Nicotinic tabacum Aqueous Leaf Extract Elicit Hepato-renal and Nuerotoxicity in rats

ABSTRACT

Nicotinic tabacum (Tobacco) has been in use for several years among the human race all over the world, Nicotiana tabacum, has probably been reported to be responsible for more deaths than any other herbs. Tobacco is used in difference ways but cigrettes constitute the largest share of manufactured tobacco products in the world. The toxicological evaluation of medicinal plants is essential to ensure their safe use. Therefore, this study investigate the hepato-renal and neurotoxic potential of Nicotiana tabacum aqueous leaf extract in Wistar rats.

However, a total of forty- eight (48) healthy male rats were separated into two groups with twenty-four rats each. Group one were given 1ml of distilled water only as the control group while the other group were treated with tobacco leaf extract of 25mg/kg body weight. The treatment were given daily for 12weeks. After the first 4weeks, 8 animals each were sacrificed from both groups. 4 weeks later another 8 animals were sacrificed while the remaining animals were sacrificed at the end of the 12weeks. Serum biochemical indices, oxidative stress parameters, and histopathological alterations in liver, kidney, and brain tissues were assessed.

Results indicated a dose-dependent increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Bilirubin, urea, and creatinine levels, suggesting hepatocellular and renal damage. Decreased brain nitric oxide and antioxidant enzyme levels, along with increased malondialdehyde concentration, BCL-2, p53 and caspase 9 level indicated oxidative and neurotoxic stress. Histological evaluation revealed hepatic necrosis, tubular degeneration, and neuronal vacuolation. These findings suggest that Nicotiana tabacum aqueous leaf extract induces dose-dependent hepato-renal and neurotoxicity in rats, underscoring the need for cautious use in traditional medicine

KEYWORD: Nicotiana tabacum; Toxicity; Hepatotoxicity; Nephrotoxicity; Neurotoxicity; Oxidative stress; Rats

1.0 INTRODUCTION

Herbal remedies derived from plants are widely utilized for their perceived safety and therapeutic potential (Jamal, 2023). However, the assumption that natural products are harmless is not always valid, as many contain compounds capable of inducing significant toxicity upon chronic exposure (Schrenk *et al*., 2020). Nicotiana tabacum (tobacco), a member of the Solanaceae family, is one such plant that has long been employed in traditional medicine for treating wounds, pain, and microbial infections (Anas *et al*., 2025). The plant contains numerous alkaloids, flavonoids, terpenoids, and polyphenols, with nicotine being the most pharmacologically active compound. While nicotine and its derivatives possess biological activity, they are also associated with deleterious effects on various organs, particularly the liver, kidney, and brain (Al-Snafi, 2022). The liver and kidney serve as critical detoxifying and excretory organs, making them highly susceptible to xenobiotic-induced injury. The brain, due to its lipid-rich composition and high oxygen utilization, is particularly vulnerable to oxidative damage (Kurian *et al*., 2023).

Despite the abundant literature on tobacco smoke toxicity, relatively few studies have assessed the systemic impact of Nicotiana tabacum aqueous extracts prepared in ways consistent with traditional medicinal practices (Zhang *et al*., 2024). Given that aqueous preparations are the most common method of consumption in rural herbal medicine, it becomes imperative to investigate their toxicological implications (Naik *et al*., 2023). Therefore, this study was designed to evaluate the hepato-renal and neurotoxic effects of Nicotiana tabacum aqueous leaf extract in rats, with an emphasis on biochemical, oxidative, and histopathological outcomes.

2.0 MATERIAL AND METHOD

2.1 Materials

2.1.1 Laboratory equipment

Beakers funnels, Stock pot, Conical flask, Filter paper, Test tubes, Sieve cloth, Water bath, Volumetric flask, Measuring cylinder, Oral cannula, Spectrophometer, Excella mixing blender, Plastic containers, Examination gloves, Test tubes and racks, Foil paper, Dissecting set, Blades Pins, Bowls, Stainless, Micro-pipette and tips, curvette, Paper tape, EDTA bottles, Urine

bottles, Plastic cages, stainless plates, Liquid soap, Sponges, Centrifuge, Homogenizer, Markers, Weighing balance, Tobacco leafs

2.1.2 Chemical and reagents

40% formaladehyde, Disodium di-hydrogen, Sodium hydrogen, Phopshate buffer solution, Distilled water, Deionized water, DNA/RNA shield (ZYMO research, USA), Normal saline, Ethanol, Creatinine, Xantine oxidase, Gluthione (GSH), Uric acid, Urea, Superoxide dismutase (SOD), Malondialdehyde (MDA), Bicarbonate (HCO-3), Kidney injury molecule (KIM-1), 8-OHdG (8-hydroxydeoxyguanosine) and Nitric oxide (NO), Alanine aminotransferase, Aspartate aminotransferase, Bilirubin (Total and Direct) were purchased from Beacon and Agape. Other chemicals used were of analytical grade.

2.2 Methods

2.2.1 Plant collection

2.2.2 Source and identification of plants

Dry tobacco plants leafs were collected from okeloko, Igboho Oorelope local government, Oyo state. This plant leafs was identified and authenticated by an expert taxonomist at the Department of pure and Biology of Ladoke Akintola University of Technology, Ogbomoso. The leafs of nicotiana tabaccum were given herbarium voucher number LHO 692.

2.2.3 Extraction and preparation of Aqueous extract of *Nicotiana tabaccum*

The plant leafs collected were carefully sorted out in order to remove unwanted materials such as sticks and other species. The leafs were sliced for easy drying and air dried for at least 2weeks. Followed by, the leafs were grounded to powered form using blender. 1000g of the powered extract were soaked into 10000ml of distilled water for 72hrs. After 72hrs, the soaked extract was filtered with the sieve and then filter paper was used to refilter it, exract was obtained and residue was discarded. The extract was further freeze dried to remove the water completely, using a freezer dryer to obatain the pure extract. Solid extract was obtained which was used for analysis.

2.2.4 Experimental design

Fresh leaves of Nicotiana tabacum were harvested and authenticated. The leaves were washed, air-dried, ground into fine powder, and extracted with distilled water by maceration for 72 hours. The filtrate was concentrated using a rotary evaporator and stored at 4°C until use. However, a total of forty-eight 48 healthy Wistar rats (150–170 g) were acclimatized and randomly assigned into two groups (n=24 per group) of 25mg/kg.bw of aqueous extract and (n=24 per group) of 1ml distilled water (control). The extract was administered orally for 12 weeks. After treatment, blood samples were collected via cardiac puncture for serum biochemical assays, while organs were excised for histopathological and oxidative stress *evaluations*.

2.3 Biochemical indices studied

Serum biochemical markers of hepatic function (ALT, AST, ALP, Total protein, Bilirubin) and renal function (Urea, Creatinine) were measured using standard enzymatic methods. Brain tissue homogenates were analyzed for oxidative stress markers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), as well as nitric oxide level. Histological analysis of liver, kidney, and brain sections was carried out after fixation in 10% formalin, embedding in paraffin, sectioning, and staining with hematoxylin and eosin.

2.4 STATISTICAL ANALYSIS

Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test ($p < 0.05$).

Serum Biochemical Parameters (hepatic and renal function)

Hepatic Function Marker:

1. **Effect of aqueous extract of *Nicotiana tabacum* leaves on alkaline phosphatase activity**

The serum activities of Alkaline phosphatase (ALP) are shown in Fig. 1 the results showed observable increase in ALP activity of rats administered aqueous extract of *Nicotiana tabacum* leaves when compared with control group (P value <0.05), with

ALP activity showing significant difference after 12 weeks but no significant difference after 4 weeks and 8 weeks respectively.

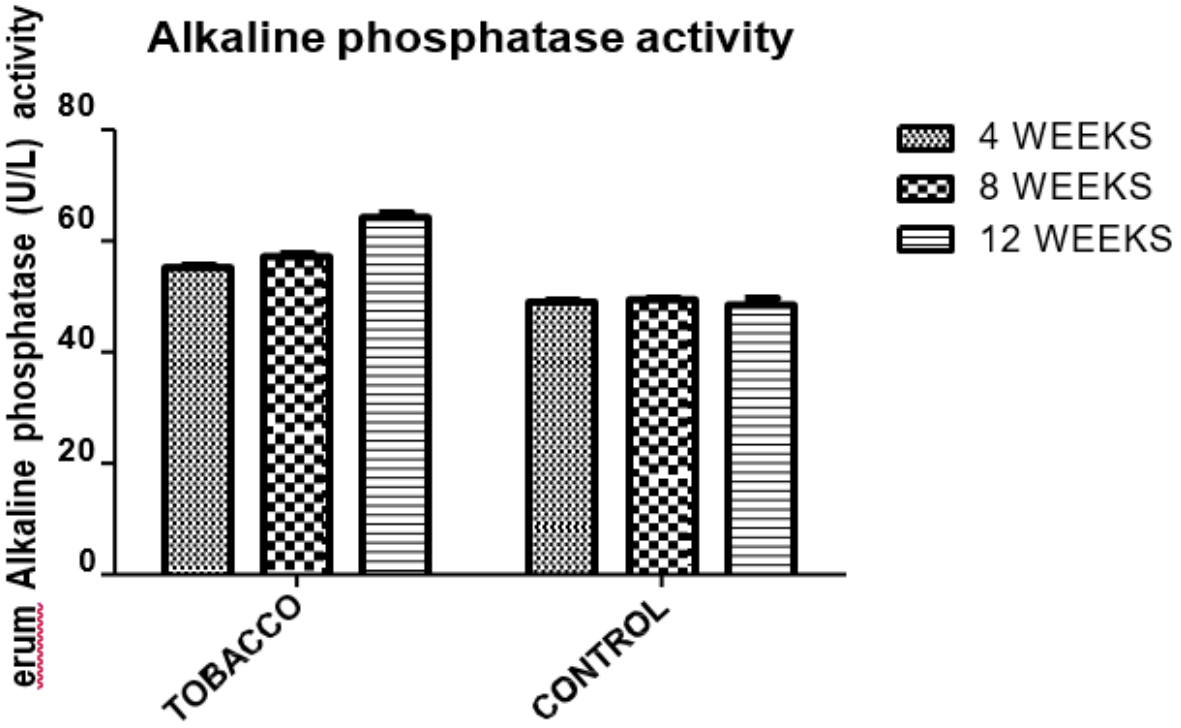


Fig 1; Serum Alkaline phosphatase activity in rats administered aqueous extract of *Nicotiana tabacum* (tobacco) leaves and control group.
($p < 0.05$) ($n = 3$)

2. **Effect of aqueous extract of *Nicotiana tabacum* leaves on Alanine aminotransferase activity**

The serum activities of Alanine aminotransferase (ALT) are shown in Fig 2, the results showed observable increase in ALT activity of rats administered aqueous extract of *Nicotiana tabacum* leaves when compared with control group (P value <0.05), with ALT activity showing significant difference after 4 weeks and 12 weeks respectively but no significant difference after 8 weeks.

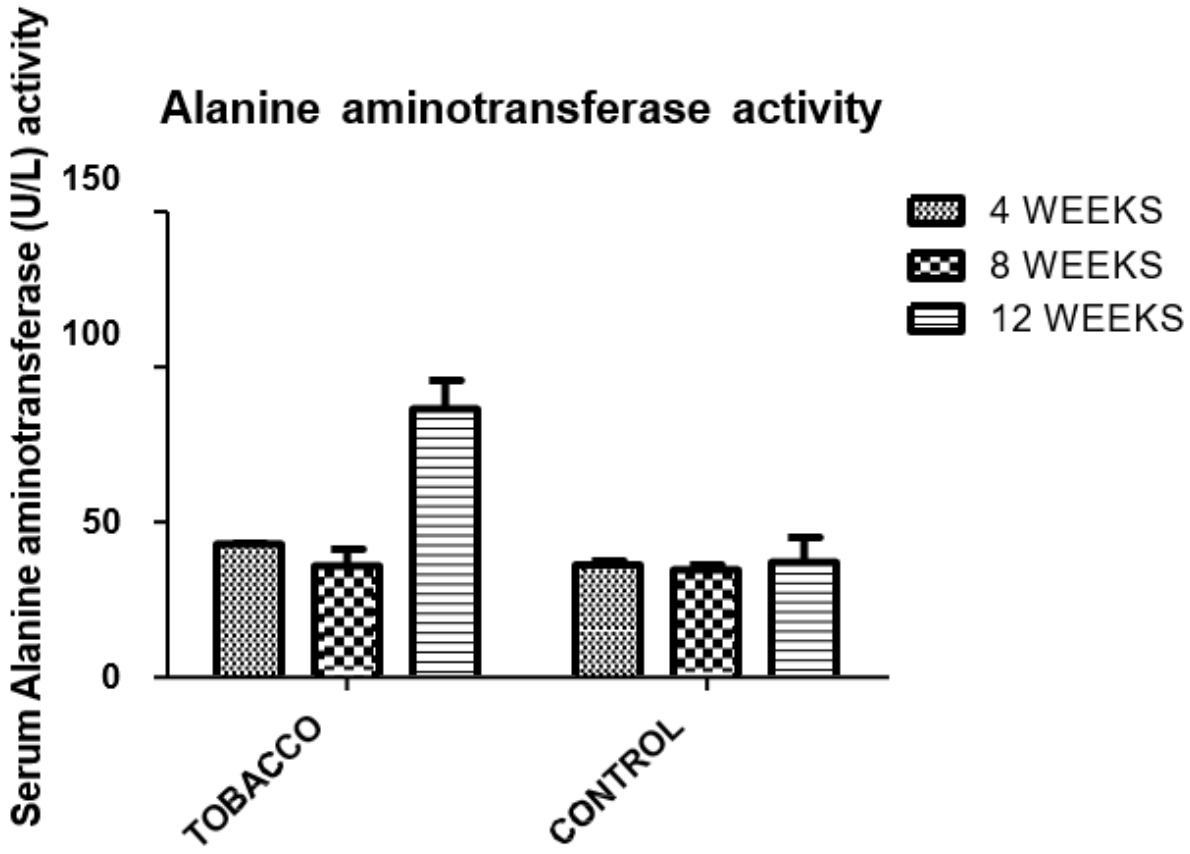


Fig. 2; Serum Alanine aminotransferase activity in rats administered aqueous extract of *Nicotiana tabacum* (tobacco) leaves and control group.

($p < 0.05$) ($n = 3$)

3. Effect of aqueous extract of *Nicotiana tabacum* leaves on Aspartate aminotransferase activity

The serum activities of Aspartate aminotransferase (AST) are shown in Fig. 3, the results showed observable increase in AST activity of rats administered aqueous extract of *Nicotiana tabacum* leaves when compared with control group (P value <0.05), with AST activity showing significant difference after 4, 8 and 12 weeks respectively.

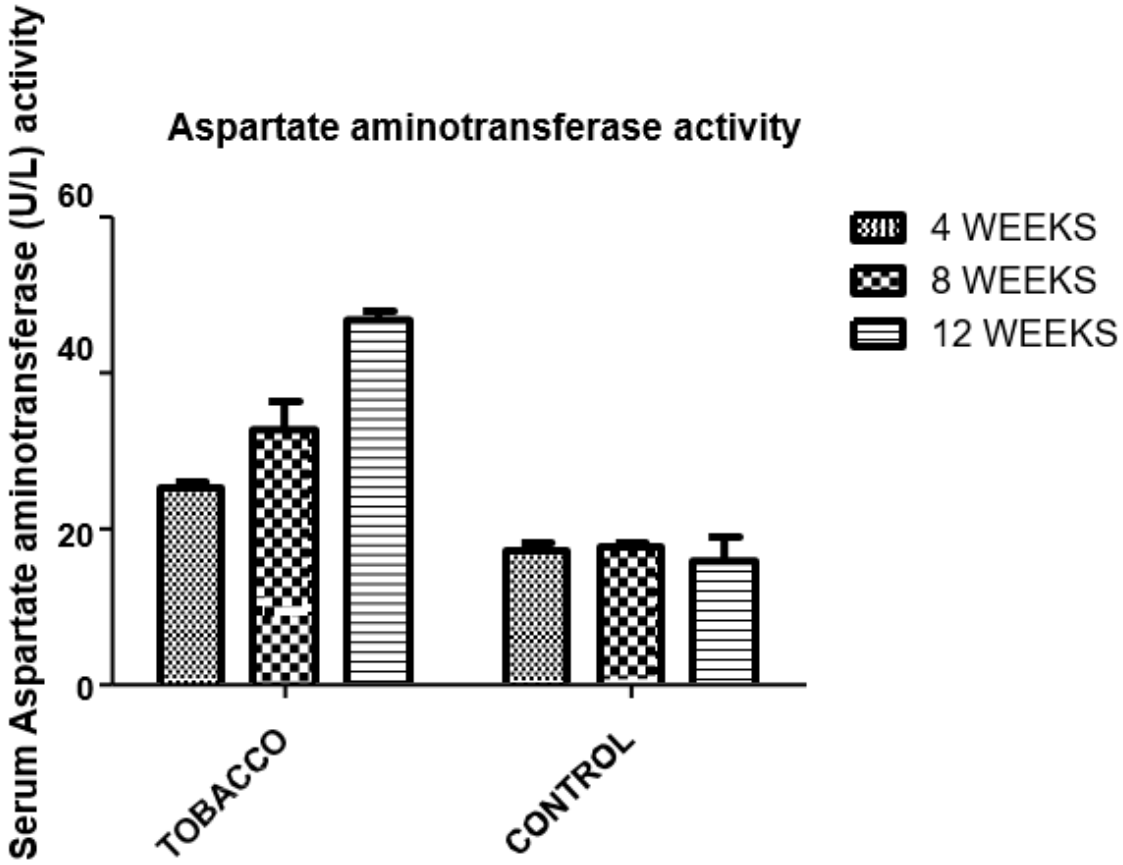


Fig 3; Serum Aspartate aminotransferase activity in rats administered aqueous extract of *Nicotiana tabacum* (tobacco) leaves and control group.

($p < 0.05$) ($n = 3$)

4. Effect of aqueous extract of *Nicotiana tabacum* leaves on serum total bilirubin concentration

The serum total bilirubin concentrations are presented in Fig 4, it showed observable increase in rats administered aqueous extract of *Nicotiana tabacum* leaves when compared (P value <0.05) with the control group, with total bilirubin concentration showing a significant difference after 4, 8 and 12 weeks respectively.

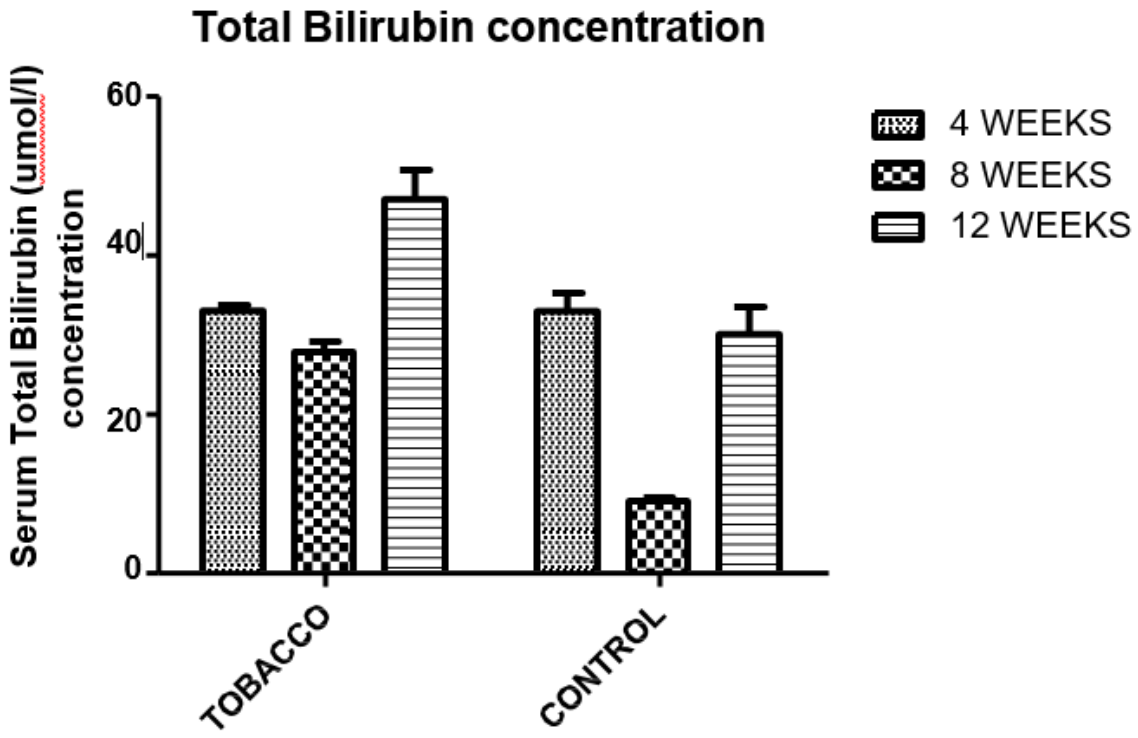


Fig 4; Serum Total bilirubin concentration in rats administered aqueous extract of *Nicotiana tabacum* (tobacco) leaves and control group.
($p < 0.05$) ($n = 3$)

5. **Effect of aqueous extract of *Nicotiana tabacum* leaves on serum direct bilirubin concentration**

The serum direct bilirubin concentrations are presented in Fig 5 respectively showed observable increase in rats administered aqueous extract of *Nicotiana tabacum* leaves when compared (P value <0.05) with the control group, with direct bilirubin concentration showing a significant difference after 4 weeks and 12 weeks respectively.

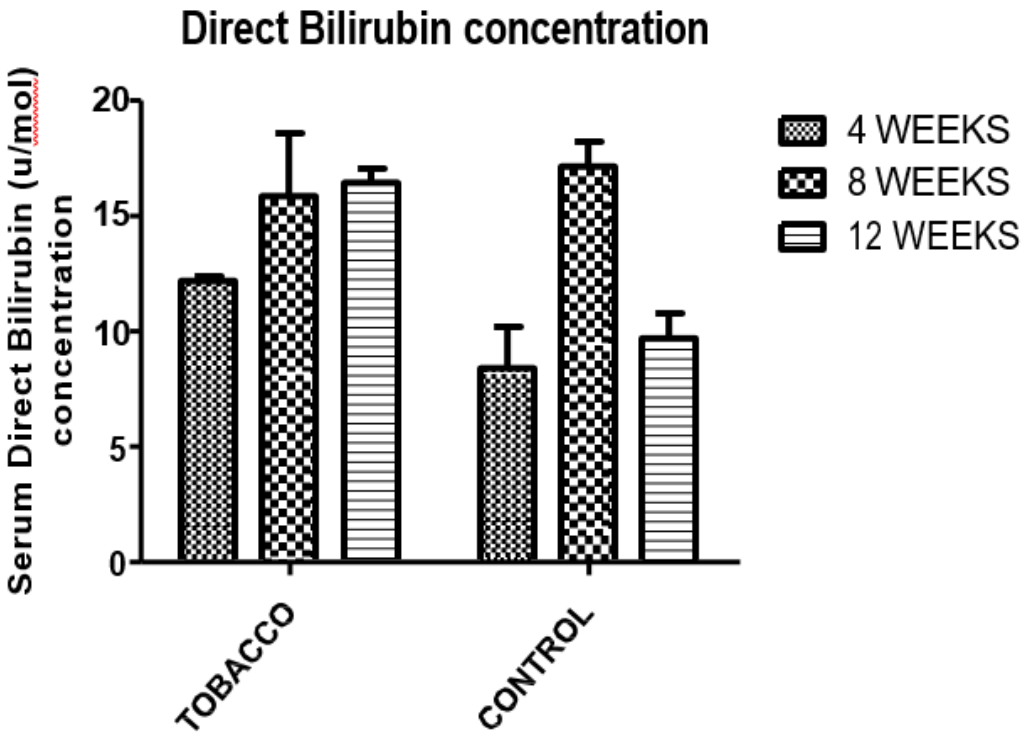


Fig 5; Serum Direct bilirubin concentration in rats administered aqueous extract of *Nicotiana tabacum* (tobacco) leaves and control group.
($p < 0.05$) ($n = 3$)

1. **Histological changes in Liver of rats in the control group**

The liver sections of control groups are showing a normal central vein, and lining endothelial, no bleeding observed, normal hepatocytes radiating arrangement from central vein and blood sinusoids appear between the hepatocytes. Hepatocytes are granular and acidophilic cytoplasm, normal arrangement of hepatic cords, normal sinusoids between the hepatocytes and containing a number of kupffer cells. In portal area, a typical portal canal contains branches of portal vein, hepatic artery and bile duct, the triad is clearly bordered by the surrounding normal hepatocytes and interlobular septa. Normal structure of functional metabolic zones between central vein and portal tract, zone, normal hepatic sinusoids connecting the zones (Fig. 6**.)**

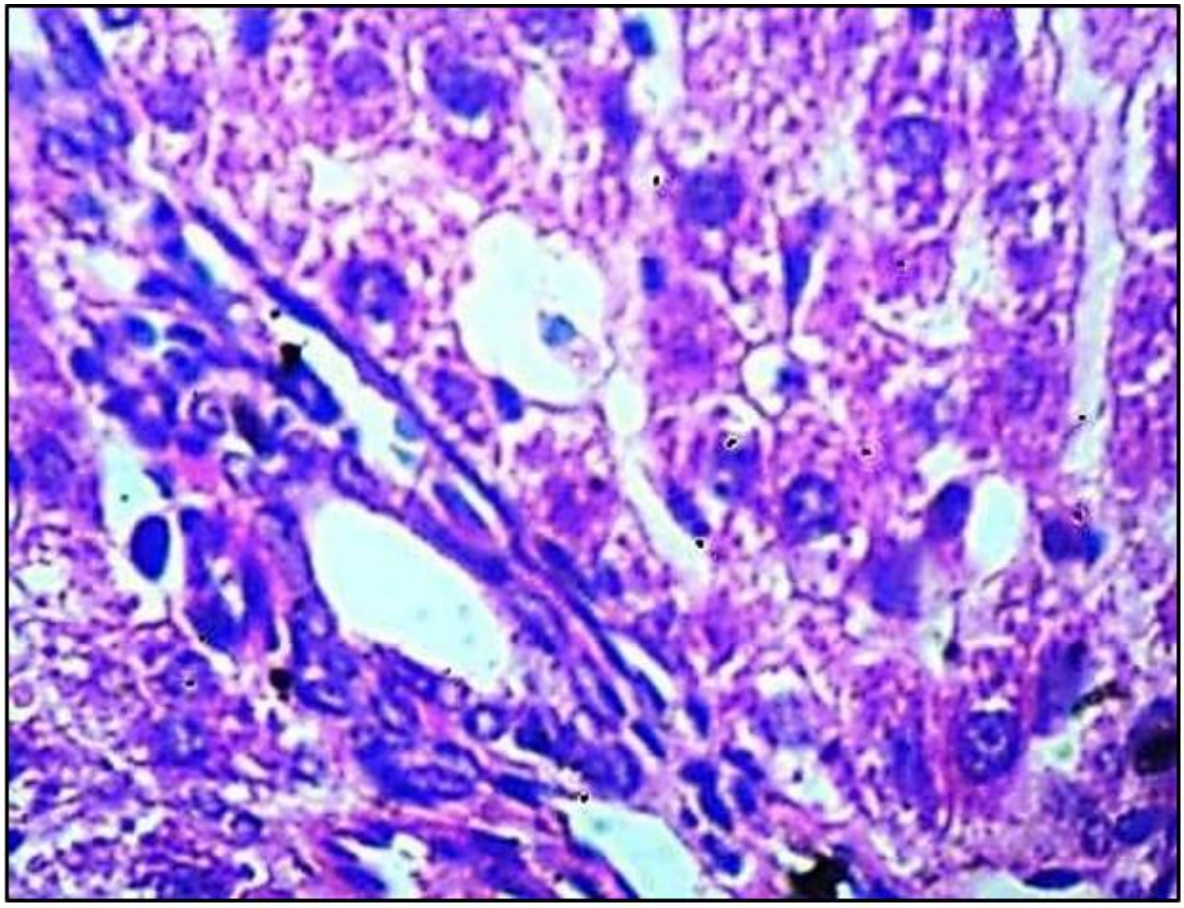


Fig 6. (x400) Microscopic representation of the liver of rats in control group

2. **Histological changes in Liver of rats administered aqueous extract of *Nicotiana tabacum* Leaves**

The liver sections of nicotine treated group shows a marked injury in central vein with hemorrhage, a marked congestion of portal triad, congestion of blood sinusoids in the 3 zones of acinus, degeneration disorder of hepatocytes especially zone 3 and adjacent area, and congestion with RBC's between hepatocytes (Fig.7).

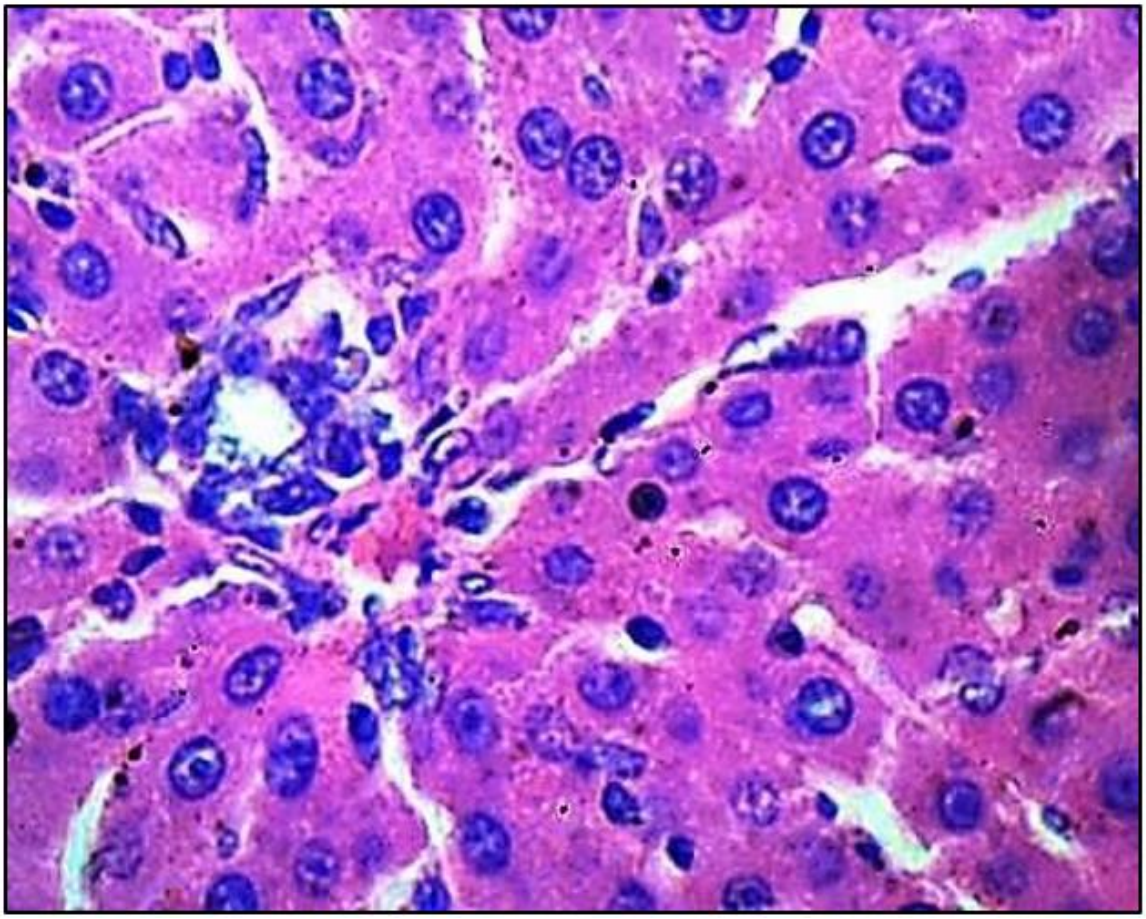


Fig. 7; (x400) Microscopic representation of the liver of rats administered aqueous extract of *Nicotiana tabacum* leaves.

Oxidative Stress and Neurotoxicity Markers in Brain Tissue

Effect of aqueous tobacco extract on the Oxidative status of the rats on brain

1. Superoxide dismutase

Table below showed the effect of tobacco extract on the superoxide dismutase level in the brain of experimental animal. The result depicted that there is significant decrease when compared with control group of each week.

Table 1 Changes in rat brain SOD level.

	Superoxide dismutase (u/ml)		
Groups	4 weeks	8 weeks	12 weeks

Tobacco	1.245±0.04803[a]	1.008±0.1196[a]	1.478±0.05022[a]
Control	1.502±0.009761	1.416±0.01893	1.793±0.08764

[a]- significant different when compared with each control group at 4 weeks, 8 weeks and 12 weeks.

(P value <0.05)

2. Nitric oxide

Table below showed the effect of tobacco extract on the nitric oxide level in the brain of experimental animal. The result depicted that there is significant decrease when compared with control group of each week.

Table 2 Changes in rat brain NO level.

	Nitric Oxide (µM)		
Groups	4 weeks	8 weeks	12 weeks
Tobacco	5.333± 0.4925*	18.26±0.2208*	13.20±0.4636*
Control	19.74±0.5604	26.79±0.05094	15.47±2.113

*- significant different when compared with each control group at 4weeks, 8weeks and 12weeks. (P value <0.05)

3. Malondialdehyde

The Malondialdehyde level results is shown in Table 3 The result obtained from malondialdehyde of the rat brain tissue administered aqueous tobacco extract showed increase when compared with the control group at each week.

Table 3 Changes in rat brain MDA level.

	Malondialdehyde (µM)		
Groups	4 weeks	8 weeks	12 weeks
Tobacco	4.391±0.06872 [a]	4.815±0.1431 [a]	2.669±0.2183 [a]
Control	2.897±0.2716	2.249±0.1163	0.8473±0.01837

[a]- significant different when compared with each control group at 4 weeks, 8 weeks and 12 weeks. (P value <0.05)

4. Glutathione

The Glutathione level results is shown in Table 4 The results of Glutathione of the rat brain tissue administered aqueous tobacco extract showed significant decrease when compared with the control group at each week

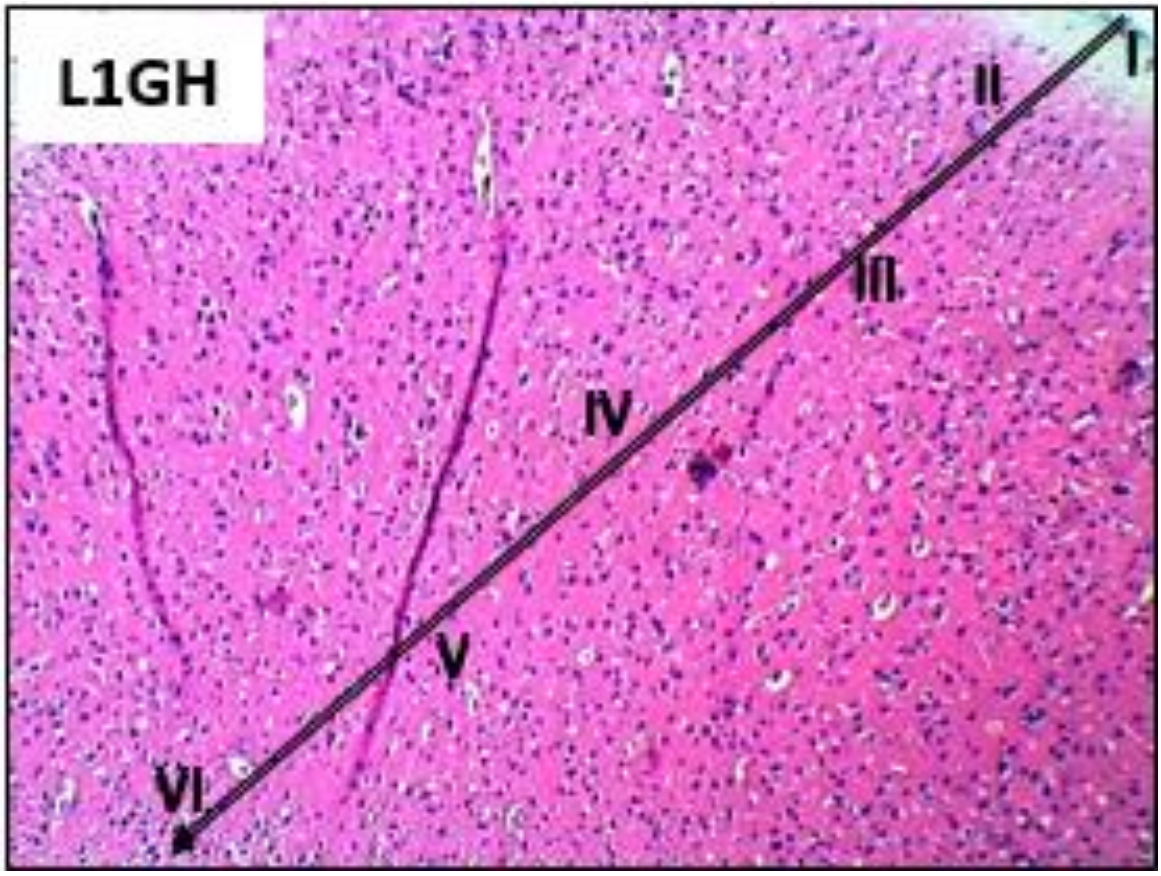
.**Table 4 Changes in rat brain GSH level.**

	Glutathione (mM)		
Groups	4 weeks	8 weeks	12 weeks
Tobacco	0.3766±0.03270*	0.4446±0.01072*	0.4078±0.01415*
Control	0.4484±0.009673	0.4707±0.06636	0.5190±0.03291

*- significantly different when compared with each control group at 4 weeks, 8 weeks and 12 weeks. (P value <0.05).

Figure 8. Histopathological Changes in Liver, Kidney, and Brain Sections

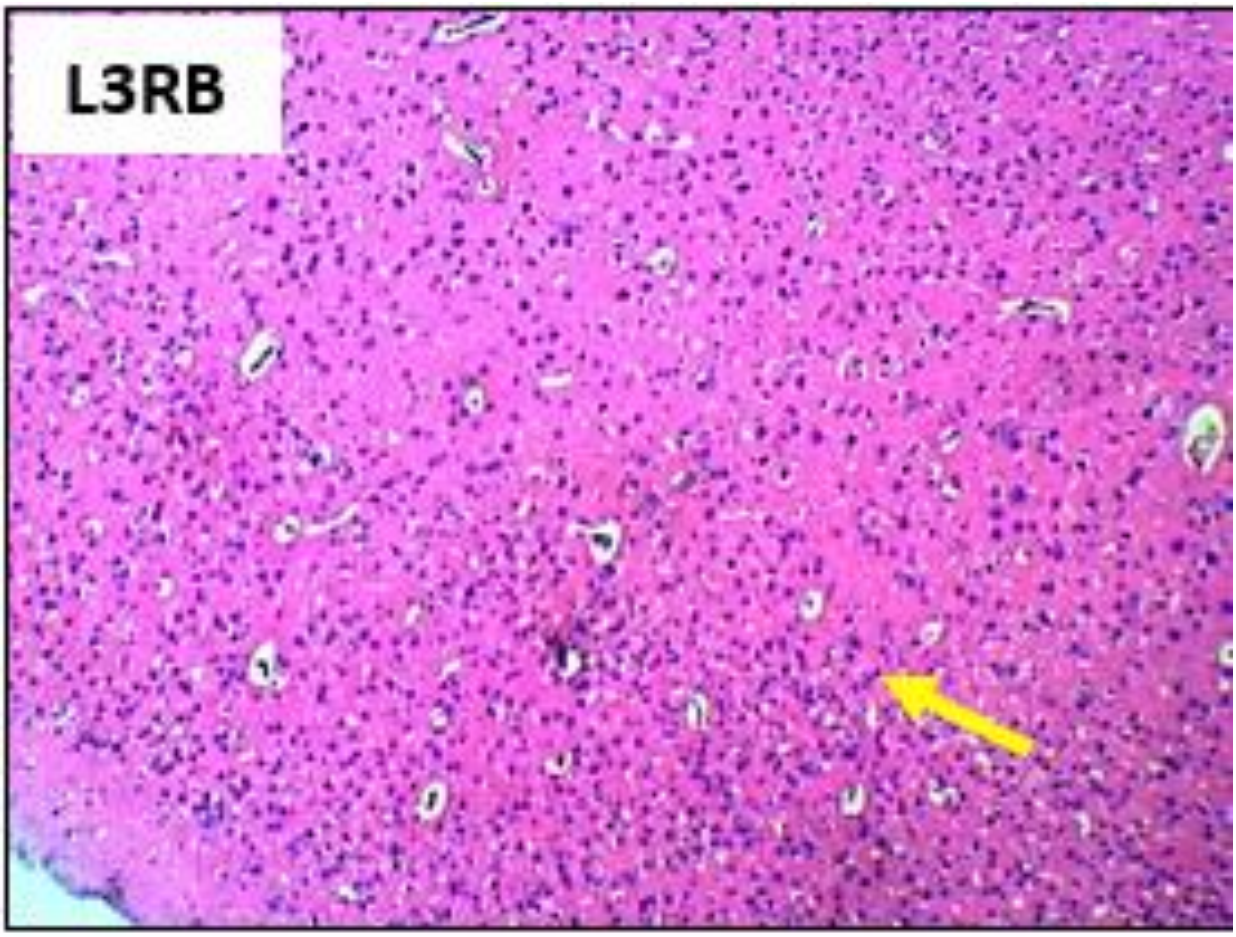
3.4 Histopathological changes in rat brain across the tobacco extract group and control.



(*X40*)

Plate 1 Photomicrographs showing panoramic views of cortex general micromorphological presentations in the brain of wistar rats across the tobacco group. H&E stain (*magnification-X40*). The molecular layer (I), External granular layer (II), External pyramidal layer (III), Internal granular layer (IV), Internal pyramidal layer (V) and the multiform layer (VI) are demonstrated across tobacco groups (white long arrow).

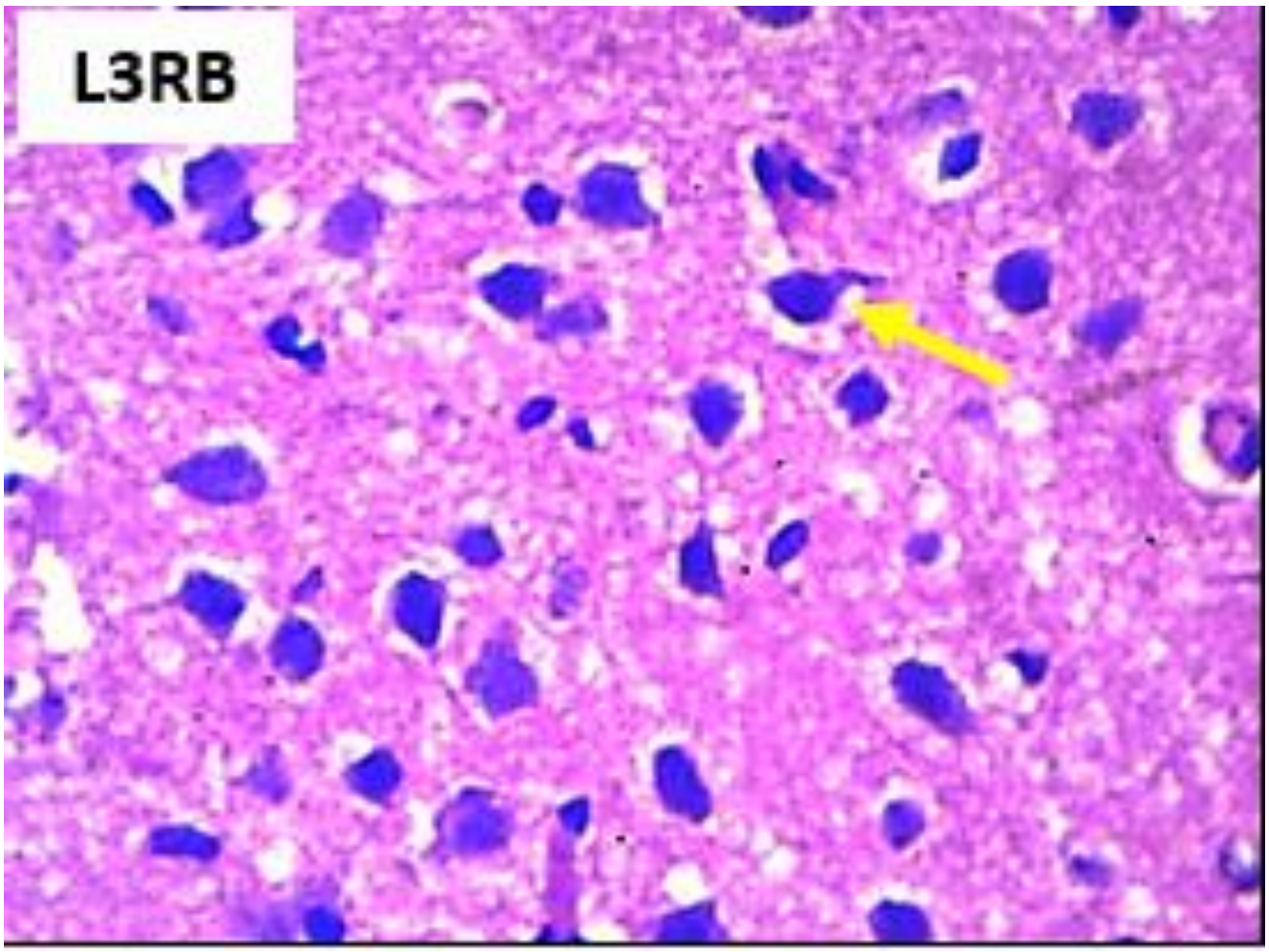
L1GB Brain tissue of rat administered tobacco extract labelled GB.



(X40)

Plate 2 Photomicrographs showing panoramic views of cortex general micromorphological presentations in the brain of wistar rats across the control group. H&E stain (*magnification-X40*). Profiles with a mild cellular alteration is indicated by yellow arrow.

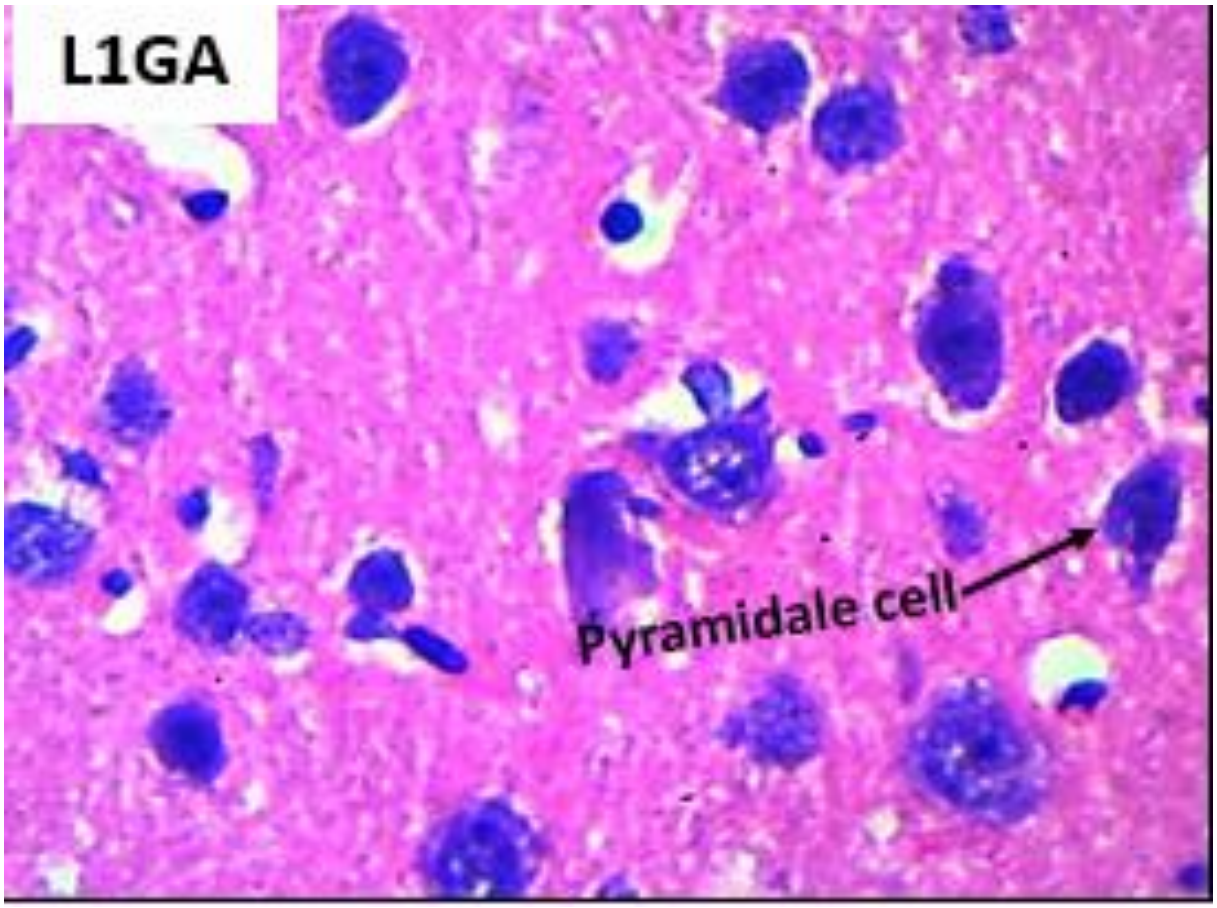
L3RB Brain tissue for control group animal labeled RB



(X400)

Plate 3 Photomicrographs showing layer three and four of the cerebral cortex micromorphological presentations in Wistar rats across the control groups. H&E stain (*Magnification- X400*). Yellow arrow denotes a mild pyknosis and degenerating neurons.

L3RB Brain tissue for control group animal labeled RB.



(X400)

Plate 4 Representative micrographs of H&E staining showing the general and magnified cytoarchitecture of the prefrontal cortex in Wistar rats across tobacco group (X400). Normal histological features of the cortex is observed in groups without red/yellow arrows, they are characterized by large pyramidal as well as granule neurons, the pyramidal cells are characterized with long axons that extend well from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extend from the well delineated soma of the pyramidal neurons in this group. Perineural space surrounding these cells appears intact, with intact nuclear and cytoplasmic content.

L1GA Brain for Tobacco extract group administered animal labeled GA.

Effects of tobacco extract on Caspase-9, BCl-2, and P53 level of the rats.

The serum B-cell lymphoma 2 (BCl-2), P53 (tumor protein) and Caspase-9 level results are represented below.

1. **Effects of tobacco extract on BCl-2 level of the rats.**

The BCl-2 level of the tobacco extract group increased when compared with control group (P value <0.05) at 4 weeks, 8 weeks and 12 weeks.

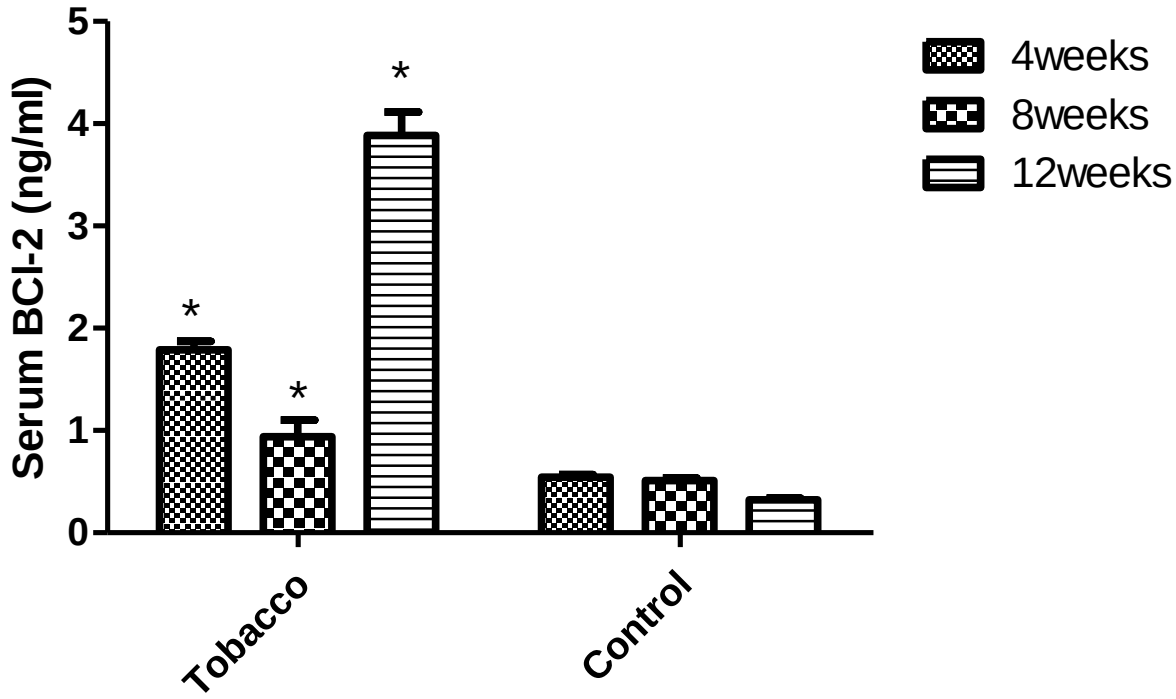


Fig 9 Serum B-cell lymphoma 2(BCl-2) level of the Experimental animals at 4, 8 and 12 weeks

*- significant different when compared with each control group at 4 weeks, 8 weeks and 12 weeks. P is significant when P <0.05

2. **Effects of tobacco extract on P53 level of the rats.**

The administration of tobacco extract increased P53 level in the serum of the rats with significant different when compared (P value <0.05) with the control group at 4 weeks, 8 weeks and 12 weeks.

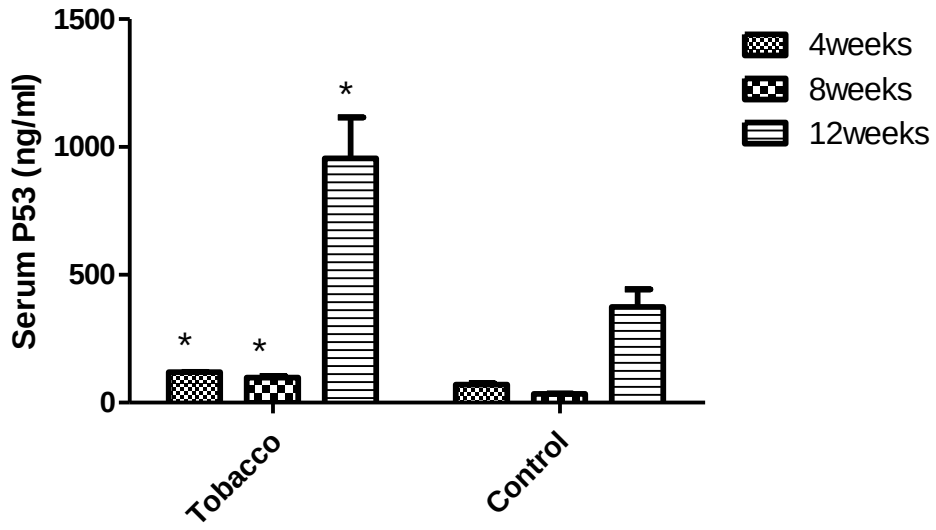


Fig 10 Serum P53 (tumor protein) level of the Experimental animals at 4, 8 and 12 weeks

*- significant different when compared with each control group at 4 weeks, 8 weeks and 12 weeks. P is significant when P <0.05

3. **Effects of tobacco extract on Caspase-9 level of the rats.**

The Caspase-9 level of the rats administered tobacco extract increased (P value <0.05) when compared with the control group at 4 weeks, 8 weeks and 12 weeks.

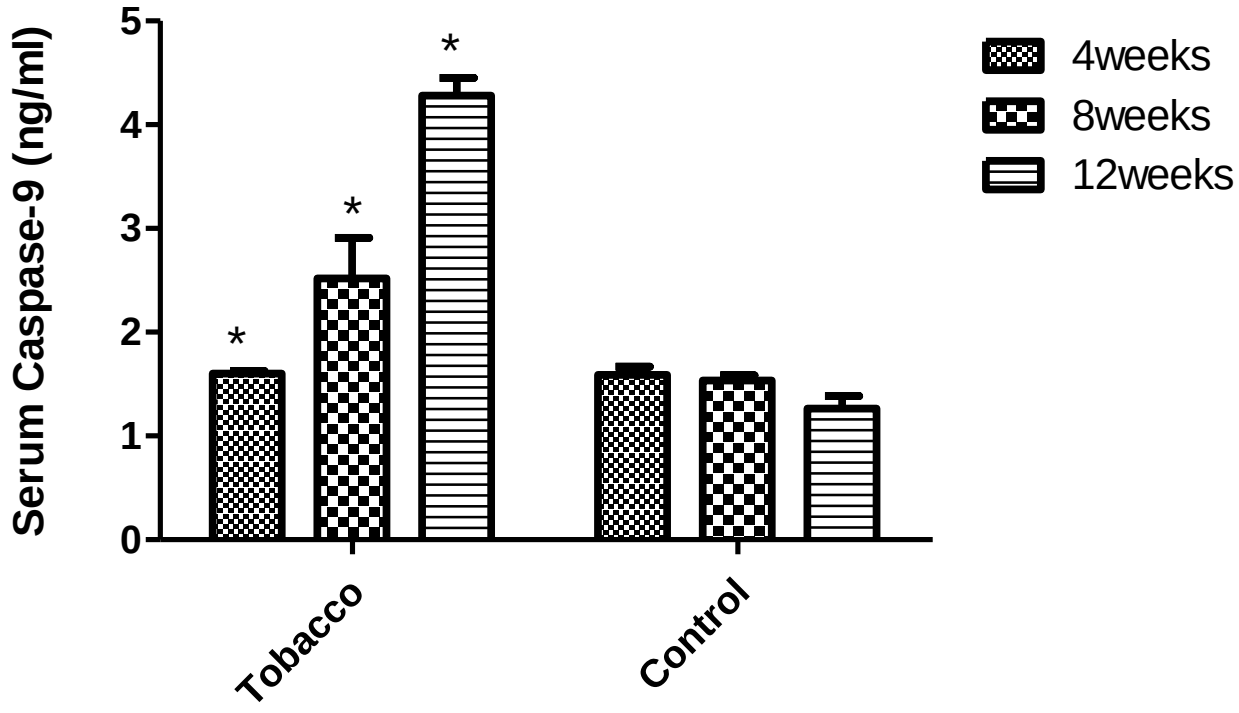


Fig 11 Serum Caspase-9 level of the Experimental animals at 4, 8 and 12 weeks

*- significant different when compared with each control group at 4 weeks, 8 weeks and 12 weeks. P is significant when $P<0.05$.

Most of the serum enzymes levels were elevated as predicted by Rudgley (2017), while the staining patterns from month to month for the tobacco treated samples were indicative of abnormal functioning of the brain, liver and kidney

3.0 RESULT and Discussion

Administration of *Nicotiana tabacum* aqueous leaf extract produced a significant dose-dependent elevation in hepatic enzymes and renal function markers. The 25mg/kg dose group exhibited the highest increase in ALT, AST, and ALP levels and direct/total bilirubin indicating hepatocellular leakage and liver dysfunction. Similarly, serum urea and creatinine concentrations increased markedly, suggesting impaired renal clearance. Brain tissue assays revealed elevated MDA levels and decreased SOD, CAT, and GSH activities, indicating oxidative stress. A decrease in nitric oxide (NO) level further confirmed neurotoxic effects. Histopathological findings correlated with biochemical alterations, showing hepatic necrosis, glomerular shrinkage, and neuronal vacuolation.

The findings of this study demonstrate that *Nicotiana tabacum* aqueous leaf extract induced hepatic, renal, and neural toxicity in a dose-dependent manner. The observed increase in serum transaminases (ALT and AST) reflects hepatocellular damage and leakage of enzymes into circulation, consistent with oxidative stress–mediated injury. Elevated ALP and bilirubin suggest cholestatic impairment, while reduced total protein may indicate compromised hepatic synthetic capacity (Andong*, et al*., 2021). The renal dysfunction observed through increased urea and creatinine levels aligns with reports that tobacco alkaloids may impair nephron integrity via reactive oxygen species (ROS) generation and mitochondrial dysfunction (Mohamed *et al.,* 2022)

In the central nervous system, nitric oxide level suggests decreases, which can affect the transmitting signals to cell in the nervous system (Garthwaite, 2008). In contrast, excessive nitric oxide is extremely toxic and will cause death of the nerve system, leading to neurotransmitter imbalance and oxidative neuronal stress (Teleanu *et al*., 2022). Increased lipid peroxidation in the brain and decreased antioxidant enzyme activity confirm the role of ROS in mediating neuronal damage (Petrovic *et al*., 2020). Studies demonstrated nicotine treatment found by up regulating BCl-2, decreased the susceptibility of cancer cells to apoptosis. In addition, according to Markouli *et al*., (2025), accumulation of BCL-2 may reflect a mechanism for counterbalancing ROS-mediated damage or it might represent the impairment of BCl-2 dependent protection from ROS.

Excessive p53 function is implicated in neural tube defects and neuronal degeneration. The p53 tumor suppressor potently limits the growth of immature and mature neurons under conditions of cellular stress (Sciaccotta *et al*., 2024). Thus, p53 function must be tightly controlled. Morrison and Kinoshita in year 2000 showed that the absence of p53 has been shown to protect neuron while increase in the level of p53 can result in extensive apoptosis in the central nervous system. The presence of nicotine in the Nicotiana tabacum activates Caspase 9. Elevated Caspase 9 in turn activates Caspase 3 and Caspase 7, which can initiate the hallmark of the degradation phase of apoptosis (Mohamed *et al*., 2022). High caspase 9 inhibition has been shown to increase the resistance of tumor cell has reported by Ping *et al*., 2017. These findings

are consistent with prior studies that reported neurobehavioral impairments and histological alterations in tobacco-treated animals.

Collectively, these results imply that aqueous *Nicotiana tabacum* extract, despite its ethno-medicinal applications, can exerted harmful systemic effects when consumed excessively or chronically (Zhang *et al*., 2024). The mechanisms appear to involve oxidative stress, inflammation, and membrane disruption across hepatic, renal, and neuronal tissues (Hajam *et al*., 2022).

3.2 CONCLUSION

Nicotiana tabacum aqueous leaf extract elicited dose-dependent hepato-renal and neurotoxicity in rats, likely mediated through oxidative stress and disruption of antioxidant defenses. The present findings highlight the potential risks associated with traditional medicinal use of tobacco extracts and advocate for cautious regulation, dose standardization, and further mechanistic investigation into its bioactive components

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