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Biochemical Analysis of *Persea americana* Extract for Mitigating Cadmium-Induced Liver Damage in Rats

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Abstract **- This research investigated the effects of methanol seed extract of *Persea americana* Mill on the liver of Wistar rats exposed to cadmium toxicity. The liver, known for its critical role in metabolism and interaction with the gastrointestinal tract, is predisposed to damage caused by drugs and other harmful chemicals. According to research, *P. americana* is rich in secondary metabolites with hepatoprotective properties. The seeds of *P. americana* were obtained from its fruits, procured from a local seller. The seeds were cut, air-dried under shade until crisp, pounded into powder, and extracted in methanol for 72 hours. Exactly 20 rats were subdivided into four groups, each containing five rats. The normal control group was given normal saline. The second group received 20 mg/kg bw CdSO4, the third group received 20 mg/kg bw CdSO4 with 400 mg/kg bw extract, and the fourth group received 20 mg/kg bw CdSO4 with 100 mg/kg bw silymarin. The results showed a significant difference in AST, ALT, ALP, and GGT levels in group 1 compared to the second group, which exhibited an increase. The treated groups demonstrated a considerable decrease in these marker enzymes relative to the induced group. There was no statistically significant difference in the protein levels among the groups. However, albumin levels decreased in group 2 compared to the other groups. MDA levels increased significantly in group 2, while SOD and CAT levels showed a significant decrease in group 2. The plant extract exhibited a promising hepatoprotective effect on the liver of animals induced with cadmium (CdSO4).**

Keywords: **Methanol Seed Extract, Persea Americana, Cadmium Toxicity, Hepatoprotective Properties, Liver Enzyme Markers**

I. INTRODUCTION

The liver is a crucial internal organ in various animals, performing numerous essential functions. It is involved in detoxifying harmful substances, synthesizing proteins, and producing biochemicals vital for digestion [1]. The liver also plays significant roles in the synthesis and breakdown of biomolecules. It is responsible for glycogen storage, erythrocyte catabolism, and the synthesis of various proteins, including albumin and globulin.

Additionally, it facilitates hormone biosynthesis and removes harmful substances from the body. The liver generates bile, which is essential for digesting fats through emulsification. This specific organ manages a wide range of bodily reactions, both in the synthesis and breakdown of molecules, which are critical for maintaining normal physiological functions [2].

When the liver sustains damage, its plasma membrane becomes more permeable, allowing enzymes and other substances to leak out [3]. The liver's distinct metabolic functions and its interaction with the gastrointestinal tract make it particularly vulnerable to damage from drugs and other substances. Some medications, even within therapeutic ranges, can harm the liver, especially when overdosed. Furthermore, various chemical agents used in industrial settings and laboratories, as well as traditional treatments, can cause liver toxicity. This condition, known as hepatotoxicity, highlights the liver's susceptibility to injury from a wide range of substances [4].

The use of medicinal plants dates back to ancient times, with historical references highlighting their role in clinical treatments. This knowledge is especially prevalent among rural populations in developing countries, where the traditional use of plant parts for treating various ailments is common [5]. Medicinal plants are recognized for generating diverse chemical substances that perform biological functions and offer protection against predators. Recently, these plants have gained considerable attention due to their effectiveness in treating numerous diseases with minimal or no side effects [5]. According to Yakubu et al. [6], the affordability and accessibility of medicinal plants make them a primary source of healthcare in rural areas, particularly in African nations. Studies have demonstrated that *Persea americana* (commonly known as avocado) is rich in tannins, saponins, flavonoids, alkaloids, and glycosides. These compounds are utilized for their nephroprotective (kidney-protecting) and hepatoprotective (liver-protecting) properties in countries like Nigeria and South Africa [7][8].

Metallic elements are naturally present in the environment and are challenging to eliminate completely. The extensive use of metals in industries and daily life has led to significant issues related to toxic metal pollution [9], [10]. Recently, concerns have been raised regarding the infiltration of heavy metals into the biosphere and their public health and ecological effects. Human exposure to harmful metals is increasing due to their use in homes,

industries, agricultural practices, and technologies [9]. Ingestion, absorption, and respiration are the primary routes through which these metals reach vital organs, posing serious threats to health [11], [12].

Cadmium (Cd), a toxic heavy metal, is used in pigment production, stabilizers, alloys, and chemicals, particularly in electronics such as rechargeable nickel-cadmium batteries. Consequently, cadmium's environmental persistence has increased with industrial advancements [13], [14]. Cadmium is also present in food, drinking water, and cigarette smoke. Research indicates that cadmium toxicity triggers the generation of reactive oxygen species (ROS), leading to cellular stress in various tissues [15]. Cadmium exposure damages tissues by stimulating membrane lipid peroxidation [16]. Within biological systems, defense against this injury requires a synergy between enzymatic and non-enzymatic antioxidants to alleviate oxidative stress. Cadmium induces oxidative stress by disrupting antioxidant enzyme systems and interfering with gene expression processes [17].

Disruption of cellular redox stability causes significant tissue and organ damage, impairing their function [13]. Researchers are investigating various aspects of antioxidant status under cadmium (Cd) intoxication across different biological systems. According to Otitoju *et al.,* [18], the toxicity intensity of cadmium depends on exposure levels. Analyzing enzyme activities and specific chemical markers in blood serum provides crucial diagnostic information for liver and kidney diseases. Endogenous antioxidants play a vital role in defending against oxidative damage, highlighting their importance in protecting biological functions [15], [19].

II. MATERIALS AND METHODS

A. Plant Materials Collection, Preparation and Crude Extraction with Methanol

Fresh fruits of *P. americana* were purchased from the local market in Baissa. The fruits were cut open to remove the seeds, which were then sliced into pieces. The sliced seeds were dried under shade and pounded in a clean mortar and pestle. The extraction process followed the methodology reported in [20] and [21]. Exactly 500 grams of the sample were soaked in approximately 2 L of methanol at a 1:4 ratio for 72 hours. The extracts were filtered using Whatman No. 1 filter paper to obtain the final filtrate. The obtained substance was evaporated using a rotary evaporator, and the concentrated filtrate was placed in a water bath at 45°C to remove excess moisture, yielding the desired concentrate.

B. Grouping and Exposure of Experimental Subjects (Experimental Design)

Twenty Wistar rats of both sexes were procured from Wukari, Taraba State, Nigeria. Their weights were recorded accordingly. The rats were acclimatized for one week in wire cages, providing proper ventilation and a natural light cycle (12 hours light and 12 hours dark), while being fed ad libitum with commercial feed and water. Bedding materials were replaced every three days to maintain a hygienic environment and eliminate ammonia gas.

The twenty experimental subjects were divided into four groups of five rats each. Toxicity was induced in all experimental subjects except for the normal control group (Group 1). Cadmium was administered orally at 20 mg/kg body weight as $CdSO_4$ for 21 days. Group 1 (normal control) was given normal saline, Group 2 was induced with cadmium and left untreated, Group 3 was treated with 400 mg/kg body weight of the extract, and Group 4 received the standard drug silymarin (Table I). This method was adopted from Osukoya *et al.,* [22] with slight modifications.

TABLE I EXPERIMENTAL DESIGN

Groups (n=5)	Treatments
1	Normal saline (negative control)
2	$CdSO_4$ (20mg/kg b.w) and untreated
3	$CdSO_4$ (20mg/kg b.w) + *P. americana* seed extract
4	$CdSO_4$ (20mg/kg b.w) + Sylimarin

C. Blood and Tissue Collection from Animals

On the 22nd day, the animals were subjected to an overnight fast beginning the previous night. They were anesthetized using chloroform vapor, and laparotomy was performed to expose the internal organs. Cardiac puncture was conducted to obtain blood samples, part of which was stored in EDTA tubes, while the remainder was collected in plain tubes and centrifuged at 3000 rpm for 10 minutes. The resulting supernatant was used for biochemical analyses. The blood samples collected in EDTA tubes were analyzed for hematological indices. Renal and hepatic tissues were collected for fixation, histological photomicrography [23], and tissue homogenization.

D. Tissue Homogenization

The livers of Wistar rats from the various groups were carefully harvested and preserved in normal saline, with tissue remnants removed. Homogenization was performed by weighing the tissue, pounding it, and then placing it in a prepared phosphate buffer (1:10 w/v) at pH 7.4. The resulting supernatants were collected and used for the analysis of MDA, SOD, and CAT levels [24].

E. Determination of Malondialdehyde (MDA)

To 1 mL of 15% trichloroacetic acid, 1 mL of 0.67% thiobarbituric acid was added, followed by the addition of exactly 50 µL of the tissue homogenate to the mixture. The mixture was incubated in a water bath at 80 °C and then rapidly cooled on ice for 5 minutes. Subsequently, centrifugation was performed at 3000 × g for 10 minutes.

Malondialdehyde (MDA) levels were estimated spectrophotometrically at 535 nm, and lipid peroxidation levels were calculated using the molar extinction coefficient of malondialdehyde [25].

F. Calculation

Molar extinction of MDA = 1.56 x 10^5 $M^{-1}cm^{-1}$
MDA concentration = Absorbance/1.56 x 10^5 $M^{-1}cm^{-1}$

G. Determination of Superoxide Dismutase (SOD) Activities

The determination of superoxide dismutase (SOD) activity was carried out using a method reported by Martin et al. [26] and Yakubu et al. [27]. A 0.05 M, pH 7.8 phosphate buffer was prepared, and 920 µL was added to a clean test tube. Then, 40 µL of the tissue homogenate was added and labeled as test sample A1 (this process was carried out for each of the samples A2 to A4). A blank reagent was also prepared by mixing 40 µL of the prepared buffer into a separately labeled tube. The mixture was homogenized by gently swirling and allowed to stand at room temperature for 2 minutes.

Approximately 40 µL of prepared hematoxylin was added to both the test samples and the reagent blank test tubes, which were quickly mixed to initiate the auto-oxidation reaction. Spectrometric absorbance was measured at 560 nm ten times over 5 minutes at regular intervals (every 30 seconds) against distilled water, with the reagent blank also being read. SOD activity was estimated by measuring the ratios of the auto-oxidation rates with and without the sample. The superoxide dismutase activity was calculated using:

$$\text{Absorbance}_{\text{Reagent test}}(A_R) = \text{Absorbance}_{\text{Reagent test 2}} - \text{Absorbance}_{\text{Reagent test 1}} \text{Absorbance}_{\text{Sample test}}(A_S) = \text{Absorbance}_{\text{Sample test 2}} - \text{Absorbance}_{\text{Sample test 1}}$$

$$\%\ \text{SOD inhibition} = \left(1 - \frac{A_S}{A_R}\right) \times 100$$

$$\text{SOD activity (U/ml)} = \left(1 - \frac{A_S}{A_R}\right) \times 100 \times 1.25$$

H. Determination of Catalase (CAT) Activities

Catalase activity was estimated using a method reported by Aebi [28]. A 50 mM potassium phosphate buffer, pH 7.0, was prepared as the working buffer, and 1000 µL was added to a cuvette to standardize the spectrophotometer at 240 nm. Subsequently, 950 µL of the working buffer mixture and 460 µL of 30 mM hydrogen peroxide (H_2O_2), along with 50 µL of the tissue homogenate, were measured at 240 nm every minute for 5 minutes. Catalase activity was determined and expressed as (U/mL) of the decomposition rate, represented as (ΔA240 nm/min) of the sample.

ΔA240 nm/min = Change in absorbance per minute
Catalase (U/mL) = (ΔA240 nm/min)/Volume of reaction mixture

I. Liver Biochemistry Assessment

Spectrophotometric determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total protein (TP), and albumin (ALB) was performed using their respective kits (products of Randox liver function assay). The procedures were carried out according to the manufacturer's instructions.

III. RESULTS OF THE STUDY

Results are presented as the mean ± standard deviation of group results (n = 5). Mean values ± standard deviation in the same column with different letters of the alphabet as superscripts indicate a statistically significant difference ($p < 0.05$).

TABLE II EFFECTS OF EXTRACT ON LIVER MARKER ENZYMES

Group	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)
1	30.03±4.09^{a}	2.75±0.35^{a}	3.01±1.04^{a}	134.10±6.99^{a}
2	50.61±9.00^{c}	15.04±2.09^{c}	10.47±1.27^{c}	336.05±29.71^{b}
3	33.39±2.06^{a,b}	7.71±1.94^{b}	4.63±3.47^{a,b}	173.80±11.41^{a}
4	46.34±12.95^{b,c}	8.67±2.67^{b}	6.48±1.94^{b}	147.95±89.42^{a}

The results for liver biomarkers in Table II show that AST, ALT, ALP, and GGT significantly increased in the second group compared to the normal group ($p < 0.05$). There was a statistically significant decrease in AST in group 3 compared to group 2 ($p < 0.05$).

The disparity in ALT levels was considerably lower in groups 3 and 4 compared to group 2, although still significantly higher than in group 1. Furthermore, GGT values for group 4 were significantly higher than in group 1.

TABLE III EFFECTS OF EXTRACT ON TOTAL PROTEIN AND ALBUMIN

Group	TP (g/dL)	Albumin (g/dL)
1	3.86±0.18^{a}	3.13±0.15^{c}
2	3.31±0.77^{a}	1.05±0.19^{a}
3	3.50±1.23^{a}	2.31±0.73^{b,c}
4	4.46±1.31^{a}	2.05±1.65^{b}

The group results obtained are presented as mean ± standard deviation (n = 5). Mean values ± standard deviation in the same column with different letters of the alphabet as superscripts indicate a statistically significant difference ($p < 0.05$).

The results for total protein and albumin in Table III show that protein levels did not change significantly across all groups, although there was a mild increase in group 4 ($p < 0.05$). A significant decrease in albumin levels was observed in group 2 compared to group 1 ($p < 0.05$). A significant increase in albumin was observed in groups 3 and 4 ($p < 0.05$). The change in albumin for group 4 was significantly less compared to group 1 ($p < 0.05$).

TABLE IV EFFECTS OF EXTRACTS ON *IN VIVO* ANTIOXIDANT STATUS OF THE LIVER OF WISTAR RATS

Groups	MDA (µM)	SOD (U/mL)	CAT (U/mg)
1	1.94 ± 0.41^{a}	31.45 ± 6.34^{b}	12.18 ± 4.53^{b}
2	3.00 ± 0.17^{c}	19.68 ± 9.88^{a}	3.09 ± 0.50^{a}
3	2.33 ± 0.06^{b}	$27.57\pm12.03^{a,b}$	4.00 ± 0.33^{a}
4	2.38 ± 0.03^{b}	$26.84\pm7.81^{a,b}$	3.58 ± 0.50^{a}

Results are expressed as mean ± standard deviation of the group results obtained (n = 5). Mean values ± standard deviation in the same column with different letters of the alphabet as superscripts indicate a statistically significant difference ($p < 0.05$). Group 1 results for MDA in Table IV show a statistically significant increase in the first group compared to the first group ($p < 0.05$). However, the results also show a significant decrease in the treated group compared to the second group ($p < 0.05$). Additionally, the table shows a notable decrease in the levels of SOD in animals in the second group compared to group 1 experimental subjects ($p < 0.05$). Furthermore, the differences in SOD levels in other groups show no statistically significant difference compared to both groups 1 and 2. Moreover, the results for CAT reveal a considerable decrease in the second group compared to group 1 ($p < 0.05$). There is also a significant drop in the values in all treated groups relative to the first group, although the increase in various treated groups relative to the second group is not significant ($p < 0.05$).

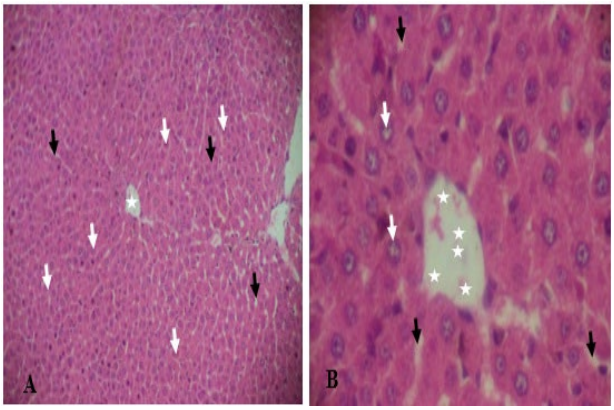

Fig. 1 (Plate 1/Group 1)

The liver of the experimental subjects exposed to normal environmental and nutritional conditions displays normal hepatocyte morphology, characterized by their typical polygonal shape and intact nuclei. The hepatocytes are interspersed by hepatic sinusoids (black arrows), which are connected to the central vein (white stars). H&E: A: X100, B: X400.

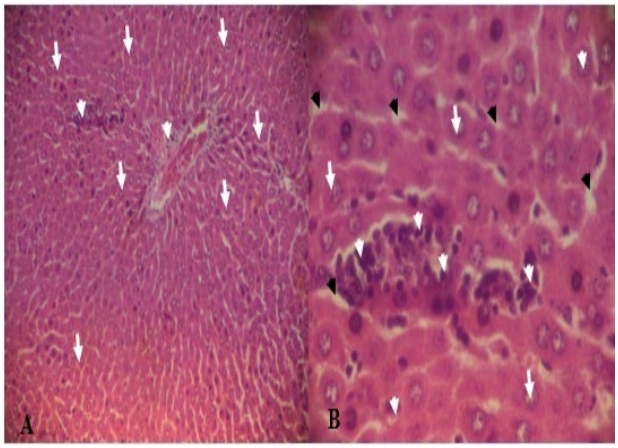

Fig. 2 (Plate 2/Group 2)

The histological micrograph of the group 2 animal reveals mild inflammation, indicated by the presence of inflammatory cells. Elevated serum enzyme activities suggest cellular leakage and a loss of functional integrity in the liver cell membrane. The hepatocytes are morphologically normal, with intact nuclei (white arrows), and are interspersed by hepatic sinusoids (black arrowheads). H&E: A: X100, B: X400.

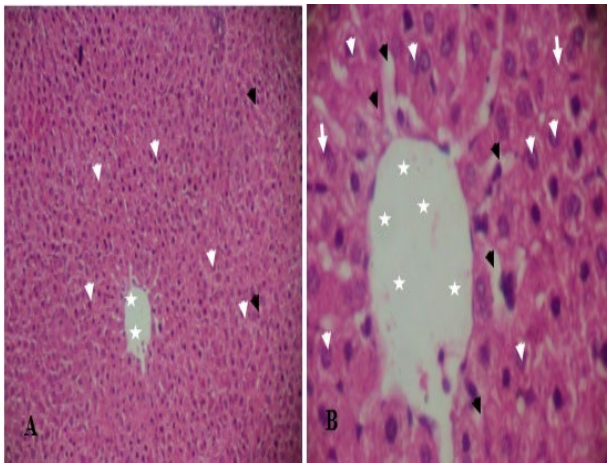

Fig. 3 (Plate 3/Group 3)

The liver of the albino rat induced with cadmium sulfate and treated with *P. americana* seed extract appears to help maintain hepatocyte integrity, as evidenced by the normal polygonal shape and intact nuclei of the cells (black arrowheads), which are linked to the central vein (white stars). H&E: A: X100, B: X400.

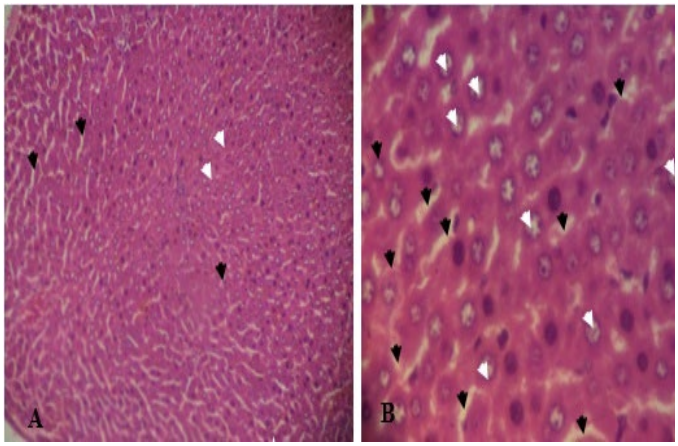

Fig. 4 (Plate 4/Group 4)

The liver of the albino rat induced with cadmium sulfate and treated with silymarin shows normal morphology, with hepatocytes displaying their characteristic polygonal shapes and intact nuclei (white arrowheads), interspersed by hepatic sinusoids (black arrowheads). H&E: A: X100, B: X400.

Group 1: Normal Control
Group 2: Positive control (induced with cadmium, 20 mg/kg body weight)
Group 3: Induced with cadmium (20 mg/kg body weight) and treated with seed extract of P. americana (400 mg/kg body weight)
Group 4: Induced with cadmium (20 mg/kg body weight) and treated with silymarin (100 mg/kg body weight)

IV. DISCUSSION

It is widely understood in biological systems that heavy metals, such as cadmium, have adverse effects on vital organelles of the cell, including the cell membrane, endoplasmic reticulum, mitochondria, lysosomes, and various important organic biocatalysts (enzymes) that function in metabolism, detoxification, and damage repair, as reported by [29]. The results in Table II support this assertion, as the values for AST, ALT, ALP, and GGT in Group 2 are significantly higher relative to Group 1 (normal control group) and other treated groups ($p < 0.05$). This is also observed in the histological conditions of the various experimental subjects, as shown in the histological micrograph of the liver. The liver of the Group 1 animal depicted in Figure 1 shows normal hepatocyte morphology, characterized by typical polygonal shapes and intact nuclei. In contrast, the histological micrograph of the Group 2 animal in Figure 2 reveals mild inflammation, indicated by the presence of inflammatory cells. Elevated serum enzyme activities suggest cellular leakage and a loss of functional integrity in the liver cell membrane [30][3].

Increased serum levels of ALT, AST, ALP, and GGT may indicate potential hepatic injury or damage [31]. This correlation with hepatic enzymes supports the likelihood of liver issues. While high blood ALP levels can result from an increase in liver production of this enzyme [32] or coronary artery disease, as noted by Johnson *et al.,* [33] and Schoppet and Shanahan [34], this is due to their role in promoting vascular calcification via the pyrophosphate pathway. However, when comparing Group 2 with Groups 3 and 4, the opposite trend was observed for AST ($p < 0.05$). Additionally, significantly decreased levels of ALT in Groups 3 and 4 were observed relative to the positive control group. This suggests that the treatments administered to Groups 3 and 4 may have mitigated liver damage, as evidenced by the lower enzyme levels. Elevated serum enzyme activities typically indicate cellular leakage and compromised liver cell membrane integrity, reinforcing th e potential for liver injury.

The histological analysis further supports these findings, showing normal liver morphology in the control group (Group 1) and mild inflammation in Group 2, characterized by the presence of inflammatory cells. This inflammation is consistent with the elevated enzyme levels observed, highlighting the impact of the treatments on liver health. The significant differences in enzyme levels between the groups underscore the importance of monitoring these biomarkers to assess liver function and the effectiveness of therapeutic interventions. Overall, the data suggest that while elevated enzyme levels can indicate liver damage, appropriate treatments can reduce these levels and improve liver health, as demonstrated by the results in Groups 3 and 4.

The seed extract of *Persea americana* has been shown to significantly lower serum ALP levels in rats with acetaminophen-induced liver insufficiency [35], aligning with the findings presented in Table II. Elevated ALP levels can indicate liver damage but may also result from dysfunction in other organs such as the bile duct and bones, or conditions like cancer and myocardial infarction [36][37]. The study also observed a significant reduction in the levels of GGT in the treated group relative to the positive control group, as shown in Table 2. The seed extract appears to help maintain hepatocyte integrity, as evidenced by the normal polygonal shape and pyknotic nuclei of the cells in Figure 3. Additionally, Figure 4 illustrates the liver histology of Group 4, which appears morphologically normal with hepatocytes displaying their distinctive polygonal shapes and intact nuclei, interspersed by hepatic sinusoids. This suggests that the seed extract not only reduces enzyme levels but also supports the structural integrity of liver cells, potentially offering a protective effect against liver damage.

The findings in Table III indicate no significant differences in total protein levels across the groups, consistent with Ogbonnaya *et al.,* [35]. However, albumin levels in Group 2 are significantly lower compared to Group 1 ($p < 0.05$). The data also show a considerable rise in albumin levels in the treated group (Group 3). This suggests that while *Persea americana* extracts may not significantly affect total protein levels, they can significantly improve albumin levels

($p < 0.05$). The liver produces many proteins, and hepatic utility is often assessed by evaluating serum total protein, albumin, and globulin levels [38]. Low total protein, or hypoproteinemia, can result from persistent malnutrition, malabsorption, or protein loss due to kidney or liver disease. This highlights the importance of monitoring these biomarkers to evaluate liver health and the effectiveness of treatments [39].

Table IV illustrates the impact of treatments on MDA levels, a marker of oxidative stress in cadmium-induced liver toxicity in rats. MDA, a byproduct of lipid peroxidation, indicates cellular damage, making its measurement crucial in chronic diseases [40]. The data show that Group 2 has significantly higher MDA levels compared to the treated and normal groups ($p < 0.05$), suggesting elevated lipid peroxidation. The treated groups exhibit significantly lower MDA levels than Group 2 ($p < 0.05$), indicating that the extracts may reduce lipid peroxidation in the liver, likely due to certain phytochemicals.

Yakubu *et al.,* [41] reported that *Persea americana* seeds contain quercetin and chlorogenic acid, potent antioxidants that inhibit free radical activity. Various secondary metabolites are important in biological functions, including anticancer, pharmacological effects, antioxidant, neurological, cardiovascular, antimicrobial, anti-inflammatory, hepatoprotective, reproductive system protective, and anti-obesity effects, all of which are associated with quercetin [42][41]. This suggests that the seed extracts could effectively mitigate oxidative stress and protect liver cells from damage.

Table IV shows a significant difference in SOD levels between Groups 1 and 2 ($p < 0.05$), with Group 2 exhibiting lower levels than Group 1. Superoxide dismutases (SODs) are metalloenzymes present across all life forms, acting as an essential defense, the first line of defense against ROS-induced damage [43]. The enzymes are associated with the conversion of molecular oxygen superoxide anion radicals (O2-) into molecular oxygen and hydrogen peroxide (H2O2), thereby reducing the harmful concentration of O2- that can damage cells [44][45]. This process involves the alternate oxidation and reduction of metal ions at the active site of SODs [46][47][45]. The significant drop in SOD levels in Group 2 suggests a reduced capacity to neutralize ROS, potentially leading to increased cellular damage.

As the body ages, natural levels of superoxide dismutase (SOD) decrease, making individuals more susceptible to diseases related to oxidative stress [48]. This decline in SOD levels increases vulnerability to oxidative damage caused by free radicals. The results indicate that animal groups with lower SOD levels experience higher oxidative stress, leading to increased lipid peroxidation, compared to groups with higher SOD levels. This suggests that maintaining higher SOD levels can help mitigate oxidative stress and its associated cellular damage.

The results in Table IV show that the normal group has the highest catalase (CAT) levels, significantly different from the other groups ($p < 0.05$). Catalase is an enzyme that decomposes H2O2, carrying out an important biological function in the defense of cells from oxidative stress. It neutralizes hydrogen peroxide (H2O2), preventing cellular damage. Catalase activity is a common test to assess the cell's antioxidant defense system [49]. The findings suggest that catalase activity significantly decreased in the hepatocytes of both the induced and treated animal groups, indicating a reduced ability to counteract oxidative stress in these groups. Phenols and flavonoids have the potential to protect against liver injury [50], as suggested in this study.

V. CONCLUSION

This research showed that cadmium can induce toxicity to the liver, as demonstrated by both the tables and the figures. However, the devastating effects of the heavy metal (cadmium) were greatly reduced in the experimental subjects treated with the plant extract used in this study. This is likely due to the availability of certain phytochemicals and secondary metabolites inherent in this plant material. The outcome of the research indicates that the plant extract has the potential to protect the liver from the harmful and toxic effects of cadmium.

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