



Effect of Calcium Carbide-Ripened Bananas and Protective Role of Glycine and Blackseed Oil on Liver and Kidney Function

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ABSTRACT

Background: Artificial fruit ripening with calcium carbide remains widespread in low- and middle-income countries because it is inexpensive and readily available, despite being banned for human consumption. Given the growing presence of chemically ripened fruits in many communities, there is an urgent need to identify affordable dietary interventions that may mitigate organ damage.

Method: Sixty male Wistar rats were divided into ten groups and fed composite diets containing either naturally ripened (control), calcium carbide-ripened, arsenic-contaminated, or market-ripened bananas, with some groups receiving glycine or black seed oil supplementation for four weeks. After treatment, blood, liver, and kidney samples were analyzed for biochemical markers, heavy metal content, and tissue histology.

Results: Results showed that rats fed calcium carbide-ripened or arsenic-contaminated bananas had significantly elevated liver and kidney biomarkers such as AST, ALT, ALP, creatinine, and urea ($p < 0.0001$), indicating organ damage. Histological analysis revealed severe tissue alterations, including hepatic hemorrhage, fibrosis, glomerular shrinkage, and tubular dilation. Heavy metal analysis confirmed high arsenic and cadmium accumulation in these groups.

Glycine supplementation markedly improved biochemical profiles, reduced tissue damage, and lowered heavy metal accumulation, demonstrating strong hepatoprotective and nephroprotective effects. Black seed oil also offered protective benefits, particularly in the market-ripened group, though it was less effective under heavy arsenic exposure. Periodic Acid-Schiff staining showed significant glycogen depletion in affected groups, with partial restoration in those treated with glycine or black seed oil.

Conclusion: Artificially ripened bananas, especially those treated with calcium carbide or arsenic, pose serious risks to liver and kidney health. Glycine consistently mitigated these toxic effects, while black seed oil provided moderate protection. These findings emphasize the urgent need for strict regulation of artificial fruit ripening and highlight glycine as a promising dietary intervention against food-related organ toxicity.

INTRODUCTION

Bananas, *Musaceae* e.g *Musa acuminata*, *Musa balbisiana*, *Musa ornata*, are among the most popular fruits worldwide, valued for their rich nutritional content, affordability, and widespread availability across diverse cultures and economic groups. As a

staple fruit in many tropical and subtropical regions, bananas are consumed daily and make a substantial contribution to the dietary energy intake of millions. However, due to their climacteric nature and short postharvest shelf-life, artificial ripening techniques are often employed to meet consumer demand and

minimise losses during transportation and distribution¹. One of the most common artificial ripening agents in low- and middle-income countries is calcium carbide (CaC₂). When exposed to moisture, calcium carbide releases acetylene gas, which imitates the natural action of ethylene thereby initiating fruit ripening processes such as starch degradation, chlorophyll breakdown, and cell wall softening². Although acetylene effectively induces ripening, calcium carbide is not food-grade and frequently contains hazardous impurities such as arsenic and phosphine (phosphorus hydride). These contaminants are well-known environmental toxicants and have been linked to serious deleterious health effects in both humans and animals³. Organs primarily involved in detoxification such as the kidneys and liver are particularly vulnerable to the toxic effects of environmental agents. The kidneys, which play a vital role in filtering blood, excreting metabolic waste, and maintaining electrolyte balance, are especially susceptible to chemical insults⁴. Chronic exposure to heavy metals like arsenic and cadmium has been shown to impair renal function by inducing oxidative stress, inflammation, glomerular degeneration, and tubular necrosis^{5,6}. Experimental studies have demonstrated that animals fed calcium carbide ripened fruit exhibited elevated serum urea and creatinine levels, along with histological changes such as glomerular atrophy and tubular necrosis consistent with nephrotoxicity^{7,8}. Similarly, the liver, which functions as the body's primary metabolic centre, is another target of chemical toxicity. The liver is responsible for xenobiotic metabolism, enzyme detoxification, and bile production. Exposure to chemical contaminants from ripening agents can disrupt hepatic enzyme activity and compromise membrane integrity, leading to hepatocellular injury. Biomarkers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are frequently elevated in cases of liver damage, reflecting oxidative stress and hepatocellular degeneration⁹. In light of the increasing concerns about food safety, attention has shifted toward the use of natural protective agents that may mitigate organ toxicity caused by chemically ripened fruits. Glycine, a simple non-essential amino acid, has garnered interest due to its antioxidant, anti-inflammatory, and cytoprotective properties. It acts as a free radical scavenger and modulates cellular stress responses, thereby shielding renal and hepatic tissues from chemically induced injury³. Previous studies have demonstrated that glycine supplementation can reduce oxidative damage, decrease inflammatory cytokine expression, and restore structural integrity in organs affected by heavy metals like arsenic¹⁰.

Although glycine has been widely documented for its antioxidative, cytoprotective, and anti-inflammatory roles in mitigating renal and hepatic injury caused by various toxicants, there is limited evidence regarding its effectiveness against calcium carbide-induced toxicity. Given its ability to stabilize cell membranes, attenuate oxidative stress, and modulate inflammatory responses, glycine was selected in this study as a potential dietary intervention¹¹. Similarly, black seed oil (*Nigella sativa*), a natural remedy extensively studied for its antioxidant, anti-inflammatory, and immunomodulatory properties, has demonstrated protective effects against hepatic and renal toxicity induced by heavy metals and xenobiotics. Its bioactive component, thymoquinone, is particularly noted for scavenging free radicals, preserving organ architecture, and improving biochemical markers of liver and kidney function¹². Therefore, its inclusion in this study provides a comparative evaluation of a phytotherapeutic agent alongside glycine, offering insight into two distinct but potentially complementary protective strategies against calcium carbide-induced organ damage.

MATERIALS AND METHODS

Sample Collection

Unripe banana (for controlled ripening) (*Musa acuminata*) samples were obtained from Idi-Oro Market (6.5240981°N, 3.3614536°E) within the Mushin Local Government Area of Lagos state, Nigeria, a major wholesale fruit market in Lagos known for banana sale, while ripe banana samples (*Musa acuminata*) (for the market ripened group) were acquired from retailers at Ojuwoye Market (6.5281536°N, 3.3555239°E), a smaller market compared to Idi-Oro that also retails bananas and is also situated in Mushin Local Government Area of Lagos state, Nigeria. The bananas were all obtained in November 2024.

Feed Preparation

Unripe bananas were purchased from wholesalers at the Idi-Oro Market in Mushin Local Government, Lagos, Nigeria. 3.8kg of bananas were arranged inside a plastic container with a sack lining it. 250g of Calcium Carbide was placed in the center of the container and left for 72 hours. At the end of the 72 hours, there was noticeable condensation in the container, and the bananas were soft to the touch, indicating that they were ripe.

For naturally ripened bananas, 3.8 kg of similarly sourced unripe bananas were put in a plastic container and left for 72 hours to ripen without any artificial ripening agent.

The bananas were then cut and oven dried at 28°C until crisp to touch.

Animals

Male wistar albino rats were purchased from the Animal Centre, College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed under standard environmental conditions (25°C, 12h/12h light/dark cycle) and were fed pelletized with rat chow and tap water *ad libitum*. The rats were acclimatised over four weeks to reach a weight of 150 g-200 g before beginning the experiment. All procedures adhered strictly to the Guidelines for the Care and Use of Laboratory Animals in Research with ethical approval sought from the Animal Ethics Committee of the College of Medicine, University of Lagos. (CMUL/ACUREC/11/24/1735).

Determination of Body Weight

Rats were weighed on a Mettler electronic balance PC 2000 Switzerland before treatment (Day 0) to record initial weight, then weekly for four weeks.

Experimental Design

Sixty male albino rats were divided into 10 groups (n = 6) as follows: GROUP 1(NRB): CONTROL DIET – feed containing 30g naturally ripened banana; GROUP 2 (CRB): feed containing 30g carbide-ripened banana only; GROUP 3 (ARB): ARSENIC DIET – feed containing 0.03ppm arsenic plus naturally ripened banana; GROUP 4 (MRB): MARKET-RIPENED DIET – feed containing 30g market-ripened banana; GROUP 5 (CRBG): feed containing 30 g carbide-ripened banana plus 0.0342g of glycine; GROUP 6 (ARBG): 0.03 ppm arsenic diet plus 0.0342 g of glycine; GROUP 7 (MRBG): 30 g market-ripened diet plus 0.0342 g of glycine; GROUP 8 (CRBB): feed containing 30 g carbide-ripened banana plus 0.2 ml black seed oil; GROUP 9 (ARBB): 0.03 ppm arsenic diet plus 0.2 ml black seed oil; GROUP 10 (MRBB): 30 g market-ripened banana diet plus 0.2 ml black seed oil.

The feed composition is provided in the table 1.

Table 1: Feed composition of animals

S/N	Composition	Normal Ripened Diet (G)(Control)	Carbide Ripened (G)	Arsenic Diet(G)	Market Ripened(G)
1	Dried Naturally Ripened Banana	30	-	30	-
2	Dried Market-ripened Banana	-	-	-	30
3	Carbide Exposed Banana	-	30	-	-
4	Arsenic salt	-	-	0.03 ppm	-
5	Growers Mash				
6	Limestone	50	50	50	50
7	Ferobin (multivitamin) plus	2	2	2	2
8	Palm Oil	5	5	5	5
9	Sugar	10	10	10	10
10	Crayfish	5	5	5	5
11	Groundnut Husk	8	8	8	8

For groups 5, 6 and 7, 0.0342 g of glycine was added to the feed, group 8, 9 and 10 received 0.2 ml of blackseed oil.

The feeding and treatment occurred over four weeks. At the end of this period, blood samples 1 ml were collected from each animal in each group via the retro-orbital venous plexus into blue-cap heparinised bottles. Subsequently, the kidneys,

brains, and livers were harvested and fixed in 4% paraformaldehyde solution for histological analysis and biochemical testing.

Histological Procedures

The tissues were fixed in 4% paraformaldehyde solution and stained with Haematoxylin and Eosin (Bancroft, Layton, and Suvarna, 2019). Sections were cut at five micrometers (5 μm), and slides were examined under magnifications of x100 and x400.

Atomic Absorption Spectrophotometry

One gram of each brain sample was weighed into a 250 ml beaker, followed by the addition of 15 ml of nitric acid (HNO_3). The mixture was heated on a mantle at 105°C for 1 hour and 30 minutes until fully digested. The digest was then diluted to 100 ml with distilled water in a standard flask¹³. Metal concentrations in the solution were measured using Microwave Plasma Atomic Emission Spectroscopy (MPAES), with blank (nitric acid and distilled water) and standard solutions (digested solution of the brain) run for calibration before sample analysis.

Statistics

Data from all groups were compiled and analysed using Two-Way ANOVA and Tukey's Multiple Comparison Test. Results are expressed as Mean \pm SEM (n=6) using GraphPad Prism 6. Significance was set at $p < 0.0001$.

RESULTS

Effects of Feed on Body Weight

There was a noticeable change in average body weight across the ten treatment groups from Week 1 to Week 4. The control group maintained the lowest average weight throughout the four weeks, starting from just above 140 g and increasing slightly over time.

In contrast, (CRB) consistently recorded the highest average weight, peaking around 220 g in Week 1 and maintaining a relatively stable trend throughout, suggesting a potential positive influence of this treatment on body weight.

Groups (ARB) and (ARBG) exhibited lower average weights compared to the other groups, with noticeable dips observed particularly in Week 2 and Week 3, indicating a possible negative effect on weight gain or maintenance.

Groups (MRB), (CRBG), (MRBG), (CRBB), and (ARBB) maintained moderate weight values with minor fluctuations, staying within the 180 g–210 g range. Notably, Group 7 showed a peak in Week 4, suggesting improved weight gain toward the end of the treatment period.

Group (MRBB) experienced a gradual but consistent decline in weight from Week 1 to Week 4, ultimately reaching around 180 g, which may indicate a detrimental effect of the treatment on weight over time.

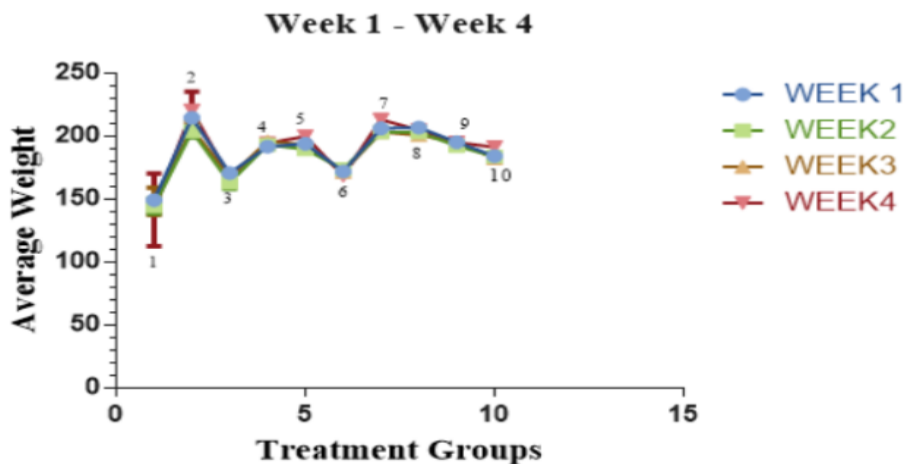


Figure 1: Effect of carbide and arsenic-based diet on body weight after 4 weeks of administration, Values expressed as a chart. 1=normal ripened banana-fed group (NRB), 2= Carbide-ripened banana-fed group (CRB), 3= Arsenic-ripened banana-fed group (ARB), 4=Market ripened banana group (MRB), 5= Carbide-ripened banana + glycine-fed group (CRBG), 6=Arsenic ripened banana + glycine-fed group (ARBG), 7=Market ripened banana + glycine-fed group (MRBG), 8=Carbide ripened banana + black seed oil (CRBB), 9= Arsenic-ripened banana + black seed oil (ARBB), 10= Market-ripened banana + black seed oil (MRBB).

Effect of various diets on Blood biochemistry

Animals in the control group (Group 1), which received naturally ripened bananas, maintained normal liver and kidney profiles, serving as the baseline for comparison. The group that was fed only carbide-ripened bananas showed elevated levels of AST, ALT, and ALP, indicating liver injury likely due to the hepatotoxic effects of calcium carbide ($p < 0.0001$). Similarly, Group 3, which received arsenic with naturally ripened bananas, showed mild liver enzyme elevations ($p < 0.0001$), pointing to hepatic stress induced by arsenic. Notably, Group 4, fed market-ripened bananas, showed less severe liver dysfunction, suggesting a comparatively lower toxicity than arsenic or carbide alone.

Intervention groups that included glycine (Groups 5–7) or black seed oil (Groups 8–10) demonstrated varying levels of protection. Group 5 (Carbide + glycine) recorded significantly reduced ALP compared to control and a notable increase in

albumin ($p < 0.0001$), indicating improved liver synthetic function and reduced cholestasis. Group 8 (Carbide + black seed oil) also showed lowered ALP ($p < 0.0001$), suggesting hepatoprotection by black seed oil. Among the arsenic-fed groups, Group 6 (Arsenic + glycine) maintained more stable liver enzymes than Group 9 (Arsenic + black seed oil), highlighting glycine's superior protective effect in arsenic toxicity.

Regarding kidney function, elevated creatinine levels in Groups 6, 8, and particularly Group 9 (Arsenic + black seed oil) point to renal impairment. Group 9 had the highest creatinine value, suggesting that black seed oil was less effective in mitigating arsenic-induced nephrotoxicity. Urea levels were moderately elevated in these groups as well, reinforcing signs of reduced renal clearance. Electrolyte values remained within physiological ranges, though slight variations were noted, such as elevated sodium in Group 10.

The values are represented in Table 2a and 2b.

Table 2a: Blood biochemistry results of the analysed blood samples

Enzyme	(NRB)	(CRB)	(ARB)	(MRB)	(CRBG)
AST	26.33±6.84	37.67± 9.94	23.67± 4.18	27.33± 4.91	34.67± 4.70
ALT	18.00±0.58	21.33± 3.28	17.00±2.52	12.00± 1.16	16.67± 1.21
ALP	43.00±9.54	35.00± 3.79	34.00±4.16	29.00± 1.53	25.00± 3.46 ^A
PROTEIN	70.43±5.33	82.10± 3.97	79.40± 5.68	78.57± 3.33	79.27± 1.53
ALBUMIN	39.03±0.93	41.23± 0.79	41± 0.35	36.70± 2.31	52.53± 9.54
T BIL	3.97± 0.18	4.17± 0.15	3.7±0.15	3.9 ±0.1	3.47± 0.19
D BIL	0.67± 0.67	0.8±0.0	0.67± 0.09	0.7±0.06	0.77± 0.09
UREA	5.10± 0.69	6.07±0.09	6.23±0.32	4.87±1.02	5.03± 0.43
CREATININE	68.43±10.89	66.47± 6.37*	67.40± 6.09*	57.67± 8.0*	72.37± 8.0
Na	143.03 ± 0.41	139.37± 1.80	139.63± 1.67	138.90± 2.42	140.57± 2.67
K	4.90 ± 0.1	4.93± 0.12	4.8± 0.25	4.6±0.1	4.77± 0.07
CL	102.57±1.96	103± 1.29	101.63± 1.59	102.20± 1.22	102.57± 1.61
HCO ₃	19.17 ±0.33	19.167 ± 0.69	19.7 ± 0.98	20.83±1.15	19.07± 0.48

Values are expressed as mean ± Standard Error of Mean (SEM), and two-way ANOVA and Dunnett's & Tukey's multiple comparison test was used to check the level of significance and analysis of variance, where n=6 normal-ripened banana-fed group (NRB), carbide-ripened banana-fed group (CRB), arsenic-ripened banana-fed group (ARB), market-ripened banana group (MRB), carbide-ripened banana + glycine-fed group (CRBG), arsenic-ripened banana + glycine-fed group. T BIL= total bilirubin, D BIL= Direct bilirubin, HCO₃= Bicarbonate ion

A: $p < 0.0001$ significant compared to control, B: $P < 0.0001$ significant compared to control, C: $P < 0.0001$ significant compared to control D: $P < 0.0001$ significant compared to control, D: $P < 0.0001$ significant compared to control, E: $P < 0.0001$ significant compared to control *: $P < 0.0001$ significant compared to Group 9, **: $P < 0.0001$ significant compared to Group 4

Table 2b: Blood biochemistry results of the analysed blood samples

Enzyme	(ARBG)	(MRBG)	(CRBB)	(ARBB)	(MRBB)
AST	37.00± 4.0	32.33± 5.81	30.33± 6.12	25.00± 6.51	34.00± 2.22
ALT	17.33± 0.88	19.67± 5.67	16.67± 0.67	15.33± 0.88	20.33 ± 1.76
ALP	26.67± 1.20 ^B	28.00± 3.22 ^C	32.33± 1.33	36.67± 2.33	25.33± 1.67 ^D
PROTEIN	76.57± 2.31	74.00± 3.62	73.33 ± 1.69	80.23 ± 1.11	79.10± 4.91
ALBUMIN	39.77± 1.82	41.63± 4.71	38.83± 0.38	40.93 ± 2.32	39.47± 2.71
T BIL	3.37± 0.12	3.87± 0.24	4.07± 0.29	3.57± 0.17	3.47 ± 0.23
D BIL	0.70± 0.12	0.60± 0.06	0.80± 0.06	0.70± 0.06	0.83± 0.03
UREA	7.13± 0.81	5.63± 1.10	4.90 ± 0.42	8.20± 2.03	4.83± 0.46
CREATININE	75.30± 3.90 ^{**}	70.40± 7.40 [*]	64.53 ± 3.38 [*]	87.00± 14.18 ^E	73.30± 14.75
Na	139.47±0.75	139.47±4.33	143.30 ± 2.01	139.87± 1.94	146.33± 7.84
K	4.57±0.03	4.33± 0.24	4.73± 0.22	4.87± 0.19	4.90 ± 0.15
CL	100.03±1.98	102.90±1.01	102.00± 1.30	102.60± 1.39	103.80± 2.44
HCO ₃	21.10±2.21	19.83± 0.29	20.83 ± 1.42	20.77± 0.92	20.50 ± 0.75

Values are expressed as mean ± Standard Error of Mean (SEM), and two-way ANOVA and Dunnett's & Tukey's multiple comparison test was used to check the level of significance and analysis of variance, where n=6 Arsenic-ripened banana + glycine-fed group (ARBG), market-ripened banana + glycine-fed group (MRBG), Carbide ripened banana + black seed oil (CRBB), Arsenic-ripened banana + black seed oil (ARBB), and market-ripened banana + black seed oil (MRBB).

T BIL= total bilirubin, D BIL= Direct bilirubin, HCO₃= Bicarbonate ion

A: p<0.0001 significant compared to control, B: P<0.0001 significant compared to control, C: P<0.0001 significant compared to control D: P<0.0001 significant compared to control, D: P<0.0001 significant compared to control, E:P<0.0001 significant compared to control *: P<0.0001 significant compared to Group 9, **: P<0.0001 significant compared to MRB.

HEAVY METAL ANALYSIS OF ORGANS

As shown in the Bar chart below (fig 2.), there were significantly elevated (p<0.0001) levels of arsenic and cadmium observed in the groups administered calcium carbide-ripened bananas, arsenic-contaminated feed, and market-ripened bananas, with the highest accumulation of arsenic recorded in (MRBB) (66.67 ± 18.75 mg/kg). ARBB also showed the highest cadmium level (29.56 ± 8.76 mg/kg), indicating a marked toxic burden in the organs.

Groups treated with glycine (Groups 5–7) and black seed oil (Groups 8–10) exhibited relatively lower

concentrations of most metals like zinc, copper, nickel, iron and chromium compared to their respective toxicant-only groups, suggesting a partial protective or detoxifying effect. Other metals, such as nickel, copper, and lead, showed moderate variations across groups but remained elevated in carbide and arsenic-exposed rats. Zinc and iron levels fluctuated mildly without a consistent pattern of significance.

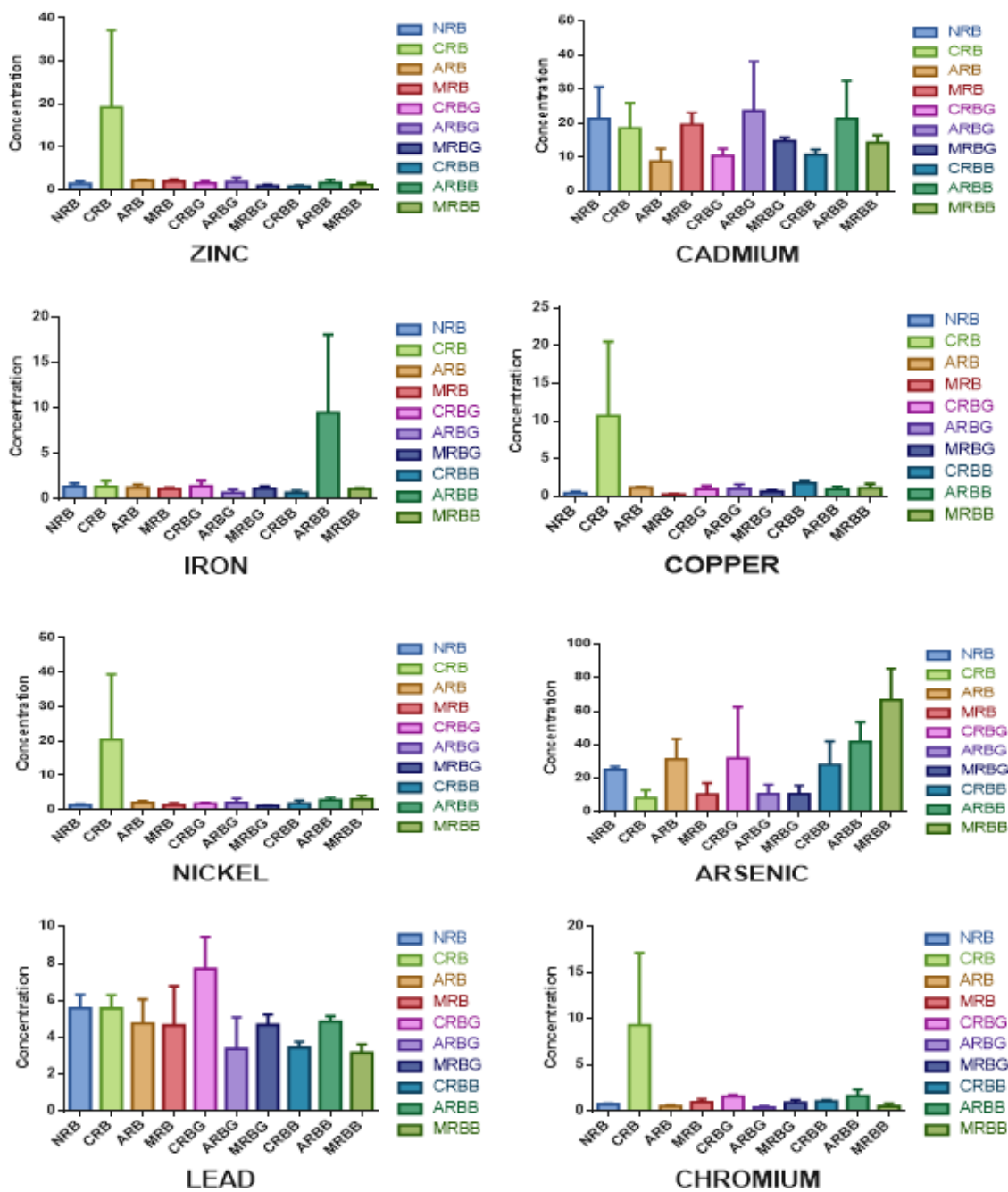


Figure 2: Bar chart showing heavy metal contents in the brain.

Values are expressed as mean \pm Standard Error of Mean (SEM), and two-way ANOVA and Dunnett's & Tukey's multiple comparison test was used to check the level of significance and analysis of variance, where $n=6$.

Histopathological Assessment of Liver (H&E, x400)

Histological examination of liver tissues stained with hematoxylin and eosin (H&E) at $\times 400$ magnification revealed distinct structural variations across treatment groups. In the control group, liver sections exhibited normal hepatic architecture with well-arranged hepatocytes, visible Kupffer cells, and an intact central vein. No pathological lesions were observed.

The group fed with carbide-ripened banana showed mildly dilated sinusoids but preserved tissue organisation, with no evidence of necrosis or fibrosis, suggesting only minor structural alterations. In contrast, Group 3 (arsenic + naturally ripened banana) displayed marked histopathological disruptions, including haemorrhagic sinusoidal spaces, dense mononuclear infiltrates, and extensive fibrosis within the portal area, indicative of significant arsenic-induced hepatotoxicity.

Liver sections from the group fed market-ripened banana revealed areas of parenchymal degeneration and haemorrhage, suggesting hepatocellular damage possibly linked to chemical residues from artificial ripening agents. The group that received carbide + glycine demonstrated dilated, congested blood vessels alongside focal areas of fibrosis, indicating partial amelioration of structural damage by glycine.

The groups fed with arsenic + glycine and market + glycine showed dilated congested vessels but lacked extensive fibrosis or necrosis, suggesting glycine may exert moderate hepatoprotective effects against arsenic and market-ripening-associated injury.

The group fed with carbide + black seed oil presented with similar vascular congestion, accompanied by focal parenchymal vacuolation and the presence of greenish-brown pigments, which may reflect mild hepatic stress or pigment accumulation due to oxidative metabolism.

In the group that received arsenic + black seed oil, focal parenchymal degeneration and fibrosis were still observed, suggesting that black seed oil conferred limited protection against arsenic-induced liver damage. However, the group that received the market-ripened feed + black seed oil showed normal hepatocyte arrangement with only mild central vein dilation and no observable lesions, indicating substantial histological preservation and hepatoprotection by black seed oil in the context of market-ripened banana exposure.

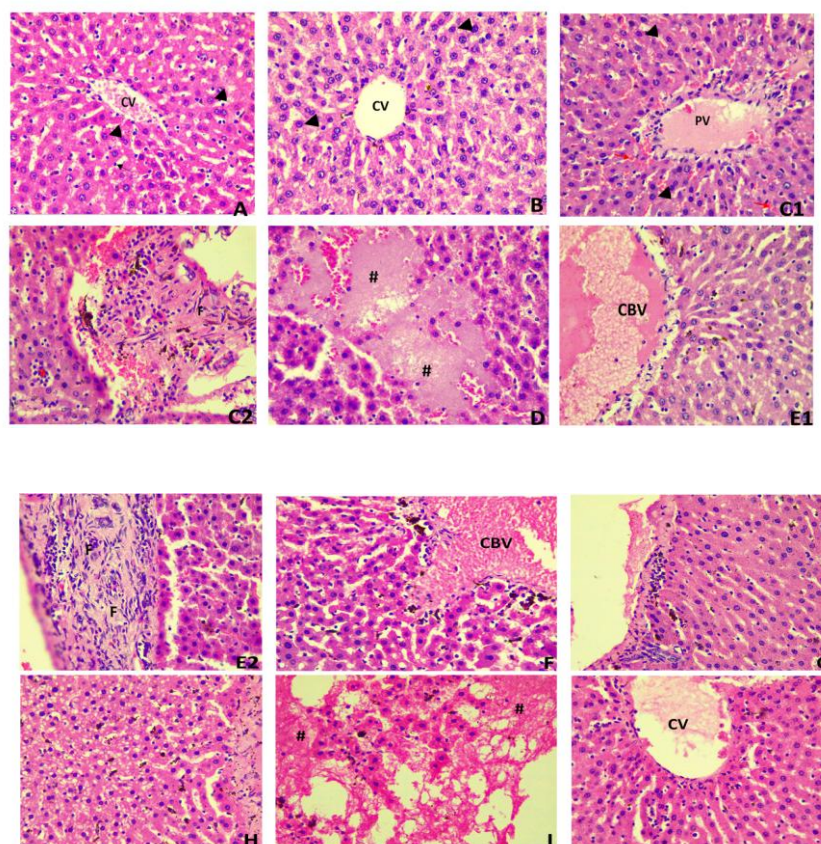


Plate 1 (A-J): Photomicrograph of Sections from the liver showing Hepatocytes(arrowhead). A=no lesion observed in the Kupffer cells (Thin Black arrow), and Central vein (CV) in Control group, B= no lesion observed in the Central vein (CV), and mild dilation of sinusoid in the carbide ripened banana fed group, C1= The sinusoidal spaces were haemorrhagic in the group fed arsenic diet, C2=Mononuclear infiltrates (asterisks) and portal area(PT) with extensive fibrosis (F), D=areas of parenchymal degeneration (#)and haemorrhage in the market ripened banana fed group E1 & E2= dilated congested vessels (CBV), and a focal area of extensive fibrosis (F) in the (carbide + glycine) fed group, F=dilated congested vessels (CBV) in the (arsenic+ glycine) fed group, G=dilated congested vessels (CBV) in the (market banana + glycine) fed group, H=dilated congested vessels (CBV),A focal area of vacuolation in the parenchymal and greenish-brown pigments seen in the (carbide+blackseed oil) fed group, I= A focal area of parenchymal degeneration (#) fibrosis in the (Arsenic+ blackseed oil) fed group, J=no lesion observed in Kupffer cells (Thin Black arrow), and mildly dilated Central vein (CV) in the market ripened banana group + black seed oil. H&E x400.

Histopathological Assessment of Liver (PAS, x400)

The Periodic acid-Schiff-stained liver sections were examined across all groups to evaluate glycogen content in hepatocytes, and the result is given below: In (NRB), (CRB), (ARB), MRB, ARBG, MRBG, CRBB, and MRBB-fed groups, no magenta staining was observed in the hepatocytes, indicating glycogen depletion. While in the CRBG-fed group, the

presence of occasional mild cytoplasmic tint may reflect residual or non-glycogenic PAS-reactive substances.

The ARBB-fed group showed clear magenta staining in hepatocyte cytoplasm, indicating the presence of glycogen and suggesting the preservation or restoration of liver glycogen.

The images of the slides are shown in Plate 2.

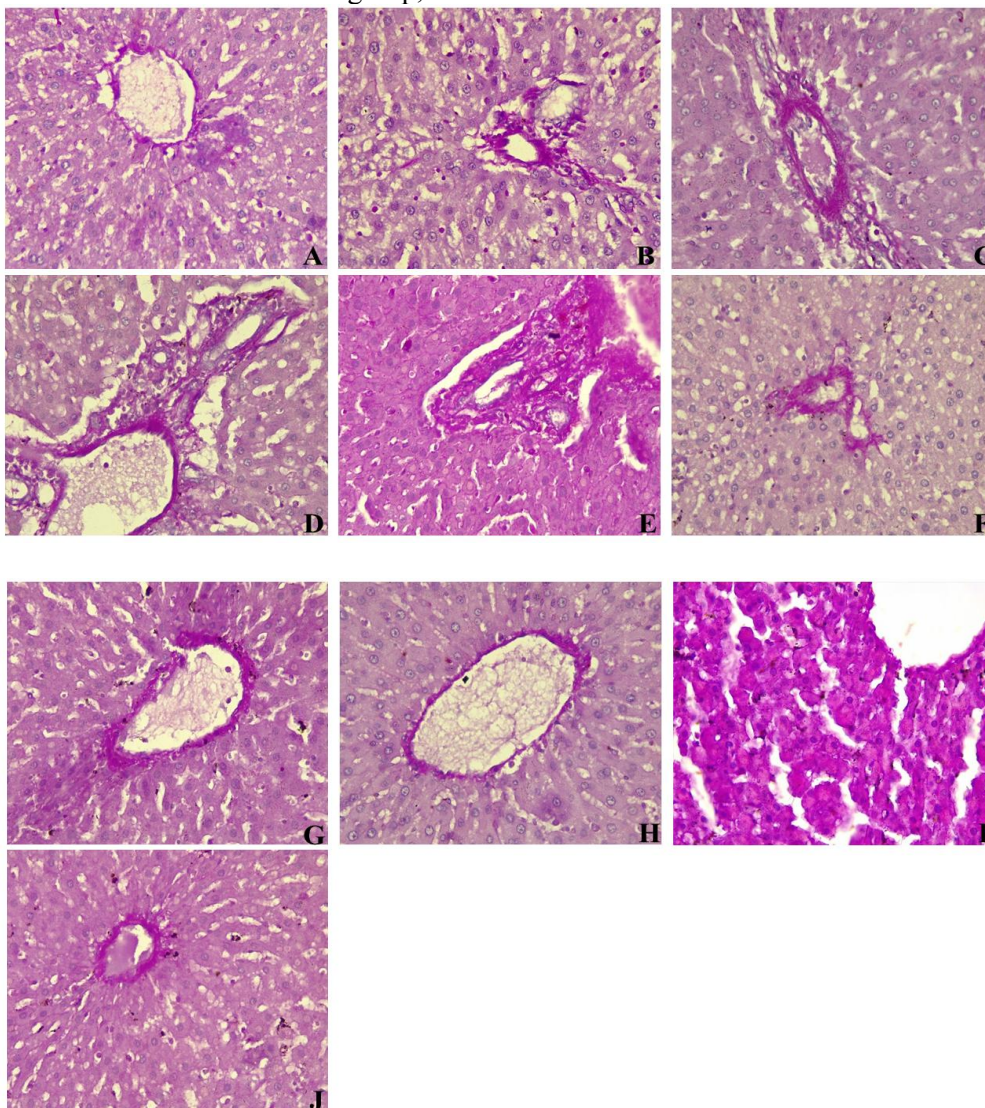


Plate 2 (A-J): Photomicrograph of Sections from the liver showing absence of magenta staining in hepatocytes, indicating glycogen depletion, E= Minimal to absent PAS reflecting presence of residual or non-glycogenic Periodic Acidic Schiff reactive substances in CRBG fed group, I= Presence of magenta cytoplasmic deposits indicative of glycogen accumulation in hepatocytes in the ARBB fed group. PAS x400.

Histopathological Assessment of the Kidney

Light microscopic examination of hematoxylin and eosin stained renal sections at $\times 400$ revealed treatment-dependent intrinsic alterations in glomerular and tubular architecture, interstitium, and vasculature.

Group 1 (Control — naturally ripened banana). Renal parenchyma exhibited normal architecture: well-

formed glomeruli with open capillary tufts, intact Bowman's spaces, and orderly proximal and distal tubules. The interstitium was unremarkable, with no congestion, inflammatory infiltrates, fibrosis, or necrosis.

Group 2 (Calcium carbide-ripened). Sections showed early nephrotoxic changes characterized by glomerular shrinkage and mild tubular dilatation.

Occasional interstitial vascular congestion and capillary engorgement were seen. There was no widespread tubular necrosis or established interstitial fibrosis, consistent with mild-to-moderate injury.

Group 3 (Arsenic + naturally ripened). Pronounced renal damage was evident: markedly shrunken glomeruli, extensive degeneration of tubular epithelial cells with loss of brush border, prominent interstitial edema, and dense mononuclear inflammatory infiltrates. Focal areas of interstitial fibrosis were present, indicating more advanced and chronic injury.

Group 4 (Market-ripened). Renal changes were moderate and focal, consisting of tubular epithelial degeneration, mild glomerular atrophy, and patchy interstitial congestion. These features suggest chemical-induced stress without the severe fibrosis or widespread necrosis seen in arsenic exposure.

Group 5 (Carbide + glycine). Glycine co-treatment attenuated carbide-associated damage: glomerular and tubular abnormalities were milder than in Group 2, with reduced vascular congestion and minimal interstitial fibrosis. Residual, mostly mild, tubular changes persisted.

Group 6 (Arsenic + glycine) and Group 7 (Market + glycine). Both groups exhibited attenuated injury

relative to their toxin-only counterparts. Tubular and interstitial alterations were present but less severe, with decreased inflammatory infiltrates and limited fibrosis, indicating partial nephroprotection by glycine.

Group 8 (Carbide + black seed oil). Mild glomerular and tubular alterations were present alongside focal interstitial congestion and occasional pigment-laden cells, suggesting low-grade oxidative or pigment-related changes.

Group 9 (Arsenic + black seed oil). Renal sections revealed focal glomerular and tubular degeneration with limited interstitial fibrosis, consistent with partial mitigation of arsenic toxicity by black seed oil.

Group 10 (Market + black seed oil). Kidneys were largely preserved, showing near-normal glomerular and tubular architecture with only mild vascular congestion, indicating substantial protective effects of black seed oil against lesions associated with market-ripened banana exposure.

Overall, the histology demonstrates a spectrum of intrinsic renal injury from minimal (control) to severe (arsenic), with both glycine and black seed oil providing varying degrees of structural protection, with black seed oil showing particularly marked preservation in the market-ripened model.

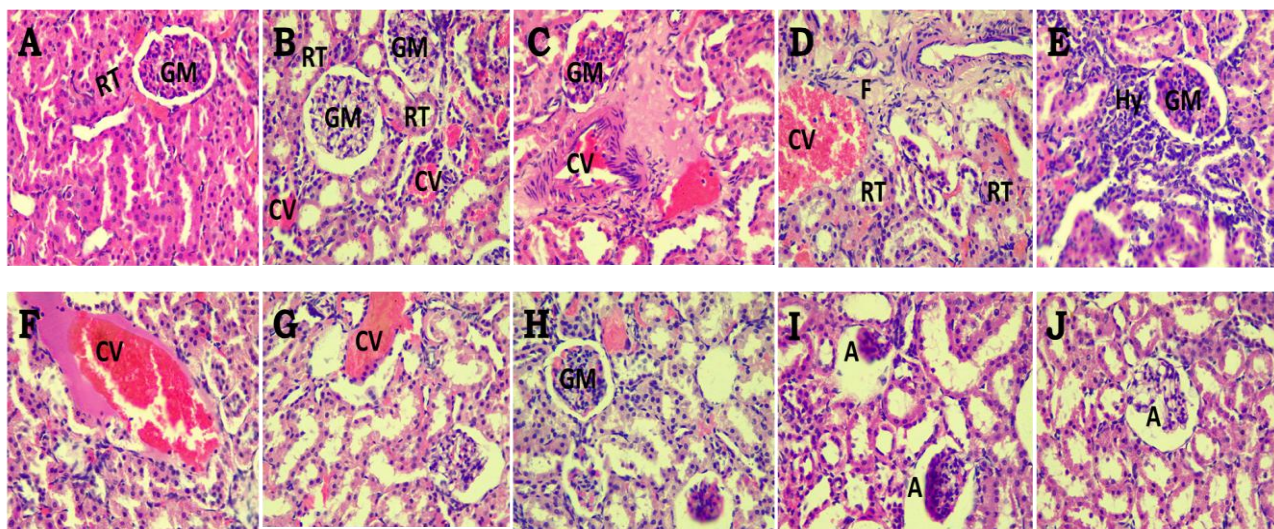


Figure 5(A-J): Photomicrograph of kidney section showing Glomeruli (GM), renal tubule (RT). A section showing that no lesion was observed. B section showing dilated renal tubules (RT) and congested blood vessels (CV). C-section showing congested blood vessels (CV) with thickened wall. D section showing congested blood vessels (CV) with thickened wall and perivascular fibrosis (F). E section showing congested blood vessels (CV) and area of hyperplasia (Hy) in the renal parenchyma. F section showing dilated congested blood vessels (CV). G section showing distended renal tubules (RT), dilated congested blood vessels (CV). H section showing dilated congested blood vessels (CV). There was focal atrophic glomerulus (A). I section showing dilated congested blood vessels (CV). There were focal atrophic glomeruli (A). J kidney section showing that there was a focal atrophic glomerulus (A). Haematoxylin and Eosin stain, x400 magnification.

DISCUSSION

The liver, as the central organ responsible for xenobiotic metabolism, glycogen storage, and protein synthesis, is highly vulnerable to toxic insults from contaminated or chemically altered food sources. The present study demonstrates that exposure to artificially ripened bananas, particularly those treated with calcium carbide or contaminated with arsenic, induces significant hepatic dysfunction. In contrast, co-administration of glycine and black seed oil provided varying degrees of hepatoprotection, as supported by biochemical, histological, and heavy metal profiling.

Biochemical markers such as AST, ALT, and ALP were markedly elevated in animals fed with carbide-ripened bananas and arsenic-contaminated diets, reflecting hepatocellular injury and impaired biliary function. These findings are consistent with an earlier study as reported in Nigeria,¹⁴ where comparable enzyme elevation and histological changes were observed following consumption of artificially ripened fruits, further confirming the toxicological relevance within local contexts.

Histological assessments revealed severe architectural disruption in the arsenic group, characterised by hemorrhagic sinusoids, mononuclear infiltration, and fibrosis, thereby confirming arsenic's strong hepatotoxic potential. Market-ripened bananas caused moderate degeneration and haemorrhage, likely due to a mixture of artificial agents, while carbide-ripened bananas produced only mild sinusoidal dilation, suggesting an early stage of hepatic stress. This implies that the liver retains some compensatory capacity under lower exposures, but persistent or higher doses could precipitate more extensive injury. Intervention groups receiving glycine demonstrated marked biochemical and histological improvement. Reduced ALP levels, increased albumin, and minimal fibrosis in the carbide + glycine group indicated glycine's restorative role in liver function. Literature supports these findings: as it has been shown that glycine attenuates liver injury through Nrf2 activation and NF- κ B inhibition, hence enhancing glutathione synthesis, and prevents apoptosis¹⁵. A Nigerian data further reinforce these protective effects, reporting similar enzyme normalization in toxin-exposed models treated with glycine¹⁴.

Black seed oil supplementation also produced hepatoprotective outcomes, especially in animals fed market-ripened bananas where near-normal histology and enzyme levels were maintained. This observation agrees with Danaei et al. (2022)¹⁶, who attributed hepatoprotection to thymoquinone's ability to preserve hepatocyte integrity. Opute et al. (2024)¹⁷ further confirmed similar benefits in Nigerian rat

models. However, under high arsenic burden, black seed oil provided only partial protection, as evidenced by persisting fibrosis and degeneration. This suggests that while effective under moderate toxicity, its protective capacity may be overwhelmed at higher toxic loads^{16,18}.

Heavy metal analysis using Atomic Absorption Spectrophotometry (AAS) corroborated these patterns. Elevated hepatic arsenic and cadmium were detected in arsenic-exposed groups, while glycine- and black seed oil-treated groups exhibited reduced accumulation, supporting their detoxification potential. Both agents are known to enhance antioxidant activity and facilitate chelation of harmful metals, which explains the reduced tissue burden observed¹⁷.

Beyond structural preservation, biochemical markers also reflected improved protein synthesis and carbohydrate metabolism. Glycine treatment was associated with restored albumin levels and better glycogen storage, reflecting preserved metabolic functions. These effects may stem from its support of mitochondrial stability and metabolic pathways, as previously reported by Heidari et al. (2022)¹⁹. The preservation of albumin synthesis and glycogen metabolism underscores the broader therapeutic potential of glycine in maintaining liver function.

Glycogen content was further confirmed by Periodic Acid-Schiff (PAS) staining. Most groups exposed to arsenic and carbide showed little or no PAS-positive staining, suggesting glycogen depletion and impaired carbohydrate metabolism. This aligns with reports that loss of PAS staining indicates hepatocellular injury and metabolic stress²⁰. Interestingly, the arsenic + black seed oil group displayed clear PAS-positive staining, suggesting partial restoration of glycogen stores. This highlights the combined antioxidant and membrane-stabilizing effects of glycine and black seed oil, which support metabolic recovery²¹. In contrast, the carbide + glycine group displayed only faint PAS staining, indicating that while glycine offers significant protection, it may not fully restore glycogen metabolism in isolation.

Taken together, these findings confirm that chemically ripened bananas, particularly those treated with calcium carbide or arsenic, induce significant hepatic dysfunction through oxidative stress, heavy metal accumulation, and structural damage. Glycine emerged as a potent hepatoprotective agent, functioning through antioxidant, anti-inflammatory, and metabolic stabilizing mechanisms. Black seed oil also contributed meaningful protection, although its efficacy was more limited under heavy toxicant burden. These results validate both local and international reports and highlight the promise of dietary interventions as practical safeguards against

food-related liver toxicity. The kidney, as the body's primary organ for blood filtration and xenobiotic excretion, also exhibited pronounced vulnerability to chemically ripened bananas and arsenic contamination. Rats fed calcium carbide-ripened bananas developed early nephrotoxic changes, including shrunken glomeruli, dilated tubules, and vascular congestion, resembling previous reports in rodents⁷. With arsenic exposure, the renal damage worsened, showing tubular degeneration, oedematous interstitium, and patchy fibrosis, consistent with heavy-metal nephropathy described by Sanches and Andrade (2023)⁸.

Serum biochemical indices supported these observations, with arsenic and carbide-exposed groups showing elevated creatinine and urea levels, indicating compromised glomerular filtration. The most severe creatinine increase was seen in the arsenic + black seed oil group, suggesting that protective capacity diminishes under higher toxic burdens. AAS results reinforced this link, revealing the highest arsenic and cadmium accumulation in toxin-only groups. These metals generate reactive oxygen species, disrupt mitochondrial respiration, and activate fibrogenic signalling, explaining the observed pathology²².

Glycine supplementation provided substantial nephroprotection, as shown by improved renal histology, lower creatinine and urea levels, and reduced arsenic and cadmium accumulation. These findings are consistent with its known antioxidant role, glutathione enhancement, and suppression of oxidative cascades²³. Black seed oil also provided protective effects, though primarily in moderate exposure contexts. In animals fed market-ripened bananas, kidney structure and function were largely preserved, consistent with prior reports²⁴. However, its protective effect was inadequate under heavy arsenic load, suggesting dose-dependent limitations. Collectively, the evidence indicates that calcium carbide-based ripening and arsenic contamination of bananas pose significant hepatic and renal hazards through heavy-metal accumulation and oxidative stress. Glycine which can be sourced from legumes, cereals and grains, animal sources locally consistently mitigated these effects, offering robust and broad-spectrum protection, while black seed oil which can be sourced from seeds of *Nigella sativa* offered partial but valuable benefits under moderate exposures. These results underscore the urgent need for stricter regulation of fruit-ripening practices and highlight the potential of low-cost nutraceutical strategies, particularly glycine supplementation, as practical interventions in populations exposed to chemically ripened produce.

Conclusion

This study confirms that consumption of artificially ripened bananas, particularly those ripened with calcium carbide or contaminated with arsenic, can induce significant liver and kidney damage. However, dietary supplementation with glycine and black seed oil demonstrated varying degrees of organ protection, with glycine showing stronger efficacy, especially against arsenic-induced toxicity.

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