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Effect of *Diospyrus mespiliformis* Hochst (Ebenaceae) Stem Bark Extract on Some Biochemical and Hematological Parameters in CCl_4 Induced Oxidative Stress in Albino Mice

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ABSTRACT

Diospyros mesopiliformis is a species of the family Ebenaceae. In Nigeria, the stem-bark mixture is utilized in ethno-medical practice to treat and manage a variety of illnesses. The objective of this work was to identify the bioactive compounds and assess the potential protective effectiveness of Diospyrus mespiliformis stem-bark extracts against CCl_4 induced oxidative stress in albino mice. Fifty mice were split into five groups and given various treatments for 28 days. Olive oil and 1% tween 80 orally were administered intraperitoneally to the control group. After injecting CCl_4 into other four groups to cause oxidative stress, two of the groups were treated with Diospyrus mespiliformis stem-bark extract at 200 and 400 mg/kg bwt, one group received silymarin treatment at a dosage of 100 mg/kg bwt. and the last group was not given any treatment. Biochemical and hematological markers, such as urea, creatinine, cholesterol, HDL, LDL, triglycerides, RBC, WBC, hemoglobin, PCV, and platelet levels, were assessed using blood samples. Using One-way ANOVA, the means of a these parameters were compared. Phytochemical screening led to the identification of alkaloid, flavonoid, saponins, steroids, tannins and cardiac glycosides. The findings of this investigation also indicate the administration of CCl_4 raises blood urea and creatinine levels. The administration of the extract, however, counteracts the effects of CCl_4 on creatinine and urea. Furthermore, the CCl_4 treatment resulted in a notable alteration in the levels of CHOL and HDL. When comparing the group treated with CCl_4 with the other treatment groups and the usual control, oral administration of Diospyros mespiliformis stem bark extract as well as revealed a significant difference in CHOL and HDL levels. Red blood cells (RBC), packed cell volume (PCV), white blood cells (WBC), and platelet counts (PLT) were found to be unchanged in response to CCl_4 administration, however, hemoglobin levels were significantly reduced. The levels of Red blood cell, white blood cell, packed cell volume, and platelet count were not significantly affected following the oral administration of Diospyros mespiliformis stem bark extract but hemoglobin levels were significantly raised in response to the extract and silymarin treatments. As evidenced by the reversal of CCl_4 impact on hemoglobin and renal parameters, the study showed that the plant extract had potential ameliorative efficacy.

Keywords: *Diospyros mesopiliformis*; Kidney parameters; Hematological parameters;Lipid profile; Albino mice.

INTRODUCTION

Diospyrus mespiliformis is a member of the Ebenaceae family. It is referred to as Igidudu in Yoruba and Kanya in Hausa. It is also known as African ebony or jackalberry. Savanna and northern lowland forests are home to it. This evergreen tree can grow to a height of 12 to 15 meters, but in a rain forest, it can occasionally reach 20 meters or higher. Simple alternating dark green leaves with tiny hairs on the underside of the older leaves. The shrub is diacious and produces flowers in April and May. In Africa, the fruit of this plant is a traditional cuisine that is rich in nutrients.

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The leaf extract is used to treat syphilis and fever and also serves as an antihelmintic as well as wound dressing agent (Ebbo et al., 2021).

Numerous human illnesses have been treated using *Diospyros mespiliformis*. There have been reports of strong antioxidant, anti-proliferative, and antibacterial properties in the plant extract (David et al., 2021). Must across the planet depend on the use of medicinal herbs in their daily lives. Since the beginning of time, medicine derived from plants has typically been used as the first line of treatment in rural areas. One common explanation for the widespread usage of herbal medicine is the lack of reasonably priced medical facilities for illness treatment. Africa has a wealth of medicinal plants, and Nigeria is one of the countries in the continent that regularly uses complementary and alternative medicine in addition to traditional belief systems to treat a variety of illnesses (Malami et al., 2020).

Pharmacological actions of *Diospyros mespiliformis* could be attributed to the phytochemical components it contains (Ebbo et al., 2021). Non-nutritive plant chemicals known as phytochemicals are made by plants to defend themselves against external stresses such as heat, cold, bacteria, fungi, and so on. They provide both preventive and therapeutic qualities for a variety of illnesses that affect both humans and animals. Plant roots, bark, fruits, and leaves are rich sources of phytochemicals. Since each of these phytochemicals has a therapeutic impact, a disease may be prevented or cured by using one phytochemical alone or in combination with the others found in the plant.

Carbon tetrachloride (CCl_4) has been shown to initiate lipid peroxidation, brain, kidney, heart, and testis impairment in additional to liver pathogenesis in animal models (Khan et al., 2012). The rapid release of cytokines and CCl_4 metabolites, which propagate the inflammatory response, makes the liver particularly vulnerable to oxidative stress (Khan et al., 2012). CCl_4 is one of the xenobiotics that has been shown to cause both acute and long-term tissue damage. Peroxy trichloromethyl radicals ($OOCCl_3$) and reactive metabolic trichloromethyl radicals (CCl_3) are produced when CCl_4 bioactivates the phase I cytochrome P450 system.

These free radicals may attach themselves covalently to proteins, lipids, and nucleic acids, among other macromolecules. Free radicals have the ability to break the doubly allylic hydrogen bonds found in polyunsaturated fatty acids (PUFA); exposure to CCl_4 increases the quantities of lipoperoxide and free peroxide radicals, which are very reactive and can result in damage or necrosis (Amin, 2021). This study evaluated the effect of Diospyrus mespiliformis stem bark extract on a few hematological and biochemical markers in CCl_4 induced oxidative stress in albino mice.

MATERIALS AND METHODS

Plant Material and Extract of Bioactive Compounds

A botanist at the Herbarium Unit of the Department of Biological Sciences, Gombe State University, Gombe State, Nigeria, collected and verified fresh stem bark of *Diospyrus mespiliformis*, confirming that it was similar to the voucher specimen No. 202 that had been previously deposited at the Herbarium. The stem bark of *Diospyrus mespiliformis* was allowed to air dry at room temperature under shade. A mortar and pestle were used to reduce the dried stem bark to a coarse powder. In one liter of aqueous ethanol, two hundred (200) grams of the powdered stem bark were mixed. The resultant decoction was then evaporated using a rotary evaporator

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after the mixture had been filtered using Whatman No. 1 filter paper. Until it was used, the powder was kept in the desiccator (Abubakar and Haque, 2021).

Phytochemical Screening

Primary and secondary metabolites in plant extracts are identified by phytochemical screening methods. Tannins, flavonoids, phenols, anthraquinone, coumarins, glycosides, steroids, and other phytochemical screens were assessed using Onwuatuegwu's (2017) methodology.

Experimental Animals

A total of fifty (50) albino mice were purchased from National Research Institute VOM, Plateau State and kept in Animal house, Department of Physiology, College of Medical Sciences, Gombe State University. The mice weighed between 25g to 30g. The mice were kept in a cage at room temperature with twelve hours nigh/dark cycle. They were allowed access to their feed which was (Vital feeds), Jos Nigeria and water in a hygienic environment.

Treatment Induce in Experimental Mice

Fifty albino mice (n = 50) in total were randomly assigned to five groups (n = 10), and the following treatments were carried out over the course of four (4) weeks. Three times a week, 1% physiological saline was given orally to Group I, the normal control, and twice a week, 3 ml/kg body weight (BW) of olive oil was given intraperitoneally (IP). Twice a week, IP injections of a CCl4 and olive oil (1:1 v/v) combination at a dosage of 0.5 ml/kg BW were administered to Group II (CCl4), the hepatotoxic group.

Group III (Silymarin Si+ CCl4) received twice-weekly IP injections of a combination of 0.5 ml/kg BW of CCl4 and olive oil (1:1 v/v) in addition to daily treatment with silymarin intragastrically at a dosage of 100 mg/kg BW for five days a week. Group IV received an IP injection of a combination of CCl4 and olive oil (1:1 v/v) twice a week at a dosage of 0.5 ml/kg BW, after a daily oral treatment of 200 mg/kg BW. Group V received an IP injection of CCl4 and olive oil (1:1 v/v) combination twice a week at a dosage of 0.5 ml/kg BW, following a daily oral treatment with the extract at a dose of 400 mg/kg BW.

Blood Sampling

Blood was collected under anaesthesia with chloroform from the neck of the rats and into clean plain containers after twenty-eight days (28) days of administration of the extract powder.

Determination of Hematological Parameters

The automated hematology analyzer (Sysmex KX-21NTM) was used to measure every hematological index, including Hemoglobin (Hb) concentration, platelet count (PLT), red blood cells (RBCs), packed cell volume (PCV) and white blood cells (WBCs).

Biochemical Estimation of Kidney

The ChemRay 240 Semiautomated Chemistry Analyzer was employed to ascertain the serum creatinine and urea levels.

Serum Lipid Profile Assay

Using a semiautomated chemistry analyzer, the ChemRay 240, the blood samples were evaluated for total cholesterol, HDL, LDL, and triglycerides as part of the serum lipid profile test.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 20 was utilized to perform a one-way analysis of variance (ANOVA) test at $P < 0.05$ to see if there were any significant differences between the groups.

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RESULTS

The results of the phytochemical screening test for the *Diospyrus mespiliformis* stem bark ethanolic extract were displayed in Table 1 which indicated the existence of tannins, cardiac glycosides, steroids, alkaloids, and flavonoids.

Table 1: Phytochemical screening test for the stem bark ethanolic extract of *Diospyrus mespiliformis.*

Phytochemical group	*Diospyrus mespiliformis*
Alkaloid	+
Flavonoid	+
Saponins	+
Steroids	+
Tannins	+
Anthraquinone	_
Cardiac glycosides	+

Key: + = Present, - = Absent

The results of renal indicators, such as serum urea and creatinine, are displayed in Table 2. Table 2 showed that the normal control groups, who received just water and normal diet, had the lowest concentration of serum urea and serum creatinine, while the toxic control groups, who received CCl4 at a dose of 0.5 ml/kg body weight, had the highest levels.

Based on the findings, the group that received CCl4 treatment had considerably greater levels of creatinine and urea than the normal control group. Additionally, there was a noticeable difference in the levels of urea and creatinine between the groups treated with the extract and silymarin and the groups treated with CCl4 as well as the normal control group.

Table 2: Effect of oral administration of the stem bark ethanolic extract of *Diospyrus mespiliformis* on Serum Urea and Creatinine.

GROUPS	UREA (mmol/L)	CREATININE (mmol/L)
Normal Control	12.88 ± 0.89^{a}	28.60 ± 0.12^{a}
Toxic Control (CCl_4)	21.24 ± 0.83^{b}	39.80 ± 0.55^{b}
Silymarin + CCl_4	14.96 ± 0.11^{c}	33.40 ± 030^{b}
CCl_4 + 200 mg/kg bwt	15.06 ± 0.49^{c}	31.20 ± 0.10^{b}
CCl_4 + 400 mg/kg bwt	15.20 ± 0.19^{c}	34.00 ± 0.60^{b}

The results of the lipid profile, which includes cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (TG), are displayed in Table 3.

According to Table 3, the group administered an extract weighing 200 mg/kg body weight had the highest level of CHOL, whereas the normal control group that received standard meal and water had the lowest concentration. Additionally, table 3 shows that the normal control group, which received water and a regular diet, had highest blood HDL levels whereas the toxic control group, which received 0.5 ml/kg body weight of CCl_4 had the lowest concentration. Additionally, the LDL concentration was greatest in the group treated with 400 mg/kg body weight extract, whereas the HDL level was lowest in the group treated with 100 mg/kg body weight silymarin. The blood level of TG was greatest in the group treated with 400 mg/kg body weight extract, and lowest in the group treated with 0.5 ml/kg body CCl4. The groups' LDL and TG readings did not differ significantly from those of the normal control group. There was a discernible difference in CHOL levels between the CCl4 treated group and the other treatment groups and the normal control group. Additionally, when comparing the HDL values of the CCl4-treated group to those of the other treatment groups, there was a significant difference.

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Table 3: Effect of oral administration of the stem bark ethanolic extract of *Diospyrus mespiliformis* on Lipid profile.

GROUPS	CHOL (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TG (mmol/L)
Normal Control	4.98 ± 0.03[a]	6.37 ± 0.55[a]	3.73 ± 0.50[a]	1.30 ± 0.02[a]
Toxic Control (CCl_4)	11.02 ± 0.06[b]	3.38 ± 0.21[b]	3.83 ± 0.21[a]	1.19 ± 0.04[a]
Silymarin + CCl_4	6.31 ± 0.11[a]	5.30 ± 0.55[b]	3.61 ± 0.30[a]	1.28 ± 0.23[a]
CCl_4 + 200 mg/kg bwt	8.08 ± 0.43[a]	4.22 ± 0.42[b]	3.90 ± 0.55[a]	1.24 ± 0.03[a]
CCl_4 + 400 mg/kg bwt	6.21 ± 0.90[a]	4.68 ± 0.83[b]	3.95 ± 0.83[a]	1.46 ± 0.01[a]

Table 4 displays hematological parameters, including packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), hemoglobin (HBG), and platelets (PLT). As can be shown in Table 4, the group that received 200 mg/kg body weight extract had the greatest concentration of PCV, whereas the group that received 0.5 ml/kg body weight of CCl4 had the lowest. RBC, WBC, and HBG levels were higher in the groups treated with 400 mg/kg body weight extract; in contrast, RBC and HBG concentrations were lowest in the toxic control group, which received 0.5 ml/kg body weight of CCl4, and WBC concentrations were lowest in the group treated with 200 mg/kg body weight extract. Furthermore, as shown in Table 4, the group that received 400 mg/kg body weight extract had higher levels of platelets and the toxic control group, which received 0.5 ml/kg body weight of CCl_4 got the lowest level.

Table 4: Effect of oral administration of the stem bark ethanolic extract of *Diospyrus mespiliformis* on Hematological parameters.

GROUPS	PCV (%)	RBC (mmol/L)	WBC (mmol/L)	HBG (mmol/L)	PLT (mmol/L)
Normal Control	32.00±0.68[a]	35.80±4.55[a]	36.20±4.55[a]	56.19±0.52[a]	15.88 ± 0.89[a]
Toxic Control (CCl_4)	30.00±0.80[a]	33.82±3.21[a]	34.80±3.21[a]	42.43±0.27[b]	15.24 ± 0.83[a]
Silymarin + CCl_4	31.00±0.26[a]	34.40±2.30[a]	35.30±2.30[a]	56.31±0.46[a]	15.64 ± 2.11[a]
CCl_4 + 200 mg/kg bwt	33.00±0.68[a]	34.20±4.55[a]	34.50±4.55[a]	55.85±0.13[a]	16.46 ± 1.49[b]
CCl_4 + 400 mg/kg bwt	31.00±0.42[a]	35.80±6.83[a]	36.70±6.83[a]	57.07±0.81[a]	16.20 ± 1.19[a]

DISCUSSION

Phytochemical screening results revealed the presence of cardiac glycosides, alkaloids, flavonoids, saponins, steroids, and tannins (Adamu, 2020). Due to the physiological and pharmacological properties of these active phytochemicals, all medicinal plants have the potential to be therapeutically active. It has been observed that tannins, saponins, and alkaloids prevent bacterial development and shield plants against fungal diseases (Abba et al., 2016). In hepatotoxicity investigations using animal models, carbon tetrachloride (CCl4) is a useful substance. When CCl_4 is administered, lipid peroxidation and reactive oxygen species increase, resulting in oxidative damage (Hesham, 2019). Reactive oxygen species—which are produced in excess or at an increased rate—and the body's inability to eliminate them from the body lead to an imbalance that results in oxidative stress (Tathe et al., 2022).

In this investigation, the curative action of the extract against CCl4-induced toxicity was assessed in adult albino mice. In contrast to the normal control, CCl4 administration to mice via intraperitoneal (IP) injection three times a week for 28 days resulted in hepatocellular damage and elevated blood urea and creatinine. *Diospyrus mespiliformis* stem-bark extracts, however, reversed these changes by bringing serum creatinine and urea levels

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back into a close physiological range. The plant extracts' ameliorative effects are similar to those of silymarin, which was given to mice at a dosage of 100 mg/kg body weight. It's possible that the plant extract's protective properties stopped the harm that CCl4's administration produced. This is consistent with (MohammeDontos, 2017), who found that when extract is given, clinical indicators recover to normal levels and exhibit healing effects.

Comparing each group LDL and TG readings to those of the normal control group revealed no significant differences. Comparing the CCl4 treated group to the other treatment groups and the normal control group, however, revealed significant difference in CHOL levels. Additionally, when comparing the HDL values of the CCl4-treated group to those of the other treatment groups, there was a significant difference. The significant changes in the values of CHOL and HDL may be attributed to high proportion of phytosterols and phospholipids and significant contents of unsaturated fatty acids which is in agreement with the findings of Aremu et al., (2019). There was no significant change in PCV, RBC and WBC values across the groups when compared to the normal control group. However, there was a significant change in HBG values between the CCl_4 treated group when compared to normal control and other treatment groups. The extract may affect the hematopoiesis pathway without inducing a cellular inflammatory procedure, which might explain the alterations in HBG that was likely caused by the toxic action of CCl4. (Ebbo, et al., 2020).

CONCLUSION

In this study, phytochemical screening led to the identification of different phytochemicals found in the stem bark extract of Diospyros mespiliformis. Alkaloids, flavonoids, saponins, steroids, tannins, and cardiac glycosides are the different types of phytochemicals. As this study's results clearly show, administering CCl_4 causes blood urea and creatinine to rise. On the other hand, the extract administration reverses the effect of CCl_4 on creatinine and urea. This suggests that the creatinine and urea markers may be moderated by the plant extract. Oral administration of *Diospyros mespiliformis* stem bark extract showed a significant change in CHOL values between the CCl_4 treated group when compared to normal control and other treatment groups. Also, there was a significant change in HDL values between the CCl_4 treated group and other treatment groups in contrast to normal control. The significant changes in the values of CHOL and HDL may be due to the unsaturated fatty acids and notable amounts of phytosterols and phospholipids that may be present in the extract. The oral administration of *Diospyros mespiliformis* stem bark extract did not cause significant variations in the red blood cells, white blood cells, and platelet counts as well as the packed cell volume. However, a significant change was observed in HBG values in the group treated with CCl4 in contrast to the normal control and other treatment groups. The alterations in HBG could result from CCl_4 toxic effects, indicating that the extract may affect the hematopoiesis pathway without inducing a cellular inflammatory response.

Statement of ethical approval

The animal care and use research ethics committee at Gombe State University in Nigeria approved and provided ethical approval for this work.

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