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Effect of methanol extract of *Cajanus cajan* on lipid peroxidation in wistar albino mice

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ABSTRACT

Aim: This study was aimed to assess influence of Cajanus cajan's methanol extract (MECC) on lipid peroxidation.

Method and Materials: Eighteen Wistar albino mice (25 to 30g) were divided into three groups. The control group received distilled water, while second and third groups were orally administered 100 mg and 200 mg/kg bwt of MECC respectively, over 14 days. Control group MDA levels were measured at 13.02 ± 2.50 gmol/g. The second group exhibited levels of 3.99 ± 0.00 gmol/g, and the third group measured 4.19 ± 0.12 gmol/g.

Results: Animals treated with MECC displayed significantly reduced MDA levels compared to the control group. Histopathological analysis revealed no significant lesions in the control animals but indicated centrilobular inflammation in the MECC-administered group.

Conclusion: It was concluded that presence of bioactive agents in MECC seeds that contributed to lowered MDA levels in treated animals. Moreover, these bioactive agents appeared to induce centrilobular inflammation in the treated animals.

Keywords: Medicinal Plants, *Cajanus cajan,* Wistar albino mice, lipid peroxidation, Histopathological, malondialdehyde (MDA).

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Introduction

Throughout history, medicinal plants have provided diverse chemical compounds that serve vital biological functions and defense mechanisms for plants. These secondary metabolites, although fewer than 10% of the potential total, can influence human physiology similar to pharmaceutical drugs (Lai and Roy, 2004 and Fabricant and Farnsworth, 2001). Herbal remedies are cost-effective alternatives, particularly in non-industrialized societies. While more prevalent in Asia and Africa, their usage is rising in the West due to growing scientific support (Billing and Sherman, 1998). Plants naturally produce primary metabolites like sugars and fats, and fewer species produce secondary metabolites for specialized roles, (Springer Netherlands Jacquart, 2008).

Cajanus cajan, a versatile plant, serves various purposes, including traditional medicinal uses for conditions like diabetes, skin issues, and dysentery. Chemical analyses have identified specific compounds like globulins in the plant (Ahsan and Islam, 2009). Lipid peroxidation, the oxidative breakdown of lipids, is a significant process that damages cell membranes through free radical reactions (Taylor et al., 2007).

Cajanus cajan has been studied for its traditional uses and modern applications. It exhibits antimicrobial effects against various strains, (Zu et al., 2010). Compounds from its leaves show antibacterial properties (Kunar et al., 2014). Stilbenes from Cajanus cajan might have cholesterol-modulating effects, (Luo et al., 2008). Its methanolic leaf extract demonstrates antidiabetic potential (Jaiswal et al., 2008). Pinostrobin from the plant influences brain cells, Nicholson et al. (2010). Various extracts and compounds exhibit antioxidant properties, (Pal et al., 2010; Wu, 2005). Cajanol from its roots has anticancer activity (Luo et al., 2008). Methanol extracts of Cajanus cajan protect the liver, (Ahsan et al., 2009). Its extracts display anthelmintic properties (Pal et al., 2008; Pal et al., 2010). The

plant showcases a range of pharmacological actions supported by traditional use and scientific research

Materials and Methods

Male albino mice, each weighing between 25 to 35 g of body weight, were sourced from the Preclinical Animal House at Lead City University of Ibadan, Nigeria. These mice were acclimatized within cages at the Department of Biochemistry's Animal House at Lead City University of Ibadan, Nigeria. They received both water and feed and were maintained under controlled conditions of temperature with 12-hour cycles of darkness and light. Cajanus cajan, commonly known as pigeon pea, in the form of seeds, was procured from the Oje market in Ibadan, Oyo state, Nigeria.

Extraction Process: The Cajanus cajan seeds were ground and submerged in methanol within a large, air-covered flask for a span of 5 days. The resulting mixture was subsequently filtered using sterile Whatman No. 1 filter paper. The concentrated, thick brown methanol extract was obtained through a Rotary evaporator. The crude extract underwent further concentration through evaporation to dryness, utilizing a water bath set at 37°C for approximately one week. The extraction process concludes with distillation, where the solvent mixture is separated from the methanol extract, which remains in the flask. The final extract is then weighed and transferred into pre-weighed bottles. Known amounts of the extract are mixed with specific volumes of distilled water and administered orally to the mice at doses of 100 mg and 200 mg per kg of body weight. The control group, on the other hand, received only regular feed and water.

The percentage yield of the extract was calculated using the formula below:

Weight of initial sample =
Weight of bottle + Extract = B
Weight of extract = (B-X) = Y
Percentage yield = (Yx 100)/ A)
% Yield = (weight of extract x 100)/ weight of dried plant material)

Organ Histology: Following sacrifice, the animals' livers were collected and preserved in 100% formalin. Subsequently, the collected organs underwent processing for histopathological examination.

Isolation of Mice Liver and Preparation of Post-Mitochondrial Fraction To begin the process, the liver was homogenized using a 5% Tris-KCL buffer. The resultant homogenate was then subjected to centrifugation for a duration of 15 minutes, with a rotational speed of 15,000 revolutions per minute (rpm). Following centrifugation, the resulting supernatants were carefully transferred to clean, plain bottles and stored for further analysis.

Reagents for Protein Determination

Biuret Reagent: 3g of copper sulphate (CuS04. 5H20) and 9g of Na-K tartarate were diss61ved in 500ml of 0.2M NaOH, 5g of potassium iodide was added and the solution made up to I liter of 0.2M NaOH.

Reagents for Assessment of Lipid Peroxidation: 30% Trichloroacetic Acid (TCA), 30g of trichloroacetic acid was dissolved in distilled water and up to 100ml, 0.75% Thiobarbituric Acid (TBA), 0.75g of TBA was dissolved in 100ml of 0.1M HCL. The dissolution was aided by shaking the volumetric flask in a boiling water bath., 0.15M Tris KCL Buffer, 1.12g of KCL was dissolved in 100ml of distilled water, and 2.36g of Tris was dissolved in 100ml of distilled water, the solutions (a) and (b) were then mixed together.

Protein Determination: The protein concentration of the post-mitochondrial fraction was determined utilizing the biuret reaction, following a modified protocol based on Gomal et al. (1949). In this modified method, potassium iodide was integrated into the biuret reagent to prevent the precipitation of copper ions as cuprous oxide. This approach centers on the interaction between copper ions and proteins under alkaline pH conditions. The biuret reagent comprises copper sulfate, potassium iodide, and sodium-potassium tartrate, which serves as a stabilizer for the reagent. The resultant complex formed between copper ions and proteins displays a characteristic blue hue with maximum absorbance at 540nm.

Experimental Procedure: A 0.2ml portion of the post-mitochondrial fraction was mixed with 1.8ml of distilled water, yielding a total volume of 2ml, to achieve an appropriate protein concentration level for the biuret method's sensitivity.

In duplicate, 2ml of the post-mitochondrial fraction was combined with 3ml of the biuret reagent. The mixture was incubated at room temperature for a duration of 30 minutes. Following incubation, the absorbance was measured at 540nm using a spectrophotometer.

To determine the protein concentration, the

absorbance values were multiplied by the dilution factor, providing the actual protein amount in the post-mitochondrial fraction.

Protein determination using the Biuret method

Reagents	Blank	Tests
Biuret Reagent	3 ml	3ml
Sample	-	2000µ1

Assessment of Lipid Peroxidation

Lipid peroxidation within the post-mitochondrial fraction was quantified spectrophotometrically through the thiobarbituric acid reactive substances (TBARS) method, following the procedure outlined by Varshmey and Kale. (1990). This method centers on the reaction between malondialdehyde (MDA), an end product of lipid peroxidation, and thiobarbituric acid (TBA). Under acidic conditions and upon heating, a pink-colored complex is formed as an aqueous product, exhibiting maximum absorption at 532nm, which is subsequently measured using a spectrophotometer. The obtained results reflect the quantity of free MDA products.

Experimental Procedure:

A 0.4ml portion of the liver sample was mixed with 1.6ml of 0.15M Tris-KCL buffer. To this mixture, 0.5 ml of 30% trichloroacetic acid (TCA) was added. Following this, 0.5 ml of 0.75% thiobarbituric acid (TBA) was introduced. The resulting solution was placed in a water bath and heated at 80°C for a duration of 45 minutes. After heating, the solution was cooled in an ice bath and subsequently subjected to centrifugation at room temperature for 10 minutes at a speed of 3000 rpm. The absorbance of the clear supernatant was then measured against a reference blank (distilled water) at a wavelength of 532nm, using a spectrophotometer.

Assessment of Lipid Peroxidation

Reagents	*Blan*	*Tests*
TrisKCL buffer (0.15M)	I .6ml	1.6ml
TCA (30%)	0.5ml	0.5ml
TBA (0.75%)	0.5ml	0.5ml
Samples	-	0.4ml

Calculation

Molar extinction coefficient (or molar absorbancy index for MDA) of 1.56 × 10--S N't-lCWl was used according to the expression of Adam-vizi and Seregi (1982). MDA (units/mg Protein) = DOD × Vx 1, Molar Absorbance index x v x mg Protein, DOD = Absorbance at 532nm, V = Total volume of reaction mixture, v = volume of sample (pmf in ml).

Results and Discussion

Histopathological results of control Group mice liver (Fig. 1), Group B mice liver (Fig. 2) and Group C mice Liver (Fig. 3) were presented.

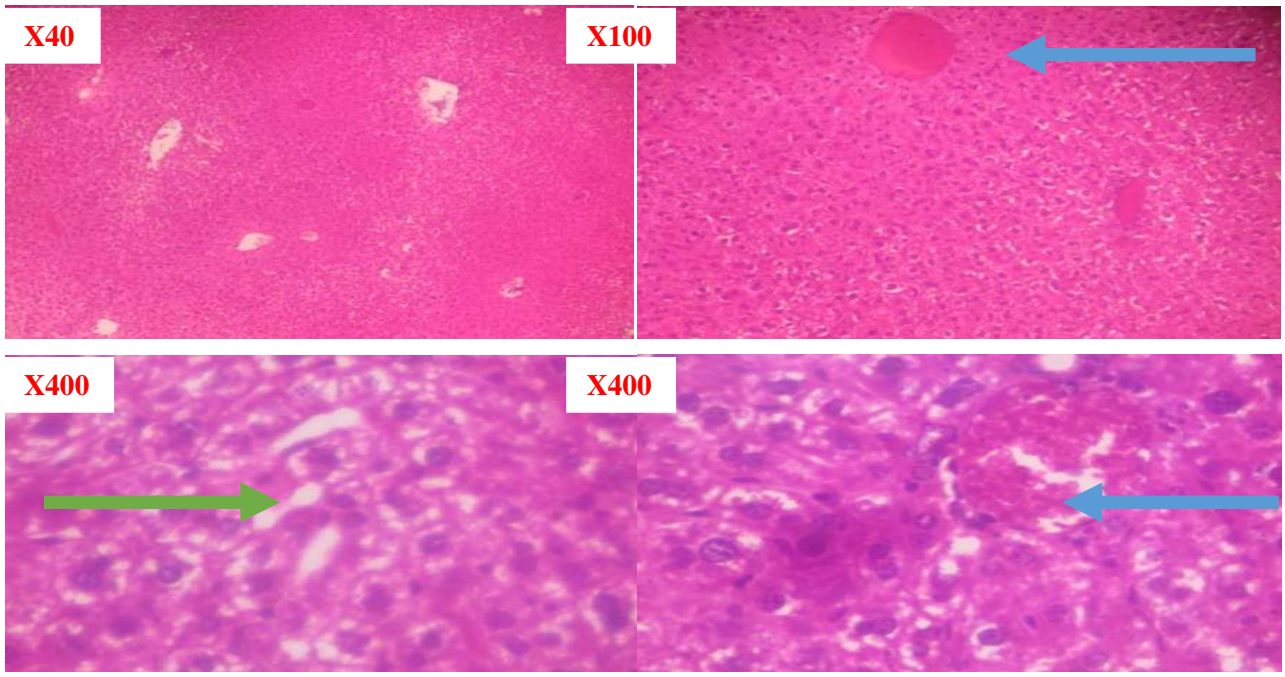

Fig 1: Plates show disseminated congestion of vessels (blue arrows) and multifocal area of mild steatosis (green arrows).

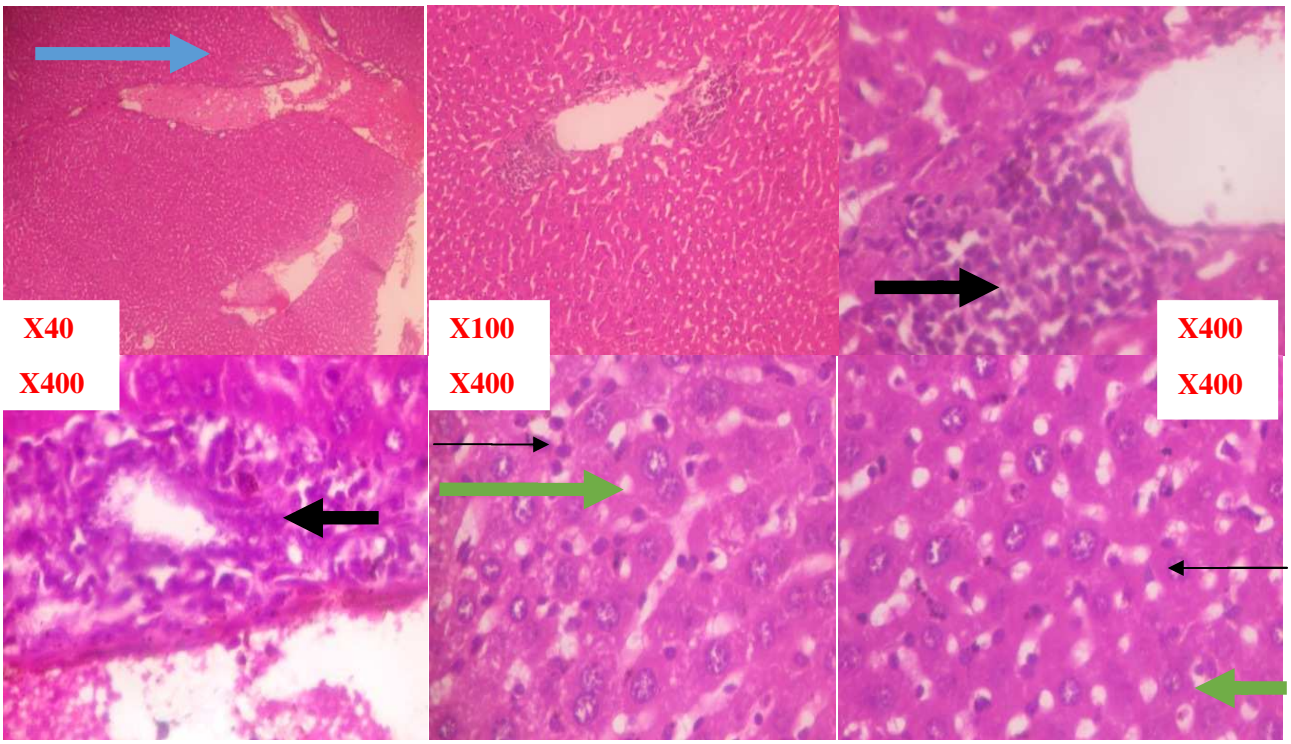

Fig 2: Plates show congestion of vessels (blue arrow), mild disseminated steatosis (green arrows), moderate centrilobular inflammation (black arrow), moderate periportal infiltration by inflammatory cells (black arrow) as well as infiltration of zone 2 (slender arrows)

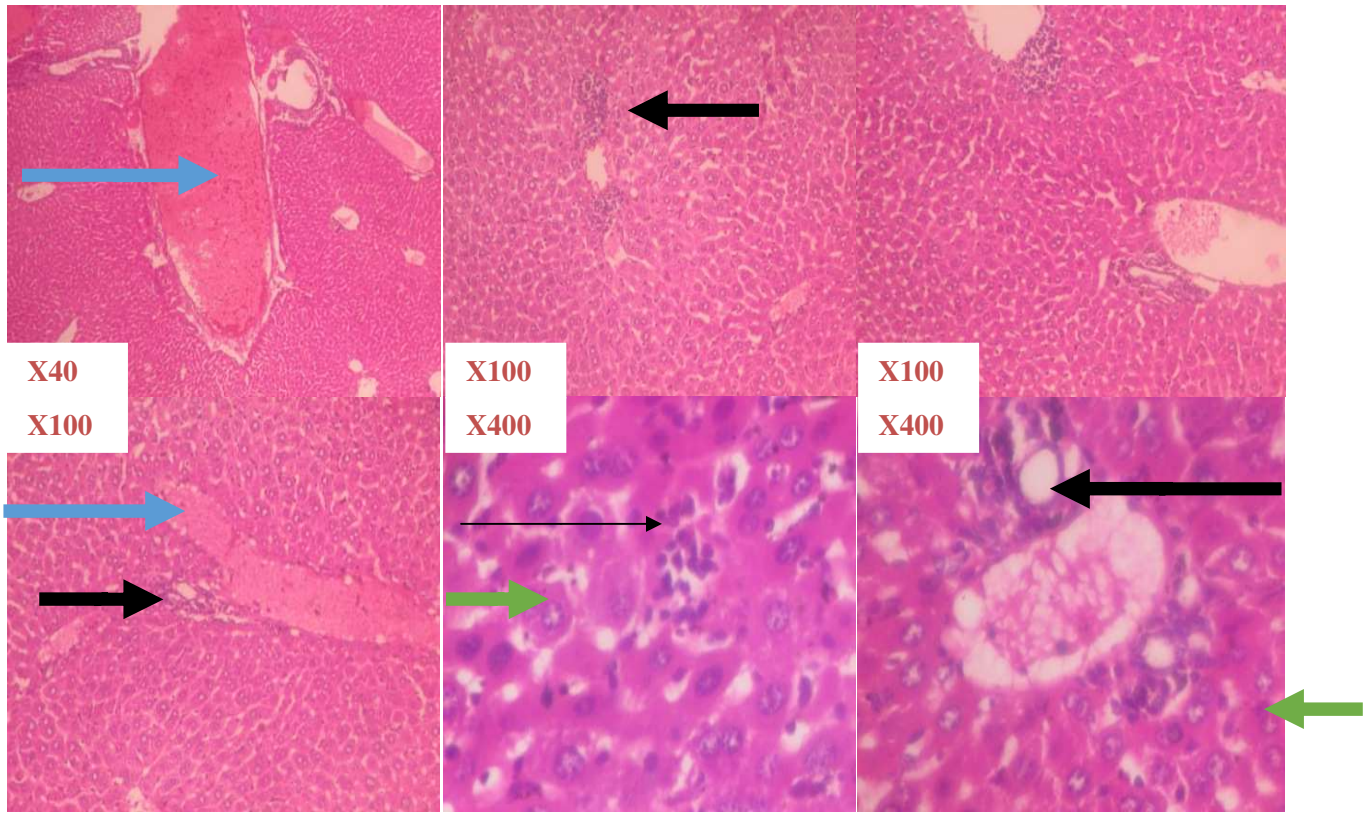

Fig 3: Plates show congestion of vessels (blue arrow), mild disseminated steatosis (green arrows), centrilobular inflammation (black arrow), periportal infiltration by inflammatory cells (black arrow) as well as infiltration of zone 2 (slender arrow).

Tissue Protein level of mice administered orally with methanol extract of C. cajan was presented (Table 1).

Table 1. The liver protein level of mice orally administered with methanol extract of C. cajan

Experimental Groups	Liver protein level (mg/ml)
Control	35.24±7.12
Group B	40.27±0.00
Group C	33.69±7.20

*= *significant when compared to control at p<0.05*

The liver protein concentration of various mice was assessed, with the groups orally administered the methanol extract of *C. cajan* displaying no significant increase or decrease in liver protein when compared to the control group at a significance level of $p<0.05$.

Lipid peroxidation (MDA) in the tissue of mice orally administered with methanol extract of C. cajan (Table 2).

Table 2. MDA level in the liver of mice administered orally with methanol extract of C. cajan

Experimental Groups	Liver MDA (µl/mol/g tissue)
Control	13.02±2.58
Group B	3.99±0.00*
Group C	4.19±0.12*

*= *significant when compared to control at p<0.05*

It was indicated that the MDA levels in the livers of mice that were orally administered the methanol extract of *Cajanus cajan* exhibited a noteworthy decrease when contrasted with the control group, achieving statistical significance at $p<0.05$.

Cajanus cajan, a medicinal plant with a rich history of use, has been a cornerstone of traditional remedies and has even contributed to the development of numerous pharmaceuticals in modern times. Throughout its historical use, Cajanus cajan has served a variety of purposes, including treating diabetes, skin irritations, menstrual irregularities, and even assisting in the expulsion of bladder stones (Zu et al., 2008). This plant material has also been utilized as a component of animal feed.

The extensive utilization of different parts of Cajanus cajan in various biological activities, backed by both traditional knowledge and experimental validation, underscores its significance. Beyond its traditional applications, contemporary scientific research has shed light on the diverse biological and pharmacological activities exhibited by Cajanus cajan.

The liver plays a pivotal role in the cardiovascular, digestive, excretory, and metabolic systems. Its functions encompass plasma protein synthesis, hormone production, detoxification, and participation in lipid metabolism, including cholesterol and lipogenesis. Oxidative stress has emerged as a crucial factor in the development and progression of numerous diseases, including diabetes, atherosclerosis, and acute and chronic liver injury(Clarke et al., 2005). Synthetic compounds often lead to toxicity through excessive production of reactive oxygen species (ROS) or a deficiency in cellular antioxidant systems. Antioxidants counteract oxidation in molecules, and certain antioxidant enzymes serve as liver biomarkers.

The present study investigated the impact of the methanol extract of Cajanus cajan beans on Wistar mice over a 14-day oral administration period. Phytochemical screening identified protein and amylase inhibitors in the extract, affecting digestive enzyme activity and leading to digestive losses (Zu et al., 2008). Lipid peroxidation and histopathological changes were employed to assess the extract's effect. It's important to note that indiscriminate administration of medicinal plants can induce toxicity in both animals and humans.

The study employed 18 adult albino mice, categorized into three groups, with Groups B and C receiving oral administrations of the methanol extract of *C. cajan* seed mixed with distilled water. The liver was then subjected to biochemical analysis to estimate tissue protein and malondialdehyde (MDA) levels, and a histopathological examination was conducted.

Lipid peroxidation entails the oxidative breakdown of lipids, culminating in the production of malondialdehyde (MDA) and 4-hydroxynonenal (HNE). Reactive oxidative stress (ROS) triggers lipid peroxidation, disrupting the lipid bilayer arrangement in cell membranes and affecting membrane-bound receptors, enzymes, and tissue permeability (Grene et al., 2004).

It was illustrated a significant reduction in MDA levels in the livers of mice from Groups B and C, administered the methanol extract of *Cajanus cajan* beans, compared to the control group ($p<0.05$) (Table 2). Meanwhile, it was demonstrated that the liver protein concentration in Groups B and C remains unaffected compared to the control group ($p<0.05$) (Table 1).

Histopathology, the microscopic examination of diseased tissues, aligned with the biochemical analysis results. Figure 1 depicts the

histopathological plate of the control group mice, revealing congestion of vessels and mild steatosis. Figures 2 and 3 display the histopathological plates of Groups B and C mice, respectively, demonstrating various levels of congestion, steatosis, centrilobular inflammation, periportal infiltration, and zone 2 infiltration.

Conclusion

It was concluded that this study sheds light on the potential toxicity of the methanol extract of *Cajanus cajan* beans. Despite its historical use and traditional knowledge, the study highlights the importance of comprehensive research on the potential adverse effects of medicinal plants. The 14-day oral administration of the extract resulted in decreased mouse numbers in Groups B and C, underscoring the need for cautious use.

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