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Alterations in Biochemical Parameters and Antioxidant Enzymes in Male Mice as Biomarkers of Exposure to Pollution with Cadmium

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ABSTRACT

There is currently a significantly larger concentration of toxins in our environment than there was in the past. This is mostly attributable to the expansion of modern industry. This investigation was conducted in order to investigate various haematological and biochemical changes in order to determine the effects of Cd on the liver and kidney. Because of its long biological half-life, it is considered hazardous to human health. The effect of sub-lethal doses (40, 80 and 120 mg\Kg) of Cadmium (Cd) on male mice were evaluated for 4 weeks, and analysis was done to estimate their biochemical parameters and antioxidant enzymes. The results showed that Cd-treated mice had considerably lower packed cell volume, red blood cells, and haemoglobin. White blood cells, on the other hand, showed a considerable rise at the higher dosages. All of the treatment groups showed considerable improvements in kidney functions compared to the control group, especially with respect to creatinine and urea. All dosages significantly increased aspartate aminotransferase and alanine transaminase levels. Contrarily, only the second and third treatments showed a substantial rise in malondialdehyde levels and antioxidant enzymes (catalase and superoxide dismutase). In conclusion, Cd-induced oxidative stress in an organism alters various biochemical parameters and antioxidant enzymes, which can be employed as biomarkers for Cd contamination.

Keywords: Antioxidant enzymes, Renal dysfunction, Liver function, Cadmium toxicity.

Introduction

Heavy metals are utilized by people for centuries and introduced into the environment, through natural and man-made sources. Cadmium (Cd) is widely available, non-essential element that is of special importance because of its environmental accumulation as a result of industrial processes as well as its presence in a variety of human and animal tissues, where it causes significant illnesses.[1] The expansion of contemporary industry has led to a significant rise in the quantity of pollution that is present in our surroundings. Cadmium (Cd) is one of these elements, and since it is so persistent in both the natural environment and the human body, it poses a threat to human health.[2] Pollutants have the potential to induce organisms to undergo a range of physiological changes. Finding biomarkers, which are indicators of exposure to the impacts of environmental contaminants, and using them to track these alterations is possible and can be done. These biomarkers make it feasible to conduct a thorough examination of an organism's health in a short amount of time and to identify potential dangers in the surroundings.[3] Degradation of soil and water due to Cd has caused issues since this metal bio-accumulates in the highest trophic levels [4]

In contrast to exposure that occurs on the job, the most major causes of Cd exposure that occur outside of the workplace are the use of cigarettes and the decisions that are made about one's diet. As cadmium levels in the human body continue to rise, it is possible that Cd-related illnesses, such as hypertension, hepatotoxicity, diabetes, cardiovascular disease, nephrotoxicity, osteoporosis, and cancers of numerous organs, may become more prevalent in the future.[5]Curiously, the vascular endothelium proposed to be a basic objective of Cd poisonousness, which prompts numerous cardiovascular confusions.[6] Cd is accounted for the potential gamble figure of trial studies of hypertension, for example, renal rounded harm and brokenness brought about by ecological Cd openness.[7]

Biomarkers show potential as sensitive indicators of toxicants entering organisms, dispersing throughout the body and exhibiting a harmful effect at important sites.[8]

As a result of pollution, organisms may experience biological alterations. These alterations can be recognized and used as biomarkers, which are indicators of environmental pollutant exposure or consequences. [3] Biomarkers have the potential to be sensitive indicators of toxicants entering organisms, spreading between tissues, and indicating toxic effects in key areas.[8] To understand the specific causes of Cd toxicity, numerous *in vivo* and *in vitro* research have been done. Even though neither metal is a Fenton's metal, the current body of research implies that oxidative stress is an important of toxicity causes for both metals.[9] Haematological markers, which are employed in disease diagnosis, usually represent the animal's physiological response to both exterior and interior environments and hence represented a useful indicator for the monitoring of animal health.[10] According to earlier studies, Cd exposure damages the liver and kidneys, which are vital organs.[11]

Cd may produce oxidative stress by interacting with the thiol groups of enzymes that function as antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase. This can happen when Cd generates reactive oxygen species, which then react with the thiol groups of the enzymes (CAT). (ROS), which results in a reduction in the amount of glutathione (GSH) found inside the cell.[12] This study seeks to find biomarkers of several biological parameters following exposure to cadmium using mice as a model organism.

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Methodology

Ethical approval

The College of Science Research Ethics Committee gave its clearance to this particular piece of study (Ref. CSEC/0921/0097).

Chemicals and animals

All the reagents and compounds used in this study were of analytical grade or greater in purity. Intraperitoneal injection solutions were made using cadmium chloride ($CdCl_2xH_2O$, Merck, Germany). The two-month-old male Swiss albino mouse has a bodyweight of 24.0 ± 2.0 g were donated by the Ministry of Health's National Center for Drug Control and ResearchThe animals were housed under regular, regulated circumstances throughout the experiment (temperature 25 ± 3°C, relative humidity 35-55%, 12-hour light-dark cycle) and were given free access to ordinary food and drink.

Experimental procedure and study design

After acclimatization duration of 14 days, mice were separated into three experimental groups and a control group. For four weeks, all groups received sub-lethal doses of $CdCl_2$ (40 mg, 80 mg, and 120 Cd/kg b.w.) intraperitoneally three times a week. Water was the only treatment given to the control group. The experimental doses were chosen based on previous studies. Animals were dissected 24 hours after receiving mild anaesthetic therapy. After the animals had been put to death for the purpose of the experiment, a blood sample from their trunks was taken and then frozen.

Biomarker assay

A haematological–analyzer was used to measure haematological parameters (Mindray BC-3000 Plus). Several other blood parameters, such as hemoglobin (Hb), the number of white blood cells (WBC), the number of red blood cells (RBC), and the packed cell volume, were assessed (PCV). In Patton and Crouch's test, blood urea nitrogen (also known as BUN) and serum creatinine (also known as CRE) were measured (1977 [13] technique. The Reitman and Frankel method was applied in order to determine the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in rat serum (1957).[14] The level of malondialdehyde (MDA) was evaluated using the Buege and Aust technique.[15] The MDA was determined using the thiobarbituric acid method, which results in a pink color at a wavelength of 535 nm due to an interaction with the thiobarbituric acid. Using a Catalase Activity Assay Kit (Colorimetric, ab83464) from sigma-Aldrich (Germany), the catalase activity of the serum was determined.

Statistical analysis

The mean and standard deviation were provided for every data. At the 5% level of significance, they were analyzed using the F test. The statistical analysis in this study was performed using Duncan's Multiple Range Test. The significance level for the test was set at P= 0.05.

Results and Discussion

Regarding the haematological measurements, all of the treated groups had substantial decreases in RBC, Hb, and PCV when compared to the control group, particularly the second and third treatment groups (Table 1). Due to an increase in immunogenic response, a malfunction in the signaling system, or a change in cell maturation that affects RBCs, anemia may be connected to the lowest RBC counts and Hb concentrations.[16]

Because we found high Cd concentrations in the blood of experimental groups in comparison to the control, we may assume that intravascular haemolysis is the source of the RBC and Hb decrease. Reduced erythrocyte size, poor haemogenesis in bone marrow, or an acceleration in decline production of erythrocytes or even their destruction leading to a decrease in Hb concentration. In general, "haemolytic anaemia," or more precisely "oligocythemia," is caused by the uncontrolled RBCs destruction via the action of chemical toxins, illnesses, elevation RBC fragility, hydration, or the shortening of RBCs' life span.[10] WBC are active functioning cells of the immune system, both specific and non-specific, and their number reflects the overall health of the immune system.[17] WBC counts increased in all treatment groups following injection with Cd in comparison to the control group that, more likely, because of the acute immunogenic response. When compared to untreated mice, the renal profile parameters BUN and CRE increased considerably in all experimental dosing regimens (Table 2). BUN and CRE are products of protein metabolism. Urea is generated by amino acids' oxidative deamination with the generation of ammonia which is converted to urea in the liver by the urea cycle. Creatinine and urea changes suggest that the kidney's excretory function may be affected even when a single dose of hazardous metals is administered. Changes in urea and creatinine levels were seen in other investigations with similar results.[18,19]

ALT and AST enzymes represent the most important; yet, highly specific and sensitive indicators of liver function. The term "liver function tests" is misleading because many of the tests do not assess the liver's function but rather identify the source of the damage.[20] In the current investigation, a single dose of Cd given after four weeks dramatically raised ALT and AST activity (Table 2). Such findings are consistent with other authors[21,22]

Table 1: Effect of different doses of Cadmium on haematological parameters

Groups	RBC (Cell * 10^6) Mean ± SE	Hb (mg\dl) Mean ± SE	PCV (%) Mean ±SE	WBC (Cell * 10^3) Mean ±SE
Control	5.46 ±0.37^a	10.67 ±1.32^a	31.60 ±3.56^a	5.32 ±2.34^d
Treatment 1 (40 mg\ kg)	4.32 ±0.78^a	9.56 ±0.95^a	30.53 ±2.54^a	11.53 ±3.00^c
Treatment 2 (80 mg\ kg)	4.04 ±0.56^b	8.0 5 ±1.63^b	25.31 ±2.78^b	16.38 ±2.80^b
Treatment 3 (120 mg\ kg)	3.63 ±0.47^c	6.44 ±0.53^c	20.62 ±1.58^c	20.33 ±3.83^a

Columns with different lower-case letters represent insignificant differences (P<0.05). RBC, Hb, PCV, WBC, and SE denote red blood cells, haemoglobin, packed cell volume, white blood cells, and standard deviation, respectively.

Table 2: Effect of different doses of Cadmium on Liver and Kidney function

Groups	ALT (mg\dL) Mean ± SE	AST (mg\dL) Mean ± SE	B. Urea (mg\dL) Mean ± SE	Creatinine (mg\dL) Mean ± SE
Control	32.65 ± 1.76^d	22.67 ± 0.63^c	41.32 ± 1.56^d	0.37 ± 0.04^b
Treatment 1 (40 mg\ kg)	60.53 ± 1.20^c	25.74 ± 2.21^c	50..33 ± 2.53^c	0.65 ± 0.05^a
Treatment 2 (80 mg\ kg)	76.46 ± 3.54^b	58.90 ± 2.06^b	58.45 ± 3.06^b	0.85 ± 0.03^a
Treatment 3 (120 mg\ kg)	90.42 ± 3.45^c	65.94 ± 3.96^a	70.42 ± 2.96^a	0.97 ± 0.04^a

Different lower-case letters within a column represent means with significant differences. ALT, AST, and SE denote Alanine aminotransferase, aspartate aminotransferase, and standard deviation, respectively.

The sensitive biomarker of liver damage is an increase in serum enzymes. The detection of several liver enzymes in serum, such as the blood lipid profile, which consists of lipoproteins, triglycerides, and cholesterol, as well as the enzymes ALT and AST, are used to evaluate the functional status of the liver and identify any damage that may have been done to it. As a symptom of hepatotoxicity, high transaminase levels, in conjunction with a bilirubin level that has increased by more than twofold, are considered to be present. Because of this, the levels of ALT and AST activity in the blood are the ones that are looked at the most frequently when evaluating liver damage.[23] It is believed that oxidative stress arises whenever the ratio of biological oxidants to antioxidants is not maintained in a state of equilibrium. When compared to the controls, the blood levels of SOD, CAT, and MDA enzymes were significantly different after the second and third treatments. These differences were statistically significant (Table 3). An earlier study suggested that this trend upwards could be associated with elevated oxidative stress.[24] The reaction of increased ROS with polyunsaturated fatty acids results in the production of toxic and reactive aldehyde metabolites such as MDA, which is one of the end products of the lipid peroxidation process.[25] Endogenous antioxidants called SOD and CAT shield cells from oxidative damage.[26] On the other hand, elevated levels of catalase, superoxide dismutase, and malondialdehyde in treated groups may indicate a cellular response to oxidative stress in these groups.

Heavy metal poisoning is a worldwide and imminent threat to all living things. This toxicity is determined by a variety of factors, including the route of exposure, genetic makeup, and nutrition, and age. Flora *et al.* [27] found that Industrialization led to expanded worldwide pollution in addition to rise in the heavy metals production like Cd. Cd is a hazardous heavy metal that exerts detrimental effects on animals and humans. Around 13,000 t of Cd is produced annually, primarily from nickel-cadmium batteries, chemical stabilizers, alloys, and metal coatings.[28] Cd-induced oxidative stress causes lipid peroxidation as well as DNA damage (mutations). A link between lipid peroxidation and Cd exposure has been established in several investigations.[29] Antioxidants have been proven to be extremely beneficial to our bodies in scientific research. These chemicals combine with and stabilize free radicals, preventing free radical-induced cell damage.[30] Short- and long-term contact with Cd causes renal failure, cardiovascular illness, hypertension, osteoporosis, hepatotoxicity, pancreatic dysfunction, and changes in organ function, among other things.[31-33] The current research was intended to identify biomarkers of some biological parameters following exposure to cadmium by using mice as model. From the study's findings it can be concluded that the toxicity by Cd-induced oxidative stress in an organism leads to alteration in some biochemical parameters and certain antioxidant enzymes which can be used as biomarkers for Cd contamination. Cadmium was found in Silurus triostegus Heckel, 1843 (Siluriformes, Siluridae) and Contracaecum sp. larvae (Rhabditida, Anisakidae).[34]

Table 3: Effect of different doses of Cadmium on antioxidant enzymes SOD, CAT and MDA

Groups	**SOD (U\mL) Mean ± SE**	**CAT (U\mL) Mean ± SE**	**MDA (U\mL) Mean ± SE**
Control	1.60 ± 0.0^{a}	1.70 ± 0.04^{a}	1.67 ± 0.02^{a}
Treatment 1 (40 mg\ kg)	1.90 ± 0.02^{a}	1.90 ± 0.03^{a}	1.95 ± 0.05^{a}
Treatment 2 (80 mg\ kg)	2.34 ± 0.05^{b}	2.58 ± 0.04^{b}	2.88 ± 0.08^{b}
Treatment 3 (120 mg\ kg)	2.97 ± 0.06^{b}	$3.5\ 1 \pm 0.07^{c}$	3.94 ± 0.09^{c}

Means with different letters in the same column differ significantly. SOD, CAT, MDA, and SE denote superoxide dismutase, catalase, malondialdehyde, and standard deviation.

Conclusion

The findings of this study reveal that *Syzygium polyanthum* (bay leaf) extract has anti-hyperlipidemic activity and acts as an antioxidant by inhibiting the production of cholesterol. Bay leaf extract may play a significant role in the prevention of diseases caused by free radicals. The research helps to understand the mechanism of action of bay leaf extract as a hyperlipidemia treatment by inhibiting the HMG CoA reductase enzyme. The bay leaf extract is a very strong antioxidant and has the potential to inhibit the HMG CoA reductase enzyme.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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