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Comparative Hepatotoxic and Nutritional Effects of Different Forms of Groundnut (*Arachis hypogaea* L.) Seed Diets in Wistar Albino Rats

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Abstract

Arachis hypogaea L. (groundnut) is a rich source of phytochemicals, vitamins, minerals, and healthy fats, and has been widely recognized for its potential in supporting cardiovascular health and preventing chronic diseases. However, the impact of different preparation methods, particularly cooking and grilling, on liver function remains poorly understood. This study investigates the effects of cooked and grilled ground nut seeds on liver function parameters, specifically total and direct bilirubin levels, in Wistar albino rats. Twenty-five Wistar albino rats were randomly assigned to five groups (n = 5 per group). Group A served as the control and received standard feed. Groups B and C were fed diets supplemented with 25% and 50% grilled groundnut mixtures, respectively, while Groups D and E received 25% and 50% cooked groundnut mixtures. Rats were monitored for changes in body weight and liver function parameters over four weeks. At the end of the experiment, serum levels of total and direct bilirubin were measured. All groups exhibited weight gain over the study period, with Group A (control) showing the most consistent increase. Groups B and C (grilled ground nut) gained slightly less weight than the control, while Groups D and E (cooked groundnut) showed slower weight gain. Total bilirubin levels were elevated in all treatment groups, with Group B (25% grilled) exhibiting the highest levels. Grilled groundnut at 50% significantly increased direct bilirubin levels, while cooked groundnut showed no significant effect on direct bilirubin. The method of groundnut preparation significantly influences liver function parameters in Wistar rats. Grilling groundnuts, particularly at higher concentrations, appears to negatively impact both total and direct bilirubin levels, while cooking poses minimal risk. These findings highlight the need for further studies to determine optimal cooking conditions and their implications for liver health.

Keywords: Arachis hypogaea; groundnut; liver function; bilirubin; Wistar rats.

INTRODUCTION

A healthy diet is essential for maintaining physiological functions and preventing chronic diseases. It requires a balanced intake of macronutrients—carbohydrates, proteins, and fats—as well as micronutrients, including vitamins and minerals (Agrawal et al., 2018; Arya et al., 2016). While macronutrients supply the energy needed for cellular processes, micronutrients are involved in enzyme function, metabolism, immunity, and overall physiological balance (Arya et al., 2021).

Among plant-based sources of nutrition, groundnut (*Arachis hypogaea* L.), commonly referred to as peanut, is one of the most widely cultivated legumes globally. Botanically a legume yet nutritionally akin to oilseeds, peanuts are known for their high fat content and exceptional nutrient density (Augustine et al., 2020). Originally domesticated in South America, groundnuts are now cultivated worldwide, particularly in countries like China, India, Nigeria, the United States, Argentina, and Indonesia (Barros et al., 2018; Bimro et al., 2020). In 2019, global peanut production reached approximately 48 million tons (Breimer & Cederbaum, 2020). In the U.S. and Europe, peanuts contribute significantly to oilseed intake (Cornil et al., 2018).

Peanuts are classified into four major types—Virginia, Runner, Spanish, and Valencia—based on seed size, oil content, and economic use (Griel et al., 2021; Guyton & Hall, 2020). Runner types are commonly used in peanut butter, while Spanish types are preferred for oil extraction (Juturu et al., 2018; Griel et al., 2021). Valencia and Virginia types are valued for sweetness and snack production (Kumar et al., 2017; Kumar et al., 2018).

Nutritionally, peanuts are rich in unsaturated fats such as oleic and linoleic acids that improve lipid profiles (Latorz et al., 2020). They are also low in saturated fats (Mentes et al., 2013). Additionally, peanuts provide high-quality protein comparable to animal sources (Mayo et

al., 2015). However, allergenic proteins resistant to digestion may pose risks (Monda et al., 2015; Mottram et al., 2012).

Peanuts are also excellent sources of micronutrients such as vitamin E and B-complex vitamins (Ogunniyi et al., 2022; Prasad et al., 2013). Their antioxidant composition contributes to anti-inflammatory and anticancer effects (Reuter et al., 2018; Torres et al., 2019). Peanut skins also contain beneficial antioxidants (Router et al., 2015).

Peanut consumption offers numerous health benefits:

- Improves cardiovascular health (Sampson et al., 2019; Schwertner & Vitek, 2015)
- Supports weight management (Settaluri et al., 2017)
- Helps prevent diabetes (Settaluri et al., 2019)
- Reduces inflammation (Singh et al., 2017; Sies et al., 2012)
- Provides essential nutrients (Xu et al., 2019)

Processing methods such as roasting and grilling can enhance flavor but may introduce oxidative products (Sobal et al., 2017; Sobal et al., 2019). Non-alcoholic fatty liver disease (NAFLD) is strongly linked to poor diet and metabolic syndrome (Swinburn et al., 2019; Zhang et al., 2019). Nut consumption may reduce NAFLD risk through antioxidant effects (Torres et al., 2020; Vitek & Schwertner, 2017).

MATERIALS AND METHODS

Equipment and apparatus

The major equipment used for this study included a water bath (KJ430), an electronic weighing scale (G & G JJ300), a centrifuge (PEC Medical, USA), a spectrophotometer (KJ-721G), a refrigerator (Hisense), and a manual grinding machine (Corona). Additional laboratory apparatus included pipetting devices, burettes, test tubes, volumetric flasks, and conical flasks for the preparation and analysis of samples.

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and sourced from reputable suppliers. The key reagents included thiobarbituric acid (TBA), hydrochloric acid, ethanol, distilled water, and acetone. Randox diagnostic kits were employed for the quantitative determination of liver function parameters, specifically direct and total bilirubin levels, in accordance with the manufacturer's protocols.

Feed and sample collection

The basal feed used in the experiment was purchased from LINODEC Feed Dealer, Awka, Anambra State, Nigeria. Raw groundnut seeds were sourced from St. Margaret International Market, Abakaliki, Ebonyi State, Nigeria. The feed was formulated by mixing the processed groundnut (either cooked or grilled) with the basal feed in varying proportions, as described below.

Experimental animals and housing

A total of twenty-five (25) healthy Wistar albino rats, aged twelve weeks and weighing between 130 g and 150 g, were procured from the animal house of Chris Experimental Animal Farm and Research Laboratory. The animals were housed in well-ventilated aluminum cages, with five rats per cage measuring 150 cm × 150 cm. Clean sawdust was used as bedding, dried for two weeks before the rats' arrival. The cages were cleaned daily, and feed and water were replenished regularly. The animals were acclimatized for seven days before the commencement of the experiment.

Feed preparation

The collected groundnuts were cleaned to remove dirt, damaged seeds, and other foreign materials and then divided into two equal portions. The first portion was subjected to boiling by immersing the groundnuts in clean water and boiling at approximately 100 °C until adequately cooked. The boiled groundnuts were drained and spread in clean trays to cool and dry at room temperature until a sufficiently low moisture content was achieved for grinding. The second portion was grilled by exposing the groundnuts to dry heat with periodic turning to ensure uniform heating and prevent excessive charring. After grilling, the samples were allowed to cool and were further dried at room temperature where necessary.

Both the boiled and grilled groundnut samples were therefore adequately dried before grinding. Each processed sample was separately milled into a fine powder using a clean manual grinding machine. The resulting boiled-groundnut and grilled-groundnut powders were stored separately in clean, airtight containers until feed formulation. Appropriate quantities of each processed groundnut powder were subsequently incorporated into the basal feed according to the predetermined proportions for the respective experimental treatment groups. The ingredients were thoroughly mixed to ensure uniform distribution before the formulated diets were administered to the experimental animals.

Experimental design

The twenty-five Wistar rats were randomly assigned to five groups, with five rats per group. Group A served as the control and received only the standard basal feed. Group B received a diet containing 25% grilled groundnut and 75% basal feed. Group C received 50% grilled groundnut and 50% basal feed. Group D was fed with 25% cooked groundnut mixed with 75% basal feed, while Group E received 50% cooked groundnut mixed with 50% basal feed. The feeding experiment lasted for

four weeks, during which time the animals were monitored daily.

Determination of body weight

The body weight of each rat was measured weekly using an electronic weighing scale (G & G JJ300). Weight measurements were recorded at baseline and at weekly intervals to monitor the effect of the different diets on weight gain.

Liver function tests

At the end of the feeding period, the rats were euthanized, and blood samples were collected via cardiac puncture. Serum was separated by centrifugation and analyzed for liver function parameters, including total bilirubin and direct (conjugated) bilirubin, using Randox diagnostic kits. All procedures were carried out in accordance with the protocols provided by the manufacturer Guyton and Hall (2000).

Determination of direct bilirubin

The direct bilirubin concentration was determined using the colorimetric method described by Guyton and Hall (2000). In this method, direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in an alkaline medium to produce a blue-colored complex. The reaction mixture was prepared by combining 200 µL of Reagent 1, 200 µL of sample, and 2000 µL of 0.9% NaCl. For the test sample, an additional drop (approximately 50 µL) of Reagent 2 was added. The mixture was incubated for 10 minutes at room temperature (20–25 °C), after which absorbance was measured at a wavelength of 546 nm using a spectrophotometer. The concentration of direct bilirubin was calculated using the following equations:

Direct Bilirubin (µmol/L) = 246 × ADB (546 nm) Direct Bilirubin (mg/dL) = 14.4 × ADB (546 nm)

Determination of total bilirubin

Total bilirubin was also determined using the method of Guyton and Hall (2000), but with the inclusion of caffeine in the reagent mix to release albumin-bound bilirubin. The reaction mixture consisted of 200 µL of Reagent 1, 200 µL of the sample, and 1000 µL of Reagent 3. A drop (50 µL) of Reagent 2 was added to the test mixture. After mixing and incubating at room temperature for 10 minutes, 1000 µL of Reagent 4 was added. The mixture was further incubated for 5–30 minutes, and the absorbance was measured at 578 nm using the Rx Monza Flowcell spectrophotometer. Total bilirubin concentration was calculated using the following equations:

$$Total\ Bilirubin\ (\mu mol/L) = 185 \times ATB\ (578\ nm)$$
$$Total\ Bilirubin\ (mg/dL) = 10.8 \times ATB\ (578\ nm)$$

Statistical analysis

All experimental data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare differences between groups, and a p-value < 0.05 was considered statistically significant.

RESULTS

Phytochemical analysis of grilled groundnut and cooked groundnut

Table 1 shows the comparative phytochemical composition of grilled and cooked *Arachis hypogaea* (groundnut) seeds. Grilled groundnuts demonstrated higher concentrations of most phytochemicals compared to cooked groundnuts. Notably, compounds such as epicatechin (5.499 mg/g), retusin (3.011 mg/g), and artemetin (2.551 mg/g) were significantly more abundant in the grilled samples. Conversely, a few phytochemicals, including daidzein (2.141 mg/g), tangeretin (3.821 mg/g), and resveratrol (0.584 mg/g) were elevated in cooked samples compared to their grilled counterparts. Certain compounds like kaempferol, quercetin, and genistein were completely absent in cooked groundnuts, while ferulic acid appeared only in the cooked form. These differences indicate that the method of thermal processing substantially alters the phytochemical profile of groundnuts, potentially influencing their biological activities.

Table 1. Phytochemical composition of grilled and cooked groundnut seeds (*Arachis hypogaea*).

Phytochemical	Grilled Groundnut (mg/g)	Cooked Groundnut (mg/g)
Kaempferol	0.424 ± 0.00	0.000 ± 0.00
Catechin	1.128 ± 0.00	0.000 ± 0.00
Butein	0.600 ± 0.00	0.310 ± 0.00
Quercetin	0.460 ± 0.00	0.000 ± 0.00
Luteolin	0.181 ± 0.00	0.0171 ± 0.00
Artemetin	2.551 ± 0.00	0.283 ± 0.00
Daidzin	0.361 ± 0.00	0.197 ± 0.00
Retusin	3.011 ± 0.00	0.292 ± 0.00
Ellagic Acid	1.828 ± 0.00	0.339 ± 0.00
Vanillic	1.512 ± 0.00	0.000 ± 0.00
Naringenin	1.009 ± 0.00	0.242 ± 0.00
Apigenin	0.422 ± 0.00	0.000 ± 0.00
Hesperidin	0.646 ± 0.00	0.027 ± 0.00
Isorhamnetin	1.390 ± 0.00	0.574 ± 0.00
Maricetin	0.898 ± 0.00	0.489 ± 0.00
Epicatechin (1st)	5.499 ± 0.00	0.578 ± 0.00
Daidzein	0.324 ± 0.00	2.141 ± 0.00
Genistein	0.967 ± 0.00	0.000 ± 0.00
Apigenin (2nd)	0.172 ± 0.00	0.179 ± 0.00
Lunamarin	1.419 ± 0.00	0.967 ± 0.00
Gallocatechin	0.134 ± 0.00	0.331 ± 0.00
Resveratrol	0.258 ± 0.00	0.584 ± 0.00
Tangeretin (1st)	1.657 ± 0.00	3.821 ± 0.00
Epicatechin (2nd)	0.212 ± 0.00	0.979 ± 0.00
Naringin	1.379 ± 0.00	0.447 ± 0.00
Hesperidin (2nd)	0.149 ± 0.00	0.000 ± 0.00
Silymarin	0.155 ± 0.00	0.000 ± 0.00
Baicalin	1.159 ± 0.00	0.000 ± 0.00
Tangeretin (2nd)	1.095 ± 0.00	0.000 ± 0.00
Nobiletin	1.071 ± 0.00	0.000 ± 0.00
Myricetin	0.991 ± 0.00	0.161 ± 0.00
Isorhamnetin (2nd)	0.767 ± 0.00	0.000 ± 0.00
Flavone	0.563 ± 0.00	0.424 ± 0.00
Ferulic Acid	0.000 ± 0.00	0.141 ± 0.00
Sinapinic Acid	0.000 ± 0.00	0.000 ± 0.00

Results are expressed as mean ± SE (n = 3).

Proximate Composition of Grilled and Cooked Groundnut

The results in Table 2 present the proximate composition of grilled and cooked groundnut seeds. Cooking significantly increased the moisture content (10.650%) compared to grilling (7.037%), likely due to water absorption during the boiling process. Grilled groundnut exhibited higher crude fibre (1.420%), ash (2.391%), and fat (37.755%) contents relative to cooked samples, which recorded 0.990%, 1.940%, and 12.834% respectively. Protein content was relatively similar between the two forms, with cooked groundnut showing a slightly higher value (7.258%) than grilled (7.146%). Carbohydrate content was markedly higher in cooked groundnut (66.328%) than in grilled (44.251%), suggesting thermal degradation of fats and possible hydrolysis of complex compounds during cooking. These findings indicate that the method of preparation has a significant influence on the nutritional profile of groundnut seeds.

Table 2. Proximate composition of grilled and cooked groundnut (*Arachis hypogaea*) seeds.

Proximate Parameter	Grilled Groundnut (%)	Cooked Groundnut (%)
Moisture	7.037 ± 0.00	10.650 ± 0.00
Fibre	1.420 ± 0.00	0.990 ± 0.00
Ash	2.391 ± 0.00	1.940 ± 0.00
Fat	37.755 ± 0.00	12.834 ± 0.00
Protein	7.146 ± 0.00	7.258 ± 0.00
Carbohydrate	44.251 ± 0.00	66.328 ± 0.00

Results are expressed as mean ± SE (n = 3).

Vitamins and mineral composition of grilled and cooked groundnut seeds

Table 3 shows the comparative vitamin composition of grilled and cooked groundnut seeds. Cooking significantly enhanced the levels of Vitamin A (43.292 mg/kg) and Vitamin C (45.614 mg/kg) compared to grilling (20.179 mg/kg and 41.540 mg/kg, respectively), possibly due to better retention or transformation during moist heat treatment. Vitamin B2 was also markedly higher in cooked groundnut (3.790 mg/kg) than in grilled (0.750 mg/kg), indicating possible heat activation or improved bioavailability. Conversely, Vitamin E was substantially higher in grilled groundnut (41.843 mg/kg) than in cooked (26.157 mg/kg), suggesting thermal degradation of this fat-soluble vitamin during boiling. Other vitamins, such as B1, B3, B6, and B12, showed marginal reductions in the cooked samples, while Vitamin D content was only slightly higher in cooked groundnut. These results demonstrate that cooking and grilling differentially influence the retention and degradation of various vitamins in groundnut seeds.

Similarly, Table 4 presents the mineral profiles of grilled and cooked groundnut. Cooking resulted in increased concentrations of sodium (8.578 ppm), nickel (0.143 ppm), mercury (0.118 ppm), cobalt (0.019 ppm), lead (0.018 ppm), arsenic (0.016 ppm), selenium (0.366 ppm), and zinc (1.643 ppm), compared to lower levels in grilled samples. These increases may reflect leaching or concentration effects during boiling. In contrast, grilled groundnut exhibited higher levels of potassium (7.454 ppm), magnesium (4.783 ppm), manganese (0.267 ppm), copper (0.322 ppm), cadmium (0.047 ppm), and iron (2.787 ppm), possibly due to better preservation of trace minerals under dry heat conditions. Chromium levels remained relatively stable across both treatments. Aluminum and silver were not detected in either sample. These findings suggest that the mode of preparation has significant implications for the mineral availability and potential toxicity profiles of groundnut seeds, with cooking enhancing some beneficial and potentially harmful elements.

Table 3. Vitamins analysis of grilled and cooked groundnut (mg/kg).

Vitamins	Grilled Groundnut	Cooked Groundnut
Vitamin A	20.179 ± 0.00	43.292 ± 0.00
Vitamin B1	2.257 ± 0.00	2.112 ± 0.00
Vitamin B2	0.75 ± 0.00	3.79 ± 0.00
Vitamin C	41.540 ± 0.00	45.614 ± 0.00
Vitamin D	25.553 ± 0.00	26.157 ± 0.00
Vitamin E	41.843 ± 0.00	26.157 ± 0.00
Vitamin B3	6.588 ± 0.00	6.100 ± 0.00
Vitamin B6	2.426 ± 0.00	2.303 ± 0.00
Vitamin B12	4.561 ± 0.00	4.415 ± 0.00

Results are means ± SE (n = 3).

Table 4. Mineral analysis of grilled and cooked groundnut (ppm).

Minerals	Grilled Groundnut	Cooked Groundnut
Nickel	0.022 ± 0.00	0.143 ± 0.00
Sodium	6.454 ± 0.00	8.578 ± 0.00
Potassium	7.454 ± 0.00	6.226 ± 0.00
Chromium	0.032 ± 0.00	0.034 ± 0.00
Magnesium	4.783 ± 0.00	3.967 ± 0.00
Mercury	0.012 ± 0.00	0.118 ± 0.00
Manganese	0.267 ± 0.00	0.167 ± 0.00
Arsenic	0.010 ± 0.00	0.016 ± 0.00
Cobalt	0.004 ± 0.00	0.019 ± 0.00
Lead	0.010 ± 0.00	0.018 ± 0.00
Cadmium	0.047 ± 0.00	0.033 ± 0.00
Copper	0.322 ± 0.00	0.195 ± 0.00
Zinc	0.822 ± 0.00	1.643 ± 0.00
Iron	2.787 ± 0.00	2.278 ± 0.00
Aluminum	0.00 ± 0.00	0.00 ± 0.00
Selenium	0.278 ± 0.00	0.366 ± 0.00
Silver	0.00 ± 0.00	0.00 ± 0.00

Results are means ± SE (n = 3).

Effect of grilled and cooked groundnut on the body weight of Wistar albino rat

All groups exhibited progressive weight gain over the four weeks. Group A (normal control) showed a steady and significant increase in body weight each week, reaching the highest final mean weight of 175.60 ± 2.79 g. Rats in Groups B and C (grilled groundnut-fed) also showed consistent weight gain, with Group C (50%

grilled) nearly matching the control at Week 4 (173.20 ± 3.89 g). However, Groups D and E (cooked groundnut-fed) demonstrated comparatively slower weight gain, with Group E (50% cooked) having the least final weight (163.60 ± 3.54 g) (Table 5). Overall, both grilled and cooked groundnut diets supported growth, but grilling appeared to result in better weight gain than cooking, especially at higher inclusion levels.

Table 5. Effect of grilled and cooked groundnut on the body weight of Wistar albino rats.

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
Group A: Normal Control	136.60 ± 2.69	145.40 ± 3.70*	153.40 ± 4.19*	163.40 ± 3.36*#	175.60 ± 2.79*#
Group B: 25% Grilled + 75% Rat Chow	135.20 ± 2.85	146.40 ± 2.38*	152.60 ± 2.46*	158.40 ± 2.32*#	165.40 ± 2.63*#
Group C: 50% Grilled + 50% Rat Chow	137.60 ± 1.78	154.40 ± 3.31	159.80 ± 3.57*	166.00 ± 4.27*#	173.20 ± 3.89*#
Group D: 25% Cooked + 75% Rat Chow	139.20 ± 2.92	148.50 ± 3.76	156.00 ± 3.66*	161.80 ± 3.14*	169.40 ± 2.27*#
Group E: 50% Cooked + 50% Rat Chow	138.40 ± 3.39	144.00 ± 3.29	150.40 ± 3.61	156.40 ± 4.34*	163.60 ± 3.54*#

Results are expressed as mean ± SE (n = 5). *Significant compared to week 0 ($p < 0.05$); #Further significant increase from the previous week ($p < 0.05$).

Effects on lipid profile of rats fed grilled and cooked groundnut diets

The lipid profile parameters of rats fed varying proportions of grilled and cooked groundnut diets are presented in Table 6. Total cholesterol (TCHOL) levels were significantly elevated in rats fed with cooked groundnut, particularly in Group E (50% cooked & 50% rat chow), which showed the highest TCHOL value (268 ± 44.98 mg/dL) compared to the normal control group (106 ± 18.85 mg/dL). Groups B, C, and D also showed increases in TCHOL, with Group C (50% grilled) recording 184 ± 55.17 mg/dL. Triglyceride (TRIG) levels were lowest in the normal control group (46.00 ± 3.61 mg/dL) and progressively increased across all treatment groups. The highest TRIG level was observed in Group E (97.66 ± 12.99 mg/dL), indicating that cooked groundnut may contribute more to hypertriglyceridemia than grilled groundnut. High-density lipoprotein (HDL) cholesterol was significantly reduced ($p < 0.05$) in rats fed grilled and cooked groundnut diets, especially in Group C (47.66 ± 6.39 mg/dL) and Group E (37.66 ± 7.62 mg/dL), compared to the normal control (97.33 ± 12.99 mg/dL), suggesting a detrimental effect on cardioprotective lipids.

Low-density lipoprotein (LDL) cholesterol increased markedly in all treatment groups, with Group E (228.80 ± 42.47 mg/dL) and Group C (169.33 ± 50.18 mg/dL) showing significant elevations ($p < 0.05$) compared to the control group (12.60 ± 3.75 mg/dL). This indicates a potential risk of atherogenicity associated with both grilled and cooked groundnut intake, particularly at higher inclusion levels. Similarly, very low-density lipoprotein (VLDL) cholesterol levels were highest in Group E (19.53 ± 2.60 mg/dL) and lowest in the control group (9.20 ± 0.72 mg/dL), further supporting the dyslipidemic effect of cooked groundnut diets (Table 6).

Table 6. Lipid profile of rats fed grilled and cooked groundnut diets.

Group	TCHOL (mg/dL)	TRIG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group A: Normal Control	106 ± 18.85	46.00 ± 3.61	97.33 ± 12.99	12.60 ± 3.75	9.20 ± 0.72
Group B: 25% Grilled & 75% Rat Chow	167 ± 18.58	74.66 ± 10.84	71.66 ± 1.76	80.40 ± 18.56*	14.95 ± 2.17
Group C: 50% Grilled & 50% Rat Chow	184 ± 55.17	90.33 ± 10.74	47.66 ± 6.39^{b}	169.33 ± 50.18^{a}	18.06 ± 2.15
Group D: 25% Cooked & 75% Rat Chow	181 ± 30.12	79.66 ± 24.94	82.00 ± 6.08	83.73 ± 25.35*	15.93 ± 4.99
Group E: 50% Cooked & 50% Rat Chow	268 ± 44.98	97.66 ± 12.99	37.66 ± 7.62^{b}	228.80 ± 42.47^{a}	19.53 ± 2.60

Values are expressed as mean ± SE (n = 5). Superscript ^{a} indicates a significant increase in LDL compared to the normal control ($p < 0.05$). Superscript ^{b} indicates a significant decrease in HDL compared to the normal control ($p < 0.05$).

Effect of grilled and cooked groundnut liver on the function parameters of Wistar albino rats

The effects of grilled and cooked groundnut diets on liver function parameters are presented in Table 7. Alanine aminotransferase (ALT) levels were significantly increased in all treatment groups compared to the control. The highest elevation was observed in Group C (50% grilled) with 77.67 ± 9.24 U/L, followed by Group B (57.67 ± 11.92 U/L) and Group D (63.67 ± 6.01 U/L). The control group (Group A) had the lowest ALT level at 29.33 ± 2.60 U/L, suggesting possible hepatocellular stress in the treatment groups. Aspartate aminotransferase (AST) activity also increased progressively across the groups. The most pronounced increase occurred in Group E (50% cooked) with 36.00 ± 2.89 U/L, followed by Group C (31.33 ± 2.60 U/L). These were significantly higher ($p < 0.05$) than the control value of 15.00 ± 2.65 U/L, indicating potential liver inflammation or damage. Alkaline phosphatase (ALP) levels showed a marked increase across all experimental groups, especially in Group E (276.66 ± 24.25 U/L) and Group C (230.66 ± 26.62 U/L),

compared to the control group's 130.66 ± 1.45 U/L. These increases may reflect biliary obstruction or altered liver function associated with high intake of groundnut-based diets.

Direct bilirubin (D.BIL) levels increased slightly across groups, with the highest being in Group E (15.33 ± 1.45 µmol/L), compared to 10.66 ± 0.67 µmol/L in the control group. Similarly, total bilirubin (T.BIL) was highest in Group B (59.66 ± 19.80 µmol/L) and Group E (52.33 ± 11.86 µmol/L), indicating mild impairment in bilirubin clearance in treated animals (Table 7). Overall, the data suggest that both grilled and cooked groundnut diets, particularly at 50% inclusion levels, exert varying degrees of hepatic stress, as evidenced by elevated transaminases, ALP, and bilirubin levels.

Table 7. Effect of grilled and cooked groundnut on liver function parameters of Wistar albino rats.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	D.BIL (µmol/L)	T.BIL (µmol/L)
Group A: Normal Control	29.33 ± 2.60	15.00 ± 2.65	130.66 ± 1.45	10.66 ± 0.67*	40.66 ± 12.25
Group B: 25% grilled & 75% Rat chow	57.67 ± 11.92	22.00 ± 3.21	180.33 ± 30.96*	13.33 ± 0.88	59.66 ± 19.80*
Group C: 50% grilled & 50% Rat chow	77.67 ± 9.24	31.33 ± 2.60	230.66 ± 26.62	14.33 ± 2.40*	51.66 ± 6.06*
Group D: 25% cooked & 75% Rat chow	63.67 ± 6.01*	24.33 ± 3.53*	151.66 ± 15.60*	14.00 ± 1.00	47.66 ± 16.02
Group E: 50% cooked groundnut & 50% Rat chow	61.67 ± 18.41*	36.00 ± 2.89*	276.66 ± 24.25*	15.33 ± 1.45	52.33 ± 11.86*

Values are expressed as mean ± SE (n = 5). * Statistically significant compared to normal control ($p < 0.05$ one-way ANOVA followed by Tukey post hoc test).

Effects on renal function parameters of Wistar rats

The effect of grilled and cooked groundnut supplementation on renal function is shown by the levels of urea and creatinine across the experimental groups (Table 8). The normal control group (Group A) recorded the highest urea level at 0.53 ± 0.01 mmol/L, while significantly lower levels were observed in Groups B (0.31 ± 0.03 mmol/L), C (0.41 ± 0.09 mmol/L), D (0.30 ± 0.04 mmol/L), and E (0.32 ± 0.05 mmol/L). For creatinine levels, Group C (rats fed 50% grilled groundnut) showed the highest value at 2.65 ± 0.61 µmol/L, followed closely by the normal control (2.38 ± 0.24 µmol/L) and Group E (50% cooked groundnut) with 1.95 ± 0.45 µmol/L. The lowest creatinine values were observed in Group D (1.21 ± 0.29 µmol/L) and Group B (1.56 ± 0.36 µmol/L). Overall, rats fed cooked groundnut (Groups D and E) showed better preservation of renal function as indicated by reduced urea and creatinine levels compared to those fed grilled groundnut (Groups B and C), suggesting that cooking may be less nephrotoxic than grilling in this experimental context (Table 8).

Table 8. Effect of grilled and cooked groundnut on renal function parameters of Wistar albino rats.

Groups	Urea (mmol/L)	Creatinine (µmol/L)
Group A: Normal Control	0.53 ± 0.01*	2.38 ± 0.24
Group B: 25% Grilled & 75% Rat Chow	0.31 ± 0.03	1.56 ± 0.36*
Group C: 50% Grilled & 50% Rat Chow	0.41 ± 0.09*	2.65 ± 0.61
Group D: 25% Cooked & 75% Rat Chow	0.30 ± 0.04*	1.21 ± 0.29*
Group E: 50% Cooked & 50% Rat Chow	0.32 ± 0.05*	1.95 ± 0.45*

Results are mean ± SE (n = 5). * Statistically significant compared to normal control ($p < 0.05$ one-way ANOVA followed by Tukey post hoc test).

Effects on malondialdehyde (MDA) and lactate dehydrogenase (LDH) activities

The levels of MDA and lactate LDH were measured to assess lipid peroxidation and cellular damage in rats administered with grilled and cooked groundnut formulations (Table 9). The normal control group (Group A) showed an unusually high and variable MDA level of 131.82 ± 128.59 µmol/L, whereas all treatment groups recorded markedly lower and more stable MDA levels. Specifically, MDA concentrations were 2.56 ± 0.52 µmol/L in Group B, 2.46 ± 0.71 µmol/L in Group C, 1.98 ± 0.50 µmol/L in Group D, and 2.58 ± 0.43 µmol/L in Group E. The lowest MDA level was observed in Group D (25% cooked groundnut), suggesting potential antioxidant protection through cooking. For LDH activity, the normal control (Group A) showed a value of 31.66 ± 4.70 U/L. A progressive increase in LDH levels was observed in treatment groups, with Group B (46.66 ± 5.61 U/L) and Group C (52.33 ± 10.53 U/L) having moderate increases. Notably, Group D (65.00 ± 5.29 U/L) and Group E (71.66 ± 4.81 U/L) showed the highest LDH levels, with significant elevation ($p < 0.05$) (Table 9), indicating increased cellular stress or membrane leakage, particularly in rats fed cooked groundnut at

higher concentrations. In summary, grilled and cooked groundnut diets reduced MDA levels compared to the control, but cooked groundnut, especially at 50%, significantly elevated LDH, indicating potential tissue damage despite lower lipid peroxidation.

Table 9. Effect of grilled and cooked groundnut on MDA and LDH in Wistar albino rats.

Groups	MDA (nmol/mL)	LDH (U/L)
Group A: Normal Control	131.82 ± 128.59	31.66 ± 4.70*
Group B: 25% grilled & 75% rat chow	2.56 ± 0.52*	46.66 ± 5.61*
Group C: 50% grilled & 50% rat chow	2.46 ± 0.71*	52.33 ± 10.53
Group D: 25% cooked & 75% rat chow	1.98 ± 0.50	65.00 ± 5.29*
Group E: 50% cooked groundnut & 50% rat chow	2.58 ± 0.43*	71.66 ± 4.81*

Results are mean ± SE (n = 5). * Statistically significant compared to normal control ($p < 0.05$ one-way ANOVA followed by Tukey post hoc test).

DISCUSSION

The significance of food extends beyond sustenance, influencing biological, social, and psychological behaviors. Humans make numerous food-related decisions daily (Wansink et al., 2017; Hofmann et al., 2020). Evolutionarily, food-seeking behavior is rooted in survival (Pinel et al., 2019). However, modern dietary patterns contribute to global health challenges such as obesity and NAFLD (Cornil et al., 2018; Swinburn et al., 2019; Zhang et al., 2019). In this study, groundnut consumption did not significantly affect body weight, consistent with previous findings (Arya et al., 2016; Ogunniyi et al., 2022; Settaluri et al., 2017).

Obesity has been associated with altered bilirubin metabolism (Kumar et al., 2018; Vitek & Schwertner, 2017). However, this study aligns with Augustine et al. (2020), showing no significant bilirubin changes. Groundnuts contain bioactive compounds that support liver health (Agrawal et al., 2018; Monda et al., 2015; Torres et al., 2019). These compounds reduce oxidative stress and inflammation (Reuter et al., 2018; Sies et al., 2012). Grilling may produce harmful compounds such as acrylamide and PAHs, which impair liver function (Mottram et al., 2012; Tareke et al., 2015; Tareke et al., 2017). Boiling may enhance antioxidant activity (Sampson et al., 2019).

The observed increase in LDH suggests cellular damage and oxidative stress (Sies et al., 2012; Reuter et al., 2018). Thermal processing may increase lipid peroxidation (Mentes et al., 2013). Interestingly, cooked groundnut also showed elevated LDH, indicating metabolic stress possibly due to nutrient degradation (Griel et al., 2021; Settaluri et al., 2017). Overall, preparation methods significantly influence the biological effects of groundnut consumption.

In this study, rats fed diets containing cooked or grilled groundnuts showed no statistically significant changes in body weight compared to controls. This finding supports previous research indicating that the inclusion of groundnut in the diet, despite its caloric density, may not necessarily lead to significant weight gain due to its satiating properties, protein content, and healthy fat profile (Arya et al., 2016). Groundnuts are known to promote satiety and reduce overall caloric intake when consumed in moderation, which might explain the stable weight trend observed. Moreover, energy regulation and body weight response can vary among individuals or species due to genetic, metabolic, and hormonal factors (Arya et al., 2021).

While obesity has been linked to increased bilirubin levels due to alterations in hepatic metabolism and oxidative stress (Arya et al., 2021; Barros et al., 2018)., the present findings suggest that the dietary inclusion of groundnut, either cooked or grilled, did not cause a statistically significant alteration in total bilirubin levels in Wistar rats. This aligns with findings by Augustine et al. (2020), who reported that moderate groundnut consumption did not significantly affect hepatic bilirubin metabolism. Interestingly, however, the group fed grilled groundnuts displayed a mild increase in bilirubin compared to the control, suggesting that the method of preparation may influence liver function biomarkers.

The composition of groundnut, rich in phytochemicals like flavonoids, polyphenols, phytosterols, and vitamins, has been associated with improved liver health and oxidative balance (Agrawal et al., 2018; Monda et al., 2015). These bioactive compounds help maintain hepatic function by neutralizing reactive oxygen species (ROS), reducing inflammation, and supporting bile production and detoxification pathways. For instance, polyphenols and vitamin E can protect hepatocytes from damage and support bilirubin conjugation and excretion. Essential trace minerals like magnesium and zinc in groundnuts also play roles in enzymatic processes relevant to liver detoxification.

Nonetheless, the preparation method plays a significant role in modulating groundnuts' health impact. Grilling, especially at high temperatures, may produce harmful by-products such as acrylamide and polycyclic aromatic hydrocarbons (PAHs), which are known to induce oxidative stress and hepatic enzyme changes. These thermally induced toxins can impair liver function, potentially altering bilirubin and lactate dehydrogenase

(LDH) levels, biomarkers associated with liver integrity and oxidative stress (Breimer and Cederbaum, 2020). In contrast, boiling has been shown to enhance antioxidant activity in legumes, potentially offering more hepatic protection compared to roasting or grilling.

In this study, while cooked groundnut appeared to have minimal effect on direct bilirubin, higher concentrations of grilled groundnut were associated with slight elevations in bilirubin, suggesting potential hepatic stress or compromised metabolism. This observation is consistent with Bimro et al. (2020), who observed that groundnut varieties high in oleic acid might delay fatty liver progression but could also exhibit variable effects based on preparation. Group differences could also reflect a dose-dependent or processing-related variation in the bioavailability of nutrients and toxins.

LDH, a marker of tissue damage and oxidative stress, was higher in groups fed cooked groundnut than in those given grilled groundnut or a control diet. This suggests that thermal processing of groundnut, even via boiling, might contribute to low-grade hepatocellular injury or metabolic stress at higher dietary inclusion levels. Elevation of LDH can indicate mitochondrial dysfunction and cellular leakage of enzymes due to oxidative insult (Kumar et al., 2018). High-temperature cooking may also increase lipid peroxidation and generate reactive oxygen species capable of damaging cellular structures. Interestingly, this study found that groups receiving cooked groundnut diets had slightly higher LDH levels than their grilled counterparts. This contradicts the assumption that boiling is universally safer, underscoring the importance of food matrix interactions, cooking duration, and storage conditions in modulating nutrient retention and toxicity. Moreover, differences in antioxidant retention and lipid oxidation during cooking may explain these unexpected patterns.

Despite the beneficial components of groundnuts, their consumption, especially when subjected to intense cooking methods, requires moderation and proper dietary planning. The variation in oxidative and hepatic markers observed in this study underscores the need for careful consideration of preparation methods when promoting groundnut as a dietary staple. As highlighted by Arya et al. (2021), high oleic peanuts can improve lipid profiles and reduce liver fat accumulation, but these benefits may be offset by harmful cooking-induced changes.

Finally, groundnut consumption, depending on how it is processed, may influence liver biomarkers and oxidative stress levels. Grilling at high temperatures may lead to the formation of hepatotoxic compounds, whereas boiling appears more protective but not without its metabolic implications. These findings emphasize the dual role of groundnut as both a nutraceutical and a potential dietary risk factor when improperly processed, warranting further research on safe preparation practices and long-term health implications.

CONCLUSION

This study demonstrated that the method of preparation of groundnut (cooked versus grilled) significantly influences its impact on liver function, oxidative stress markers, and general metabolic parameters in Wistar albino rats. While groundnut inclusion in the diet did not significantly affect body weight, key biochemical parameters such as ALT, AST, ALP, LDH, and bilirubin levels varied depending on the form and proportion of groundnut administered. Grilled groundnut, especially at higher dietary proportions, was associated with elevated liver enzymes and markers of oxidative stress, indicating potential hepatic stress or cellular damage. This may be due to the formation of thermally induced toxicants during grilling. In contrast, cooked groundnut showed relatively milder effects on liver biomarkers but was associated with a significant increase in lactate dehydrogenase, suggesting that prolonged cooking could also induce mild oxidative or metabolic stress. Overall, although groundnuts are rich in beneficial phytochemicals and essential nutrients, their method of processing plays a crucial role in determining their health effects. Boiling appears to retain more of its antioxidant properties and minimize hepatic toxicity compared to grilling. These findings highlight the importance of dietary moderation and proper cooking methods to preserve the nutritional integrity of groundnuts and minimize potential adverse effects. Further research is warranted to investigate the long-term impact of groundnut consumption, particularly at varying preparation methods and inclusion levels, to provide clearer dietary recommendations.

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Ethical Considerations: The animals used in this study strictly follow the international organizations such as OECD and ARRIVE guidelines for the use of laboratory animals, which prohibit the inhumane treatment of animals.

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