Effects of Aqueous Extract of Pigeon Pea (*Cajanus cajan*) Seed on the Liver of Pregnant Wister Rats

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ABSTRACT

Cajanus cajan (pigeon pea) is widely consumed for its nutritional benefits, particularly by pregnant women in western Nigeria. The liver plays a crucial role in metabolism and detoxification, making it essential to assess the effects of *Cajanus cajan* on liver function during pregnancy. This study examined the impact of an aqueous extract of *Cajanus cajan* seed on the liver of pregnant Wistar rats. Fifteen rats were divided into three groups: a control group and two treatment groups receiving 200 mg/kg and 400 mg/kg of the extract for 21 days. Liver analysis showed no significant changes in liver weight, while the high-dose group exhibited a significant decrease ($p<0.05$) in alkaline phosphatase (ALP) levels. Other liver enzymes and bilirubin levels remained unchanged. Histological analysis revealed normal liver morphology. These findings suggest that *Cajanus cajan* seed extract may be hepatoprotective and safe for consumption during pregnancy.

Keywords:*Cajanus cajan*, Pregnancy, Liver, Liver enzymes.

INTRODUCTION

Plant materials, particularly Pigeon pea (*Cajanus cajan*), hold significant importance in ethnomedicine and serve as vital reservoirs for discovering novel drugs[1]. Found in both hemispheres, from 30°N to 30°S, thriving in warm and tropical environments[2]. Pigeon pea cultivation is mainly concentrated in Nigeria's agroecological zones, such as Oyo state, covering approximately 190,000 hectares of land area[3]. Traditional medicine, especially plant-based remedies, shows how important plants are for healthcare in developing countries. According to the World Health Organization, about 80% of people in these countries rely on traditional medicine for their basic healthcare needs, and about 85% of these treatments use plant extracts[4]. *C. Cajan* was discovered as a traditional medicinal herb used to treat anaemia in certain regions, with Pigeon pea seed extracts showing potential benefits for conditions like sickle cell anaemia[5]. However, many herbal medicines remain untested, and their use is often unmonitored[6].

During pregnancy, the embryo's development is most at risk in the first trimester, a period when many women are unaware of their pregnancy[7]. Approximately 10% of birth defects are caused by teratogens, including certain diseases, drugs, alcohol, and stress[8]. The liver, like other organs, goes through different changes during pregnancy, managing liver problems during this is particularly challenging due to the complex relationship between the mother and fetus, compounded by the rarity of such conditions[9]. Liver functions can be accessed through the liver function test (LFT), which measures various enzymes, proteins and substances in the blood. The key parameters measured include Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma-glutamyl Transferase (GGT), Bilirubin, Albumin and Prothrombin time. Nutrition plays a very important role in the developmental phase of pregnancy, as it determines the outcome in both health and disease conditions, understanding how Pigeon pea consumption affects pregnancy outcomes and liver conditions is crucial for ensuring the health of both mothers and babies[10].

The study aims to investigate the effects of *Cajanus cajan* seed extract on pregnancy outcomes and liver function in pregnant Wister rats, including its impact on liver enzymes and histomorphology. This research is significant due to the widespread consumption of Pigeon peas among pregnant women for its nutritional benefits.

MATERIALS AND METHODS

Ethical Consideration

The experimental protocol involving animal subjects was approved by the Institutional Animal Care and Use Unit of Delta State University in Abraka, Nigeria (DELSU/CHS/PHS/2022/08). All procedures were conducted according to the guidelines established by the National Institutes of Health (NIH) for the care and use of laboratory animals was followed strictly.

Experimental Animals

A total of 23 Wister rats, comprising 15 females and eight males whose average weights were between 136 and 240 grams, bred in the

animal house of the College of Health Sciences, Delta State University, Abraka, were used for this study. The rats were allowed to acclimatize for two weeks before treatment, kept inside a well-ventilated iron cage and kept in a standard condition of temperature (25±5°C), relative humidity (50±5°C) and 12-hour light/dark cycle and access to feed and water *ad libitum.* The experimental animals were handled according to the protocol approved by the Institutional Animal Ethics Committee (IAEC) as adopted by the faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Collection of Plant Materials and Extraction Process

The dried seeds of *Cajanus cajan* were purchased in Bojida market, Ibadan, Oyo State, Nigeria. Then, it was washed with clean tap water and air-dried for two days to remove any moisture.

- **Extraction process**

700g of the plant sample (*Cajanus cajan*) will be macerated in 70% ethanol for 24 hours in ratio (1:6) and then filtered. The residue was re-soaked in fresh 70% ethanol for 24 hours. Afterwards, the filtrate was concentrated in vacuo using a rotatory evaporator to obtain a concentrated *Cajanuscajan* aqueous ethanolic extract. The concentrated aqueous ethanolic extract was freeze-dried to eliminate all moisture contents to obtain a powdered sample

Mating

Female rats mated with male rats for four days in the ratio (2:1). Pregnancy was ascertained using the vaginal smear method[11]. If spermatozoa were identified in the smear, the animal was considered pregnant, and that day was recorded as day one of pregnancy. A female Wister rat has an ovulation cycle of five (5) days, necessitating mating during this period.

Experimental Design

The Pregnant Wister rats were randomly divided into three groups, each consisting of five animals. Group A served as the Control Group and was provided with unrestricted access to food and water throughout the entire experimental period. Group B and C were given daily oral doses of 200 mg/kg and 400 mg/kg body weight of extracts, respectively, for 3 weeks (21 days).

Sample collection

The rats underwent an overnight fast before being euthanized via cervical dislocation. Their livers were collected, weighed, and preserved in formalin for histological analysis, including assessments of AST, ALT, GGT, alkaline phosphatase, bilirubin, and albumin levels.

Measurement of Liver Weight

After sacrificing the rats, their livers were removed and weighed using a scale, with the measurement expressed in grams (g).

Assay for Liver Enzymes (ALT, AST and ALP)

Alanine aminotransferase (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP) tests were carried out using ultraviolet (UV) kinetic test kits produced by Cypress Diagnostics. The test is based on photometric determination of the nicotinamide adenine dinucleotide (NADH) consumption rate by pyruvate and oxaloacetate, which is directly related to ALT, AST and ALP activities, respectively. The test is based on the procedure of Reitman and Frankel[12].

Assay for Albumin (ALB)

Albumin was measured using commercial Randox diagnostic kits according to the instructions of the manufacturer. The absorbance at 578 nm was measured using a spectrophotometer, and the unit was expressed in g/dL[13].

Method for Bilirubin Estimation

Bilirubin was estimated using a reaction with diazotized sulfanilic acid to produce azobilirubin. There are two reactions: direct and indirect. The direct reaction occurs with water-soluble conjugated bilirubin, while the indirect reaction involves adding methyl alcohol to solubilize unconjugated, water-insoluble bilirubin. Unconjugated bilirubin can be calculated after the indirect reaction[14].

Histological Studies

Histological sections were prepared from paraffin blocks and stained with haematoxylin and eosin (H & E) to examine changes in cell morphology[16].

Statistical Analysis

Statistical evaluation was performed using the one-way analysis of variance (ANOVA) method to compare the various experimental groups, followed by Bonferroni's test to determine groups with significant differences (SPSS for Windows, version 17). The significance level was set at $P<0.05$.

RESULTS

RESULTS

1. Effect of *Cajanus cajan* Seed Extract on Liver Weight

The liver weight of the treated rats was measured and analyzed. There was no significant difference ($p > 0.05$) in liver weight between the treatment groups and the control group. The high-dose group (400 mg/kg) showed a slight increase in liver weight, but it was not statistically significant.

Table 1: Liver Weight and ALP Levels

Group	Liver Weight (g)	ALP Levels (U/L)
Control	3.81 ± 0.05	[P>0.05]
Low Dose (200 mg/kg)	4.17 ± 0.68	[P>0.05]
High Dose (400 mg/kg)	4.22 ± 0.15	[P<0.05]

2. Effect on Liver Enzyme Levels

2.1 Aspartate Aminotransferase (AST)

AST levels were assessed across all groups. The results showed a non-significant reduction in AST levels in both the low-dose (200 mg/kg) and high-dose (400 mg/kg) groups compared to the control group.

2.2 Alanine Aminotransferase (ALT)

A non-significant decrease in ALT levels was observed in the treatment groups compared to the control group. The reduction was more pronounced in the high-dose group but remained statistically insignificant ($p > 0.05$).

2.3 Alkaline Phosphatase (ALP)

ALP levels were significantly reduced ($p < 0.05$) in the high-dose group (400 mg/kg) compared to the control group. However, the low-dose group (200 mg/kg) showed a non-significant decrease.

3. Effect on Bilirubin Levels

3.1 Total Bilirubin

A non-significant increase in total bilirubin levels was observed in the treatment groups compared to the control group.

3.2 Direct Bilirubin

Direct bilirubin levels showed a non-significant reduction in the treatment groups compared to the control group.

4. Histological Analysis

Histological examination of liver sections revealed normal liver structures in all groups. The liver tissues showed intact central veins, sinusoids, hepatocytes, bile ducts, and portal veins. The hepatocytes in all groups appeared normal under high magnification, with no significant pathological changes observed.

5. Summary of Findings

- No significant changes in liver weight.
- AST and ALT levels remained unchanged.
- ALP levels significantly decreased in the high-dose group.
- No significant alterations in bilirubin levels.
- Histological analysis confirmed normal liver morphology.

These findings suggest that *Cajanus cajan* seed extract may have a hepatoprotective effect and is safe for consumption during pregnancy.

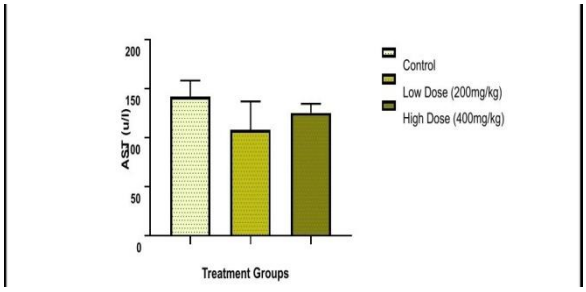

Figure 1: Effect of *Cajanus cajan* seed extract on aspartate aminotransferase

From the result above, the result showed a non-significantly reduced AST when compared to the control group. A non-significant decrease in AST levels was observed in rats treated with *Cajanus cajan* seed's aqueous extract at 200mg/kg and 400mg/kg doses, compared to the control group.

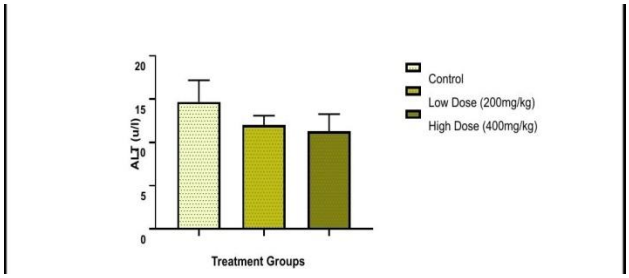

Figure 2: Effect of *Cajanus cajan* seed extract on alanine aminotransferase

From the image above, the result showed a non-significant reduction in ALT levels. There was a non-significant decrease in rats treated with the aqueous extract of *Cajanus cajan* seed at both 200mg/kg and 400mg/kg doses, when compared to the compared to the control group.

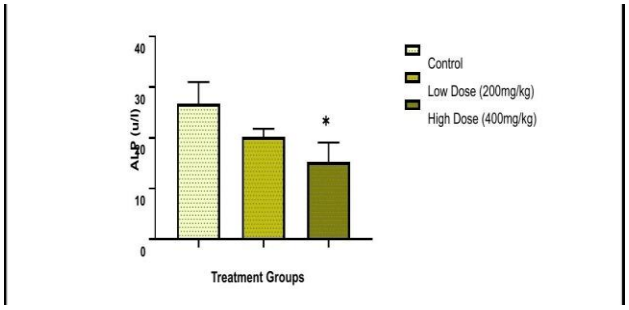

Figure 3: Effects of *Cajanus cajan* seed extract on Alkaline Phosphatase

The above result indicates a non-significant ($p>0.05$) decrease in ALP enzymes at low dosage (200mg/kg). However, a significant ($P<0.05$) decrease in ALP levels was observed in Wister rats treated with aqueous extract of *Cajanus cajan* seed at 400mg/kg, in comparison to the control group.

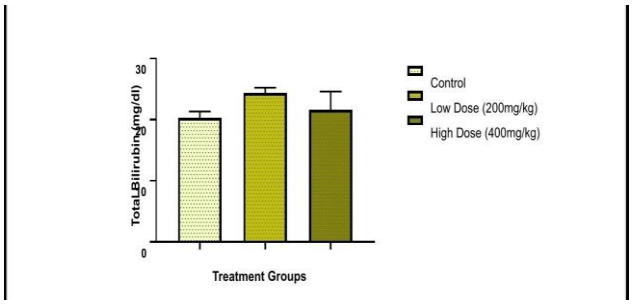

FIGURE 4: Effect of *Cajanus cajan*seed extract on total bilirubin

The results above indicate a non-significant increase in Total Bilirubin levels when compared to the control group. Specifically, there was a non-significant increase in total bilirubin levels observed in rats treated with the aqueous extract of *Cajanus cajan* seed at 200mg/kg and 400mg/kg doses, compared to the control group.

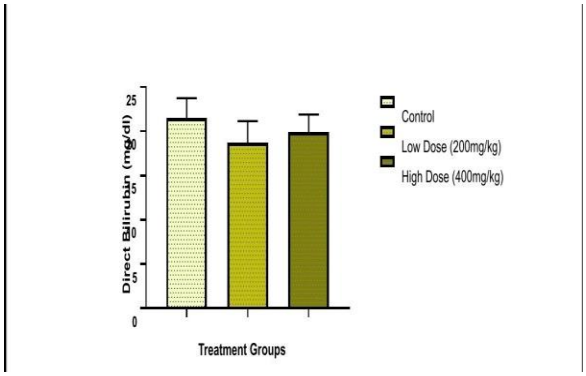

FIGURE 5: Effect of *Cajanus cajan* seed extract on direct bilirubin

The result above showed a non-significant reduction in direct bilirubin levels when compared to the control group. A non-significan reduction in direct bilirubin was observed in the rats treated with the aqueous extract of *Cajanus cajan* seed at both 200mg/kg and 400mg/kg doses compared to the control group.

Histology

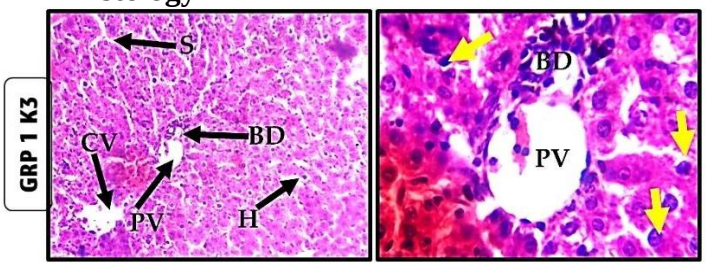

Figure 6: General histology and morphology of the liver in Group 1 L3 at 100 and 400 magnifications

The result shows the central vein (C), sinusoid (S), hepatocyte (H), bile duct (BD), and portal vein (PV). The cellular assortment and regions of the liver in this group appear normal. Furthermore, the hepatocytes with deeply stained nuclei (yellow arrows) were largely normal at high magnification.

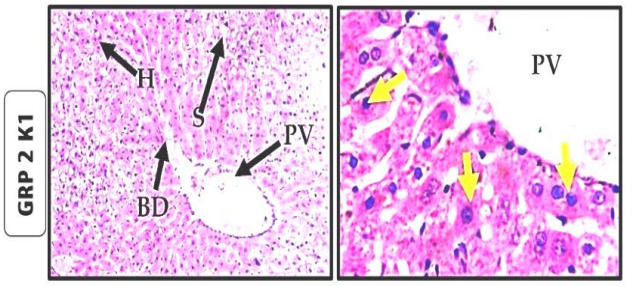

Figure 7: General histology and morphology of the liver in Group 2 at 100 and 400 magnifications

The result shows the central vein (C), sinusoid (S), hepatocyte (H), bile duct (BD), and portal vein (PV). The cellular assortment and regions of the liver of this group appear normal. Furthermore, the hepatocytes with deeply stained nuclei (yellow arrows) were largely normal at high magnification.

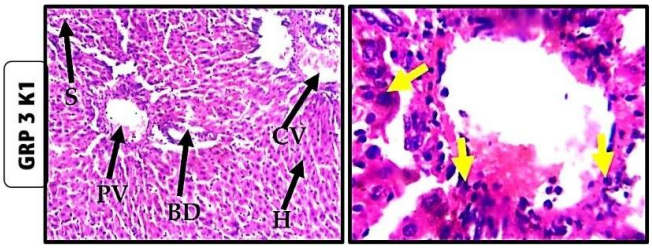

Figure 8: General histology and morphology of the liver in Group 3 L1 at 100 and 400 magnifications

The result shows the central vein (C), sinusoid (S), hepatocyte (H), bile duct (BD), and portal vein (PV). The cellular assortment and regions of the liver of this group appear normal. Furthermore, the hepatocytes were largely normal at high magnification with deeply stained nuclei (**yellow arrows**).

DISCUSSION

Hepatotoxicity means dysfunction of the liver due to an overload of chemicals, herbal supplements/drugs, solvents, and alcohol are all possible causes, It is known that DNA, lipids and proteins are the main targets of oxidative injury[17]. The liver serves to filter out toxic

substances from the bloodstream[18]. When excessive chemicals are filtering out through the liver, it becomes overloaded and can lead to hepatotoxicity[19]. Traditional testing of liver function depends on serum biomarkers like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glutamyltranspeptidase (GGT), and total bilirubin. These biomarkers are released into the blood following hepatocyte damage[21]. Treatment of rats for 21 days with 200, and 400mg/kg body weight effect of aqueous extract of *Cajanus cajan* seed caused a non-significant ($p>0.05$) increase in organ weight relative to the control. This non-significant increase in liver weight is in agreement with the previous observations[22].

From Figure 1 above, the result showed a non-significantly ($p>0.05$) reduced AST when compared to the control group. There was a non-significantly ($p>0.05$) decrease in AST levels in groups treated with 200mg/kg and 400mg/kg of aqueous extract of *Cajanus cajan* seed when compared to the control group. In line with this study agrees with the previous observation[22].

In this present study, figure 2 results showed a non-significantly reduced ALT level compared to the control group. There was a non-significant ($p>0.05$) decrease in ALT levels in groups treated with 200mg/kg and 400mg/kg aqueous extract of *Cajanus cajan* seed compared to the control group. This study does not align with the study[17] which showed a significant ($p\leq0.05$) increase in plasma ALT activity when compared to the control group. In addition, this finding corresponds to a previous study[21]. This indicates the protective effect of *Cajanus cajan*extract, revealing that it can protect the structural integrity of the hepatocellular membrane. These buttress further that natural antioxidant molecules stabilize cell membranes depending on their degree of free radicals scavenging capability[24].

From Figure 3 above, the result showed a non-significantly ($p>0.05$) decrease in ALP enzymes at 200mg/kg when compared to the control group and a significant ($p<0.05$) decreased ALP in groups treated with *Cajanus cajan* seed extract at 400mg/kg compared to control. A decrease in ALP level in the liver when previously elevated due to bone disease or liver indicates recovery or effective treatment of underlying condition[24].

In addition, the result of this present study showed a non-significantly increase in total bilirubin when compared to the control group. There is a non-significantly increase in total bilirubin of groups treated with 200mg/kg and 400mg/kg of aqueous extract of *Cajanus cajan* seed. This study is incongruent with the study of[26], but similar to the previous study[17] which showed a decrease in total bilirubin level of *Cajanus cajan* treated when compared with the control group.

This study also showed a non-significant reduction in direct bilirubin when compared to the control group. There was a non-significant reduction in direct bilirubin in groups treated with 200mg/kg and 400mg/kg aqueous extract of *Cajanus cajan* seed compared to the control group which may be due to the reduction in hepatocyte number which resulted in the reduced hepatic ability to synthesize protein[27]. This result is in agreement with a previous study indicating their protective role against liver damage[28]

From this study, the hepatoprotective effect of C. *Cajan* was substantiated further by the histopathological studies. The result shows the central vein (C), sinusoid (S), hepatocytes (H), bile duct (BD), and portal vein (PV). The cellular assortment and regions of the liver in this group appear normal. Furthermore, the hepatocytes were largely normal at high magnification with deeply stained nuclei (yellow arrows). This result does not correspond with the previous one[29], which observed several changes in the liver such as fibrosis, cirrhosis and hepatocarcinoma, possible reasons for these different observations may include differences in the composition of *Cajanus cajan* used, variation in experimental protocol or differences in timing and duration of treatment. The main compound active compounds in pigeon peas are stilbene, phenolic and flavonoid and studies have shown their hepatoprotective properties, among all the compounds, sesquiterpene hydrocarbons (phenol) are found higher in seeds constituting 93.3% of its fraction[30]. This may be responsible for a significant reduction in ALP enzyme in 400mg/kg, which is reported in this study.

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Nil.

Conflict of Interest

Nil.

REFERENCES

1. Pal D, Sachan N, Ghosh A, Mishra P. Biological activities and medicinal properties of *Cajanuscajan* (L) Millsp. J Adv Pharm Technol Res. 2011;2:207.
2. Pigeon pea (*Cajanus cajan*) forage | Feedipedia. Available from: https://www.feedipedia.org/node/22444.
3. Fatokimi EO, Tanimonure VA. Analysis of the current situation and future outlooks for pigeon pea (*Cajanus cajan*) production in Oyo State, Nigeria: A Markov Chain model approach. J Agric Food Res. 2021;6:100218.
4. Okaiyeto K, Oguntibeju OO. African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. Int J Environ Res Public Health. 2021;18:5988.
5. Abebe B. The dietary use of pigeon peas for human and animal diets. Sci World J. 2022;2022.
6. vanWyk AS, Prinsloo G. Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. S Afr J Bot. 2020;133:54-62.
7. The First Trimester. Available from: https://www.hopkinsmedicine.org/health/wellness-and-prevention/the-first-trimester#:~:text=The%20fetus%20is%20most%20vulnerable.
8. Verywell Health. What is a teratogen? [Internet]. 2021 [cited 2024 Oct 13]. Available from: https://www.verywellhealth.com/teratogens-5118058
9. Sharma AV, John S. Liver disease in pregnancy. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482201/ (2023).
10. Marshall NE, Abrams B, Barbour LA, Catalano P, Christian P, Friedman JE, et al. The importance of nutrition in pregnancy and lactation: Lifelong consequences. Am J Obstet Gynecol. 2021;226(5):607-632.
11. Choi SH, Cho YS, Kim MJ, Lee CH, HwanHoo S, Hwa BS, Lee J. Journal of Animal Reproduction & Biotechnology. 2020; 35(3): 232-238. https://doi.org/ 10.127 50/ jarb.35.3.232

12. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J ClinPathol. 1957;28:56-63.
13. Randox Laboratories. Albumin [Internet]. Randox Laboratories; 2023 Oct 12 [cited 2024 Oct 13]. Available from: https://www.randox.com/albumin/.
14. Microsoft. Search [Internet]. 2022 [cited 2024 Oct 13]. Available from: https://www.bing.com/ck/a?
15. Rolls G, Sampias C. H&E staining overview: a guide to best practices [Internet]. Leica Biosystems; 2010 [cited 2024 Oct 13]. Available from: https:// www.leicabiosystems.com/knowledge-pathway/he-staining-overview-a-guide-to-best-practices/
16. Bevans R. One-way ANOVA: when and how to use it (with examples) [Internet]. Scribbr; 2020 Mar 6 [cited 2024 Oct 13]. Available from: https://www.scribbr.com /statistics/one-way-anova/
17. Iweala EEJ, Evbakhavbokun WO, Maduagwu EN. Antioxidant and hepatoprotective effect of *Cajanuscajan* in N-Nitrosodiethylamine-induced liver damage. Sci Pharm. 2019;87:24.
18. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants, and functional foods: Impact on human health. Pharmacogn Rev. 2010;4:118-126.
19. Cleveland Clinic. Liver: What it does, disorders & symptoms, staying healthy. Available from: https://my.clevelandclinic. org/health/articles/21481-liver (2021).
20. Hepatotoxicity (liver toxicity). Available from: https://www.yalemedicine.org/ clinical-keywords/hepatotoxicity#:~:textHepatotoxicity 2C%20also%20known%20as%20liver%20toxicity%2C%20is.
21. Fu S, Davies MR, Buchanan-Hughes AM, Aithal GP, Williams R, Rhodes JM. Molecular biomarkers in drug-induced liver injury: Challenges and future perspectives. Front Pharmacol. 2020;10:1793.
22. Evbakhavbokun WO, Emeka IE. Protective effect of *Cajanus cajan* in hepatotoxic rats. IOP ConfSer Earth Environ Sci. 2019;331:012023.
23. Nwaechefu OO, Olaoluwa TD, Akinwunmi IR, Ojezele OO, Olorunsogo OO. *Cajanus cajan* ameliorated CCl4-induced oxidative stress in Wistar rats via anti-inflammation and mitochondrial-membrane transition pore inhibition. J Ethnopharmacol. 2022; 289: 114920.
24. Oluseyi A, Moshood O. Hepatoprotective effect of *Cajanuscajan* on tissue defense system in D-galactosamine-induced hepatitis in rats. Turk J Biochem. 2011; 36: 237-241.
25. Kalas MA, Chavez L, Leon M, Taweesedt PT, Surani S. Abnormal liver enzymes: A review for clinicians. World J Hepatol. 2021;13(11):1688-1698. doi:10.4254/wjh. v13.i11.1688.
26. Soetan KO. Antioxidant profile of graded levels of unprocessed pigeon pea (*Cajanus cajan*) seeds assayed from liver and kidneys of male Wistar strain rats. J Med PhysiolBiophys. 2018;45:51.
27. Mulla W, Salunkhe V, Bhise S. Hepatoprotective activity of hydroalcoholic extract of leaves of Alocasiaindica (Linn.). Indian J Exp Biol. 2009;47(2):148-152.
28. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective activity of Amaranthusspinosus in experimental animals. Food ChemToxicol. 2008; 46: 3417 -3421.

29. Junnila M, Rahko T, Sukura A, Lindberg LA. Reduction of carbon tetrachloride-induced hepatotoxic effects by oral administration of betaine in male Han-Wistar rats: A morphometric histological study. Vet Pathol. 2000;37:231-238.
30. Gargi B, Mehta V, Singh A, Deshmukh RK, Wazid A, Kour J, et al. Revisiting the nutritional, chemical and biological potential of *Cajanus cajan* (L.) Millsp. Molecules. 2022;27:6877.