to their economic and nutritional worth. Lead exerts deleterious impacts on the bodies of human beings and animals. Glutathione possesses antioxidant and decontamination characteristics in living systems. It is opined that glutathione might abrogate lead toxicity through its beneficial mechanisms of action. The purpose of this study was to find out the influence of glutathione on biochemical indices in goats exposed to lead. Twenty bucks were included in the research and were distributed into five groups (four animals/group) as follows: Control (CONT group, administered with distilled water); Lead (10 mg kg^{-1}) group; Lead (10 mg kg^{-1})+Glutathione (50 mg kg^{-1}) group; Lead (10 mg kg^{-1})+Glutathione (100 mg kg^{-1}) group and Lead (10 mg kg^{-1})+Glutathione (200 mg kg^{-1}) group. The animals received lead and glutathione daily *per os* for 21 days. The biochemical indices were estimated with a Clinical Chemistry Analyzer. Lead evoked considerable elevations in the activities of some of the serum enzymes and levels of urea, triglycerides and thyroid stimulating hormone. Also, the levels of albumin, triiodothyronine and thyroxine were decreased appreciably in the lead group. Glutathione ameliorated the undesirable effects of lead in the goats. The advantageous effects of glutathione in this research may be attributed to its bioprotective impacts. Therefore glutathione may be a valuable agent for the amelioration of lead intoxication in goats.

Key Words: Amelioration, Biochemical indices, Glutathione, Goats, Lead acetate

1.0. Introduction

Lead (Pb) can be found in most environmental systems and it accumulates easily in crucial organs of the body [27]. It is commonly used in various industries such as automobiles, paint, ceramics, plastics, and so on because of its distinctive attributes like softness, ductileness, increased malleability, low melting point and imperviousness to corrosion [16]. These applications of Pb are now being regulated in most countries in order to control its emissions in the environment.

Oxidative stress has been identified as a major mechanism through which Pb causes diverse dysfunctions in the body [49]. Oxidative stress occurs when the accessible supplies of the antioxidants in the body are inadequate to handle or deactivate free radicals of diverse categories [11]. Lead-induced oxidative stress has been associated with renal dysfunction manifested as increased

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levels of urea, creatinine, lactate dehydrogenase and alkaline phosphatase levels in rats [43], and elevations in liver enzyme activities, disruptions in serum lipid profile, as well as reductions in total protein, albumin and globulin concentrations in rats [2]. Additionally, oxidative stress elicited by lead exposure has been shown to cause increased activity of thyroid stimulating hormone and diminutions in thyroxine and triiodothyronine levels in rats [8].

Glutathione is an essential non-enzymatic antioxidant for reducing the levels of free radicals in living systems [33]. Low concentrations of glutathione are associated with impaired animal health because it is essential for immunity [28]. Glutathione plays a vital function in the decontamination and eradication of various heavy metals from the bodies of living organisms [41]. Therefore, glutathione may be valuable for the mitigation of Pb poisoning since it is an efficacious antioxidant that may be capable of counteracting oxidative stress evoked by Pb.

Several Pb poisoning episodes have been reported in Nigeria due to illegal mining activities, and domestic animals such as goats are vulnerable to Pb intoxication because of their closeness to human beings in various households and communities [13, 25, 32]. Therefore it is imperative to find out how to mitigate lead toxicity in goats because of their nutritional value and economic importance.

The purpose of the study was to find out the impacts of glutathione on biochemical biomarkers in Red Sokoto goats exposed to subacute Pb acetate toxicity under experimental conditions.

This study may be considered to be novel because it is the first study that reported the ameliorative effects of glutathione on biochemical indices in goats exposed to Pb.

2.0. Materials and Methods

2.1. Animals

Twenty male goats (bucks), about one year old were included in the study. They were purchased from a livestock market in Sokoto State (North Western Nigeria). The bucks were kept in pens at the Teaching and Research Farm, University of Abuja Main Campus, Airport Road, Federal Capital Territory. The animals underwent physical and clinical examinations. Subsequently, they were permitted to adjust adequately to the experimental location for fourteen days before the study started. They were fed with livestock feeds and supplied with water *ad libitum*. The bucks were treated in accordance with the guidelines endorsed by the University of Abuja Research Ethics Committee, and the principles of the National Institutes of Health (NIH) regarding the care and use of laboratory animals [18].

2.2. Chemicals

Lead (Pb) acetate was purchased from Merck®, Darmstadt, Germany. It was prepared by dissolving 8 g of Pb acetate in 20 ml of distilled water to attain a concentration of "400 mg/ml on a daily basis" [5, 7]. It was administered to the goats by oral gavage.

Pharmaceutical grade Glutathione (Glutathione Reduced, Jarrow Formulas® OPITAC™, KOHJIN Life Sciences Company, Limited, Japan) was bought from a certified Pharmaceutical outlet in Abuja, Federal Capital Territory, Nigeria. Five grams (5) g of L-Glutathione (10 capsules of 500 mg each) were reconstituted by dissolution in distilled water of 50 ml in volume thereby yielding a 100 mg/ml concentration. This was prepared for the bucks every day.

2.3. Experimental Procedure

The body weights of the bucks were determined and they were distributed into five groups (four per group). Neck tags were utilized for their identification. The bucks in the different groups received the xenobiotics once daily *per os* for 21 days as follows: CONT group (Control was given distilled water only), Pb group (Pb at 10 mg kg^{-1}, [42]), Pb+G50 group [Pb (10 mg kg^{-1}) +glutathione (50 mg kg^{-1}, [39])], Pb+G100 group

Table 1. Impacts of distilled water (CONT), lead (Pb at 10 mg/kg) and lead (Pb at 10 mg/kg) +glutathione (at 50, 100 and 200 mg/kg) respectively on the levels of serum enzymes, proteins and renal function indices of the bucks.

INDICES	CONT	Pb	Pb+G50	Pb+G100	Pb+G200
ALT (U/L)	39.25±3.50	45.33±4.26	39.33±1.33	32.67±1.76	37.50±2.53
ALP (U/L)	89.67±0.88	**180.67±7.67**$^{@}$	**159.25±12.87**$^{@}$	**151.67±15.24**$^{@}$	**127±5.2**$^{\beta}$
AST (U/L)	178±18.1	**242.67±10.48***	208±2.89	179.67±5.49	178.33±6.94
GGT (U/L)	43.12±4.82	**62.3±1.53****	50.12±8.42	47.48±1.3	44.87±1.86
LDH (U/L)	427.03±28.44	**710.4±32.39***	491.1±54.22	453.37±40.79	436.73±53.35
CK (U/L)	20.75±0.85	**82.33±9.67***	**56±11.55**$^{\beta\beta}$	36.33±6.89	22±6.93
ALB (g/dl)	39±1.47	**37.67±0.88*****	40.33±0.88	**44±0.71**$^{\#}$	**44.33±1.45**$^{\#}$
TP (g/dl)	64.75±4.03	63.67±3.38	67.67±2.19	69±7.1	75.25±2.29
GLB (g/dl)	25.75±2.93	26±2.19	27.33±2.96	25±2.14	30.92±6.23
Albumin/Globulin	1.57±0.20	1.32±0.29	1.43±0.10	1.52±0.19	**2.35±0.24**$^{\#\#}$
Urea (mg/dl)	3.68±0.39	**7.00±0.65**$^{\#\#\#}$	5.77±0.75	5.60±1.02	5.43±0.69
Creatinine (mg/dl)	69.33±4.70	80.67±7.31	77±4.67	76.50±5.68	70±2.31

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; GGT: Gamma glutamyltransferase; LDH: Lactate dehydrogenase; CK: Creatine kinase; ALB: Albumin; TP: Total Protein; GLB: Globulin

$^{@}P < 0.05$ Pb, Pb+G50 and Pb+G100 groups versus CONT respectively
$^{\beta}P < 0.05$ Pb group versus Pb+G200 group
$*P < 0.05$ Pb group versus CONT, Pb+G100 and Pb+G200 groups respectively
$**P < 0.05$ Pb group versus CONT and Pb+G200 groups respectively
$\beta\beta$ $P < 0.05$ Pb+G50 versus CONT group
$***P < 0.05$ Pb group versus Pb+G100 and Pb+G200 groups respectively
$^{\#}P < 0.05$ Pb+G100 and Pb+G200 groups versus CONT group respectively
$^{\#\#}P < 0.05$ Pb+G200 group versus Pb and Pb+G50 groups respectively
$^{\#\#\#}P < 0.05$ Pb group versus CONT group respectively

[Pb (10 mg kg^{-1})+glutathione (100 mg kg^{-1}, [3)]] and Pb+G200 group [Pb (10 mg kg^{-1})+glutathione (200 mg kg^{-1}, [31)]]. Lead was administered to the bucks first before glutathione in the Pb+G50, Pb+G100 and Pb+G200 groups. The goats were regularly examined for clinical signs.

2.4. Assessment of Serum Biochemical Parameters

Five (5) millilitres of blood samples were obtained from the jugular veins of the bucks and placed in plain tubes that lacked anticoagulants. These blood samples were allowed to clot and were processed for the assessment of biochemical parameters as described in an earlier publication [6)].

2.4.1. Estimation of Serum Enzymes and Proteins:

The levels of serum enzymes [aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK)], total protein (TP), albumin (ALB), urea and creatinine were determined from the samples of serum obtained from the blood samples collected from the bucks. The level of serum globulin (GLB) was derived from the difference between the levels of the total serum protein (TP) and the serum albumin (ALB) [6)]. The estimation of the biochemical indices was carried out with a Clinical Chemistry Analyzer (Erba Diagnostics, Mannheim, Germany).

2.4.2. Appraisal of Thyroid Function:

This was conducted by the evaluation of the levels of thyroid stimulating hormone, triiodothyronine and thyroxine through the utilization of fluorescence immunoassay kits based on immunodetection methods from FINECARE™ Incorporation (Guangzhou, China). The analytical sensitivities of the kits for triiodothyronine, thyroxine and thyroid stimulating hormone

Table 2. Impacts of distilled water (Control, CONT), lead (Pb at 10 mg/kg) and lead (Pb at 10 mg/kg)+glutathione (at 50, 100 and 200 mg/kg) respectively on the thyroid function test of the goats.

Thyroid Hormones	CONT	Pb	Pb+G50	Pb+G100	Pb+G200
Serum Triiodothyronine concentration (nmol/L)	4.22±0.54	**2.01±0.12***	2.14±0.12	3.12±0.46	6.14±0.11
Serum Thyroxine concentration (nmol/L)	61.75±4.87	**33±1.16***	45.5±3.18	56.33±6.36	69.33±6.98
Thyroid Stimulating Hormone concentration (mIU/L)	0.76±0.07	**1.54±0.26****	0.34±0.21	0.44±0.04	0.95±0.09

*$P < 0.05$ Pb group versus CONT, Pb+G100 and Pb+G200 groups respectively
**$P < 0.05$ Pb group versus CONT, Pb+G50 and Pb+G100 groups respectively

CONT: Control; Pb: Lead (at 10 mg/kg); Pb+G50 (Lead+Glutathione at 50 mg/kg); Pb+G100 (Lead+Glutathione at 100 mg/kg) and Pb+G200 (Lead+Glutathione at 200 mg/kg)

Table 3. Impacts of distilled water (Control, CONT), lead (Pb at 10 mg/kg) and lead (Pb at 10 mg/kg)+glutathione (at 50, 100 and 200 mg/kg) respectively on the serum lipid profile of the goats.

Serum Lipid Profile	CONT	Pb	Pb+G50	Pb+G100	Pb+G200
Total Cholesterol (mg/dl)	2.04±0.21	2.26±0.27	2.16±0.21	2.09±0.06	2.05±0.19
High Density Lipoprotein (mg/dl)	0.31±0.05	0.14±0.02	0.34±0.18	0.38±0.08	0.49±0.15
Low Density Lipoprotein (mg/dl)	1.68±0.15	2.04±0.22	1.76±0.13	1.65±0.17	1.50±0.19
Very Low Density Lipoprotein (mg/dl)	0.05±0.01	0.08±0.01	0.06±0	0.06±0.02	0.06±0
Triglycerides (mg/dl)	0.23±0.04	**0.40±0.03***	0.32±0.02	0.29±0.06	0.28±0.02
Atherogenic Index	5.58±0.73	15.14±5.94	5.35±3.32	4.50±1.30	3.18±0.79

*$P < 0.05$ Pb group versus CONT group

CONT: Control; Pb: Lead (10 mg/kg); Pb+G50 (Lead+Glutathione at 50 mg/kg); Pb+G100 (Lead+Glutathione at 100 mg/kg) and Pb+G200 (Lead+Glutathione at 200 mg/kg)

were 0.61 nmol/L, 12.87 nmol/L and 0.1 mIU/L respectively (FINECARETM Incorporation, Guangzhou, China).

2.4.3. Analysis of Serum Lipid Markers:

The levels of total cholesterol, triglycerides and high density lipoprotein were analyzed with the aid of a Clinical Chemistry Analyzer (Erba Diagnostics, Mannheim, Germany).

The very low density lipoprotein level was derived as follows: "very low density lipoprotein = 0.20 x triglycerides" [5, 17]

The formula used for calculating the low density lipoprotein was: "low density lipoprotein (mg/dL) = total cholesterol – high density lipoprotein– (0.20 x triglycerides)" [5, 17].

The values of the atherogenic index were obtained from this formula [5, 29]: "Atherogenic index = $\frac{\text{Total cholesterol- High density lipoprotein}}{\text{High density lipoprotein}}$"

2.5. Analysis of Data

The data were stated as mean ± standard error of the mean. The results were analyzed with the one-way analysis of variance (ANOVA) and the Tukey's test was applied afterwards. The data analysis package used in this research was the International Business Machines Statistical Package for the Social Sciences version 23.0 (New York, United States of America). The values of $P < 0.05$ were deemed significant.

3.0. Results

3.1. Impacts of Lead and Glutathione on the Biochemical Variables of the Bucks

3.1.1 Impacts of Lead and Glutathione on the Levels of Serum Enzymes, Albumin, Protein, Globulin, Albumin/Globulin and Renal Function Indices of the Goats

The ALP activity was significantly ($P < 0.05$) higher in the Pb group compared to that of the Pb+G200 (Table 1). Also, the ALP activity was remarkably ($P < 0.05$) lower in the control relative to those of the Pb, Pb+G50 and Pb+G100 groups respectively.

The AST, LDH and CK activities were significantly ($P < 0.05$) elevated in the Pb group compared to those of the control, Pb+G100 and Pb+G200 groups respectively (Table 1). Additionally, the CK activity was increased significantly ($P < 0.05$) in the Pb+G50 group compared to that of the control.

A marked increase ($P < 0.05$) was recorded in the GGT activity of the Pb group relative to those of the control and Pb+G200 groups respectively (Table 1).

A significant ($P < 0.05$) decline was recorded in the level of ALB in the Pb-exposed group in reference to those of the Pb+G100 and Pb+G200 groups respectively (Table 1). Moreover, there was augmentation of the level of ALB in the groups (Pb+G100, Pb+G200) in relation to those of the control respectively.

The albumin/globulin value was considerably ($P < 0.05$) increased in the Pb+G200 group relative to those of the Pb and Pb+G50 group respectively (Table 1).

There was a significant ($P < 0.05$) increase in the urea concentration of the Pb group compared to that of the control (CONT) group. This is depicted in Table 1.

No difference was recorded in the levels of the TP, GLB and creatinine among the groups (Table 1).

3.1.2. Influences of Lead and Glutathione on the Thyroid Function of the Goats

Marked ($P < 0.05$) reductions were observed in the level of triiodothyronine in the Pb group compared to those of the CONT, Pb+G100 and Pb+G200 groups correspondingly (Table 2). There were a notable ($P < 0.05$) diminution in the thyroxine concentration in the group exposed to Pb relative to those of the CONT, Pb+G100 and Pb+G200 groups respectively (Table 2). The thyroid stimulating hormone level was significantly ($P < 0.05$) increased in the Pb-exposed herd of goats in reference to the ones of the CONT, Pb+G50 and Pb+G100 groups respectively (Table 2).

3.1.3. Impacts of Lead and Glutathione on the Lipid Levels of the Goats

There was a considerable ($P < 0.05$) increase in the levels of the triglycerides in the group treated with Pb relative to that of the control (Table 3). The other lipid indices (total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and atherogenic index) were not different from one another among the groups (Table 3).

4.0. Discussion

In the current study, the levels of the serum enzymes were increased in the Pb group. Increments in the ALT and AST activities might be due to the induction of an upsurge in the cell membrane permeability or damage of the cell membranes of hepatocytes by Pb [10, 36]. A rise in LDH activity that entailed Pb intoxication in test animals has been documented by other researchers [10, 26]. Their results are in agreement with ours. Some investigators have reported that ALP level in the serum is augmented in conditions of liver, kidney and bone damage [21, 22]. This might offer an explanation for the increment in the activity of ALP recorded in this research. Moreover, GGT activity was increased in the goats administered with Pb, and this may be caused by the elicitation of oxidative stress by Pb in the hepatocytes [45]. On the contrary, the concentrations

of the serum enzymes were lowered in the bucks that received glutathione. The significant suppression of the levels of ALT, AST, LDH and CK by glutathione has also been documented in rabbits [23], and in rats [12]. These findings may be attributable to the capacity of glutathione to defend the body against the onslaught of stressors, for instance Pb [24, 35]. In the current research, the ALT activity was not significantly lowered in the glutathione groups compared to previous studies, and this may be attributed to differences in the species, dosages and duration of administration.

There was a notable ($P < 0.05$) upsurge in the level of urea in the goats that were treated with Pb. This may be due to the nephrotoxic effects of Pb in diverse life forms. An increment in the concentration of urea subsequent to oral administration of Pb has been observed in a variety of species including rats [40], goats [20] and sheep [37, 50]. In the present research, the ALB and albumin/globulin levels were substantially ($P < 0.05$) decreased, while the TP and GLB levels were inconsequential ($P > 0.05$) in the herd of goats that received Pb. Ezejiofor and Orisakwe [14] reported significant diminutions in the TP and ALB concentrations of rats exposed to Pb for four weeks, and the observations were attributed to the stimulation of the disorders in the bodies of the bucks by Pb [4]. Also, Mohamed et al. [34] stated that Pb intoxication caused remarkable reductions in ALB, TP, GLB and albumin/globulin levels in rats. However, the globulin level was unchanged, while the TP and ALB concentrations were significantly increased in a research conducted by Abd El-Ghffar and his colleagues [1] in which mice were treated with Pb. Our results may have differed from those of other investigators because of variations in the experimental animals, dosages and duration of administration utilized. In contrast, glutathione normalized the total protein levels in the bucks in the current study and this may be due to its critical bioprotective roles in the body [15].

Moreover, in this investigation, reductions were recorded in the triiodothyronine and thyroxine levels, while there was an increment in the thyroid stimulating hormone activity in the bucks that received Pb. These outcomes are in agreement with reports by Akande et al. [7] and Al Zadjali et al. [8] in rats that were given Pb. It has been reported that Pb elicits oxidative stress in thyroid cells [30] and initially disrupts the pituitary-thyroid pathway thereby leading to an increase in TSH and diminutions in T4 and T3 levels [8]. On the other hand, the thyroid function was stabilized in the bucks that received glutathione (Pb+G50, Pb+G100 and Pb+G200 groups). Glutathione eliminates free radicals and protects cells from destruction by oxidants [46, 47]. Therefore it may be surmised that glutathione improved the thyroid function of the bucks through its antioxidant capacity. Besides, glutathione is a necessary molecule for the activation of thyroxine to triiodothyronine [48]. Hence, this might provide an insight into why the levels of thyroxine and triiodothyronine were ameliorated in the bucks that received glutathione.

An obvious ($P < 0.05$) surge was documented in the level of triglycerides in the goats that were assigned to the Pb group. However, the concentrations of the remaining lipid indicators (total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and atherogenic index) did not vary between the groups. Lead augments cholesterol production and its conveyance to various parts of the body, and this may upset the concentrations of lipids in the body [19]. Different researchers have affirmed the effects of Pb-evoked lipid disorders in experimental animals [9, 38]. It is worth noting that in the present research, the lipid indices of the herds of goats that were administered with glutathione were normalized. This finding might be because glutathione can reduce cholesterol levels in the body as a part of its bioprotective functions [44].

Conclusion

Lead brought about disturbances in the biochemical indices of the goats. Conversely, glutathione ameliorated Pb intoxication in the

current research and this may be credited to its bioprotective attributes against the detrimental effects of Pb in the goats. Therefore glutathione may be considered for inclusion in the therapeutic procedure for lead toxicity after its mechanisms of amelioration have been elucidated.

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Declaration of Interest

The authors affirm that they have no known competing financial interests or personal relationships that could have influenced the research presented in this manuscript.

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