



Bioactive *Moringa oleifera* seed extracts attenuates cholesterol gall stones in hyperglycaemic Swiss mice

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Abstract

Gallstones (cholelithiasis) are thickened components of the lipid-modulating digestive fluid in the gall bladder. Commonly, cholesterol super saturation from excess cholesterol or inadequate solubilizing bile salts may result in cholesterol gall stone formation, while excess bilirubin because of liver damage or blood disorders may form pigment gallstones. Cholesterol gall stone formation is strongly correlated with diabetes mellitus and both diseases predispose patients to a high hospitalization rate. Recent studies have examined the potential of *Moringa oleifera* as a functional food, highlighting the need to test different parts of the deciduous tree for its treatment or adjuvant efficacy. In this study, we report the gall bladder cytoprotective ability of *Moringa oleifera* seed extracts and the hepatoprotective potential of the seed cake in hyperglycemic and cholesterol gall stone-induced Swiss mice. We show that the seed cake contains 37% protein and caused a 40% increase in body weight over 5 weeks, but the seed oil caused only a 15% increase in body weight over the same period, which supports the possible use of the seed oil as a functional food for weight loss. Seed cake treatment presented the potential for glycemic control, improved hematological and lipid profile and enhanced liver antioxidant activity. Seed oil increased blood high density lipoprotein content, and all seed extracts reduced hepatic sodium pump activity. Our results highlight the need to extract the active compounds in functional foods, as against using the whole plant parts as adjuvant therapies, and it makes a case for the antihyperglycemic and cytoprotective efficacy of *Moringa oleifera* seed extracts.

Keywords Monounsaturated fatty acid · Oleic acid · Seed oil · Weight loss · Functional food · Cholelithiasis

Introduction

The defective secretion and/or action of insulin is known to predispose mammals and even insects to a range of metabolic disorders called diabetes (Graham and Pick 2017; American Diabetes Association 2019; Olagoke et al. 2020). Hence, sustained hyperglycemia—the hallmark of diabetes mellitus—is associated with multisystem organ dysfunction, including gastrointestinal defects like the accumulation of cholesterol gall stones (Tan and Kou 2019).

Gall stones (especially cholesterol gall stones) are one of the leading causes of recorded hospital admission (Farthing et al. 2014). They are more prevalent amongst women and the senile populace, but the general gallstone incidence rate is on the rise (Novacek 2006). Cholesterol monohydrate crystals (Fig. 1) are the main constituents of cholesterol gall stones. They are known to cause pancreatitis, cholecystitis, empyema of the gall bladder, and gastrointestinal motor dysfunction amongst other prognosis (Di Ciaula et al. 2018). Symptoms of cholesterol gall stones may include colicky pain and dyspepsia, which may require medical or surgical (cholecystectomy) intervention (Di Ciaula and Portincasa 2018). The correlation of cholesterol gallstones with metabolic abnormalities such as diabetes (Tan and Kou 2019), and the reports of sustained systemic and neurological perturbations even after restoring near-euglycemic state in diabetic patients (hyperglycemic metabolic memory (Ceriello 2009)) necessitates the testing of adjuvant therapies that may manage or cure the known complications or comorbidities of diabetes mellitus (Testa et al. 2017).

Moringa oleifera is a widely cultivated, draught-tolerant, deciduous tree of the Moringaceae family (Avila-Treviño et al.

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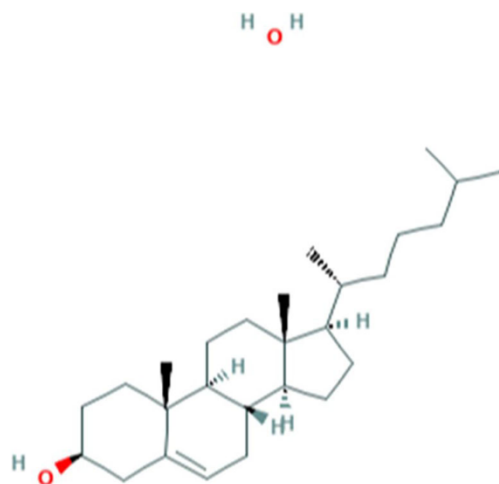


Fig. 1 2D chemical structure of cholesterol monohydrate (PubChem Identifier: CID 56841844)

2017; Al-Khalifah and Shanavaskhan 2017). It has been used as vegetables, in traditional herbal medicine, for water purification, and there are several studies into the bioactive properties of the tree's parts (Ma et al. 2020). The nutritive constituents and phytochemical properties of *Moringa* have been well characterized (Xu et al. 2016), and it is further known for it is anthelmintic, antiseptic, detergent, antiulcerogenic, antiinflammatory, antimicrobial, antioxidant, antihyperglycaemic, anticlastogenic, and antifibrotic effect (Dhakad et al. 2019; Saleem et al. 2020). Albeit, there are toxicity concerns for *Moringa* overconsumption, concerns of contraindication in pregnancy, and reports of interference with the antihyperglycemic drug; sitagliptin (Olurishe et al. 2016; Igbokwe et al. 2018; Attah et al. 2020). We therefore examined the adjuvant potential of the functional food, *Moringa oleifera* in the management of cholesterol gall stones, especially as a diabetes comorbidity.

Materials and methods

Chemicals

All chemicals used were of analytical grade. Epinephrine, ATPase, and alloxan were purchased from Sigma Aldrich, USA. Other chemicals were purchased from BDH chemicals, England.

Seed pod identification and diet preparation

Dry pods containing seeds of *Moringa oleifera* were collected from Ibadan North Local Government Area of Oyo State, Nigeria, and identified (with voucher number: FHI 110165) at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Whole seed was milled with an electric blender, seed oil was extracted as described by Folkard and colleague (Folkard and Sutherland 1994), while seed cake was the

remaining solid product after oil extraction. Milled whole seed and seed cake were mixed with the basal diet as required for each experimental group.

Protein proximate analysis of *Moringa oleifera* seed cake via thin layer chromatography

A portion of the earlier described seed cake was subjected to solvent (dichloromethane and methanol) extraction in line with established protocol (Attah et al. 2017). The extract was freeze dried and stored at 4 °C. A modified G-250 stain was prepared as described by Xu et al. (2008), while ninhydrin stain was prepared by dissolving 0.2-g ninhydrin powder in 100-ml absolute ethanol (Attah et al. 2017). Proximate analysis of the crude protein content of *Moringa* seedcake was performed according to the AOAC method (Sullivan and Carpenter 1993), and the phytochemical screening for the presence of amino acids, amines, and peptides was achieved via thin layer chromatography as described by Suryawanshi et al. (2015).

Swiss mice husbandry and experimental protocol

Swiss mice were obtained from the central animal house of the Department of Physiology, University of Ibadan, Nigeria. Male mice weighing between 18 and 24 g were acclimatized in cages for 7 days before commencement of treatment and maintained under standard conditions, including average temperature of 25 °C ± 3 °C, relative humidity of 55–65%, and a 12-h light/dark cycle. The animals had free access to water and feed (procured from Ladokun Feeds Ltd., Ibadan, Nigeria) during acclimatization and treatment. The guide for care of laboratory animals (Nih et al. 2011) was strictly adhered to and approval was gotten from the Animal Care and Use Research Ethics Committee (UI-ACUREC/19/005) of the University of Ibadan.

Diabetes induction was carried out using the established protocol of alloxan monohydrate intraperitoneal injection (Ptak et al. 1975) and animals with sustained increased blood glucose level for 72 h were pooled for the experiment. Cholesterol gallstone was induced in the hyperglycaemic mice by feeding them with basal diet containing 0.5% cholesterol (Akiyoshi et al. 1986). 35 male Swiss mice were randomly allotted to 5 groups with 7 animals each and exposed to appropriate diet for 5 weeks. The groups included intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled *M.oleifera* whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M.oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M.oleifera* seed oil (CG + seed oil). The seed oil was administered once daily via an oral cannula. Weekly blood glucose (using an Accu-chek glucometer made by Roche Diabetes Care Inc., USA) and body weight (using standard weighing scale) were monitored.

Tissue preparation

At the end of the treatment period, blood samples were collected from the retro-orbital sinus into heparinized tubes (for analysis of hematological parameters (Dacie and Lewis 1991; Salami et al. 2016)), and into plain tubes (for estimation of lipid profile and liver function tests using kits manufactured by Randox Laboratories limited, UK). Subsequently, mice were anaesthetized with 80-mg/kg b.w ketamine and decapitated. The liver and gallbladder were carefully excised, rinsed in ice cold phosphate buffer, blotted with clean filtered paper, and weighed on a digital scale (model DT 1000). Whole liver was homogenized in cold phosphate buffer (4 °C; pH 7.4) and centrifuged at 4 °C for 10 min at 3000 rpm. The resulting supernatant was then processed for biochemical assays. Sections of the gallbladder were fixed in 10% neutral buffered formalin for histological evaluation.

Biochemical assays

Liver protein content was estimated across groups by the Biuret reaction (Gornall et al. 1949). Briefly, 1 ml of diluted test tissue sample was added to 3 ml of prepared biuret solution and left for 5–10 min, a deep blue to purple color change was observed and absorbance read at 540 nm.

Superoxide dismutase (SOD) activity was estimated as described by Misra and Fridovich (1972) and Salami et al. (2020). Briefly, 0.5 ml of tissue homogenates was mixed with 2.5 ml 0.05 M carbonate buffer, pH 10.2 and left to equilibrate in glass cuvette. The reaction commenced with the addition of 0.3 ml of freshly prepared 0.3 mM epinephrine and change in absorbance at 480 nm was observed at 30 s and at 1 min. One unit of SOD activity was slated as the amount of SOD that is expected to cause 50% inhibition of the oxidation of adrenaline.

Glutathione level was estimated as a colored product (at 412 nm) of the reaction of 5,5'- dithiobis-(2-nitrobenzoate) with

sulfhydryl groups (Ellman 1959; Olagoke et al. 2020). Catalase activity was determined by reducing dichromate to chromic acetate in the presence of hydrogen peroxide, with perchromic acid as an unstable intermediate (Aebi 1984; Afolabi and Olagoke 2020). The resultant chromic acetate was measured spectrophotometrically at 530 nm. The indirect measurement of nitric oxide levels as a function of total nitrite content was done as described by Ignarro et al. (1987) and Salami et al. (2016), using the Griess reagent. Na⁺/K⁺ ATPase activity was determine by the modified method (Bewaji et al. 1985; Salami et al. 2020) of Ronner et al. (1977) and expressed as $\mu\text{mole P}_i/\text{mg Prot.}$

Histopathological examination

Gall bladder sections were fixed in 10% neutral buffered formalin, processed to wax and sections were stained with hematoxylin and eosin. Stained sections were then observed microscopically as described by Bancroft and Gamble (2007).

Statistical analysis

Data from biochemical analysis were analyzed by one way analysis of variance (ANOVA) followed by Bonferroni's post hoc test, using Graph Pad Prism 5. The mean and standard error of mean (SEM) of the variables was recorded and considered significant at $P < 0.05$.

Results

Protein proximate analysis of *Moringa oleifera* seed cake

Figure 2 shows the phytochemical screening and detection of amino acids, amines, and peptides in the seed cake. Protein

Fig. 2 Proximate analysis of *Moringa oleifera* seed protein content. Glutathione (GSH) was used as the standard peptide. Ninhydrin produced various colors in contact with *Moringa* seedcake isolates indicative of the presence of amino acids, amines, and peptides

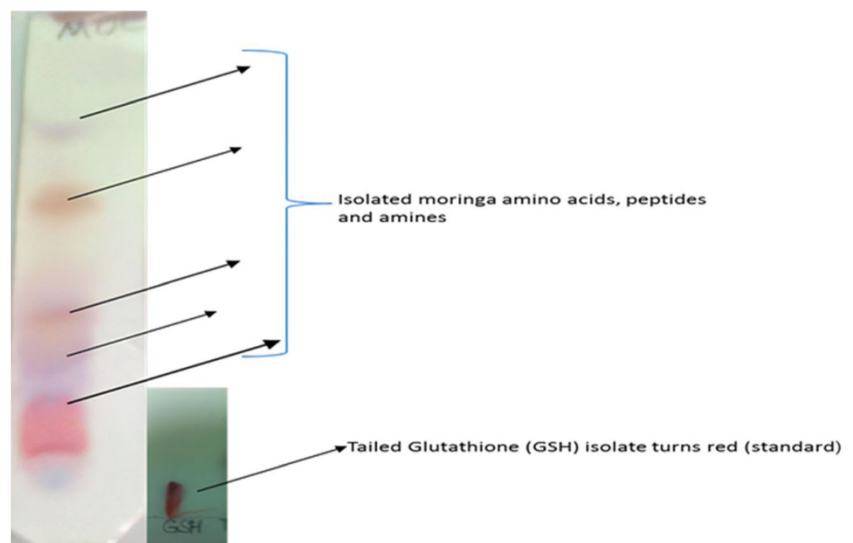
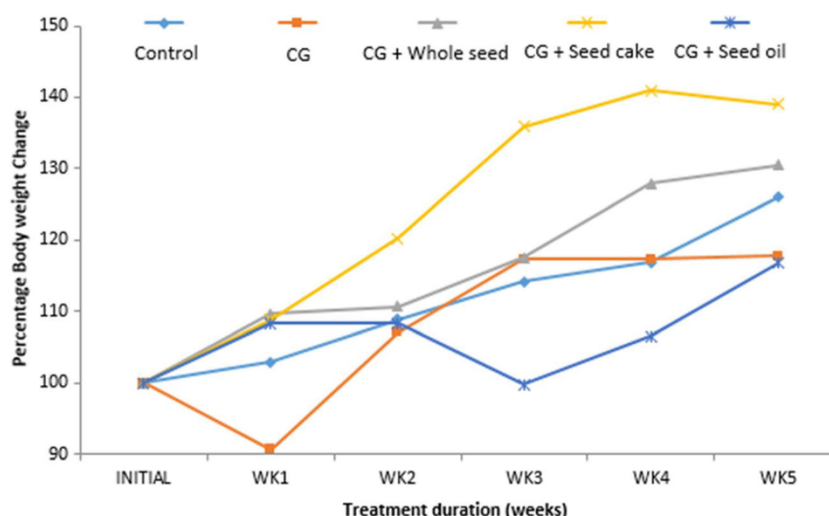


Fig. 3 Percentage body weight change in hyperglycemic and cholesterol gall stone induced mice treated with seed extracts of *Moringa oleifera*. Where: Intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled *M. oleifera* whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M. oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M. oleifera* seed oil (CG + seed oil)



proximate analysis estimated that the *Moringa* seedcake contained 37% protein.

Percentage body weight change in hyperglycemic and cholesterol gall stone induced mice treated with *Moringa oleifera* seed extracts

Figure 3 shows progressive weight gain across all groups of experimental animals. The group treated with seed cake had the highest rate of weight increase, while the group treated with seed oil showed significant weight loss. Cholesterol gall stone and cholesterol gall stone + seed oil treatment significantly reduced the rate of weight gain compared with other groups.

Fasting blood glucose in hyperglycemic and cholesterol gall stone induced mice treated with *Moringa oleifera* seed extracts

Table 1 shows significant correlation between untreated cholesterol gall stones and excessively high blood glucose levels from week 1 to week 4. The CG and CG + seed oil groups showed significant hyperglycemia. Conversely, seed cake

treatment was the most effective adjuvant therapy to reduce blood glucose levels, though the glucose readings were still double the levels recorded in the normal control group.

Hematological parameters and inflammatory markers in hyperglycemic and cholesterol gall stone induced mice treated with *Moringa oleifera* seed extracts

Table 2 shows the record of hematological variables across groups. Seed cake treatment significantly increased packed cell volume (pcv), red blood cell count (rbc), hemoglobin (hb), and neutrophil count compared with other groups. Seed oil had the exact opposite effect of seed cake treatment on pcv, rbc, Hb, and neutrophil count. Seed oil treatment further significantly increased platelet and white blood cell count compared with other groups. On the other hand, cholesterol gall stone induction significantly increased monocytes and lymphocytes count compared to other groups.

Table 3 shows blood inflammatory markers across groups. Seed oil treatment significantly increased platelet/lymphocyte ratio and platelet/nitric oxide ratio compared with other groups.

Table 1 Blood glucose levels in hyperglycemic and cholesterol gall stone induced mice treated with seed extracts of *Moringa oleifera*

Groups	Week 1	Week 2	Week 3	Week 4
Control	37.20 ± 1.587	37.20 ± 1.587	38.33 ± 0.6360 ^{bcd}	37.20 ± 1.587 ^{bcd}
CG	297.8 ± 8.976 ^{acde}	171.9 ± 18.96 ^{cd}	140.3 ± 11.26	140 ± 6.351 ^d
CG+whole seed	93 ± 5.859 ^{a,b}	105.3 ± 7.513 ^{a,b}	113.7 ± 7.33 ^a	121.3 ± 4.177 ^d
CG+seed cake	78.67 ± 3.756 ^{a,b,c}	106 ± 2.082 ^{a,b}	105 ± 5.859 ^{a,b}	78.3 ± 6.888 ^{a,b,c}
CG+seed oil	177.7 ± 30.02 ^{acd}	121.3 ± 11.32	153 ± 11.36 ^d	135.7 ± 12.68 ^d

Intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled *M. oleifera* whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M. oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M. oleifera* seed oil (CG + seed oil). Significance: ^a compared with control, ^b compared with CG, ^c compared with CG + whole seed, ^d compared with CG + seed cake, ^e compared with CG + seed oil

Table 2 Hematological parameters in hyperglycemic and cholesterol gall stone induced mice treated with seed extracts of *Moringa oleifera*

Groups	Packed cell volume (%)	Red blood cell count/mm ³	Hemoglobin (g/dl)	Platelet/mm ³	White blood cell count /mm ³	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)
Control	46.67 ± 0.33	7.35 ± 0.02	15.6 ± 0.06	92,000 ± 2000	4083 ± 650.9	24 ± 1.73	1.667 ± 0.33	68.67 ± 2.33
CG	35 ± 2.08 ^a	6.75 ± 0.23	15.77 ± 0.46	124,300 ± 3383 ^{acd}	6750 ± 419.3 ^a	27.67 ± 0.67	4.00 ± 0.577 ^{a,c,d,e}	73 ± 1.16 ^{acd}
CG+whole seed	45.33 ± 1.86 ^b	7.22 ± 0.41	15.47 ± 0.77	88,670 ± 2404	3767 ± 371.2 ^b	35.67 ± 2.03 ^a	1.00 ± 0.00	68.33 ± 1.45
CG+seed cake	54 ± 0.58 ^{a,b,c}	8.82 ± 0.02 ^{a,b,c}	17.53 ± 0.03 ^{a,b,c}	107,300 ± 2667 ^{a,c}	6200 ± 189.3 ^c	37.33 ± 2.67 ^{a,b}	2.00 ± 0.577	64 ± 2.08
CG+seed oil	44.25 ± 0.63 ^{b,d}	7.61 ± 0.13 ^d	14.93 ± 0.2 ^d	141,300 ± 3119 ^{a,b,c,d}	7213 ± 383.2 ^{a,c}	26.67 ± 0.67 ^{c,d}	1.677 ± 0.33	72.53 ± 1.45 ^d

Key: Intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled *M.oleifera* whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M.oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M.oleifera* seed oil (CG + seed oil). Significance: ^a compared with control, ^b compared with CG, ^c compared with CG + whole seed, ^d compared with CG + seed cake, ^e compared with CG + seed oil

Blood lipid profile and hepatorenal panel in hyperglycemic and cholesterol gall stone-induced mice treated with *Moringa oleifera* seed extracts

Table 4 shows blood lipid profile across groups. Cholesterol gall stone induction significantly increased total cholesterol, triglyceride, and low-density lipoprotein content in blood. Whole seed, seed cake, and seed oil restored total cholesterol and triglyceride content to near-normal values, but only whole seed and seed cake was effective in restoring low density lipoprotein to near-normal values.

Table 5 shows liver and kidney function test across groups. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and bilirubin were significantly increased in the CG and CG + seed oil groups but whole seed and seed cake treatment restored AST, ALT, and ALP to near-normal values. There was no significant difference in creatinine levels across groups.

Biochemical parameters in hyperglycemic and cholesterol gall stone-induced mice treated with *Moringa oleifera* seed extracts

Figure 4 shows biochemical parameters across groups. Superoxide dismutase (SOD) activity was significantly reduced in the CG and CG + whole seed groups, while glutathione (GSH) level and catalase activity were significantly reduced in the CG, CG + whole seed and CG + seed oil groups. Nitric oxide levels were significantly reduced in all treated groups compared with control, while Na^+/K^+ -ATPase activity was significantly reduced in the CG + whole seed, CG + seed cake, and CG + seed oil groups compared with control (Fig. 5).

Microscopic evaluation of gallbladder sections in hyperglycemic and cholesterol gall stone-induced mice treated with *Moringa oleifera* seed extracts

Figure 5 shows the photomicrograph of gall bladder sections. Induction of cholesterol gall stones correlated with significant thickening of the gall bladder mucosal layer, infiltration of inflammatory cells, and active mitotic cell division. The architecture of gallbladder in animals treated with whole seed, seed cake, and seed oil were similar to those of normal control animals as they presented with a single layer of normal tall columnar epithelial lining, no mucus secretion, and no active mitotic cell division.

Discussion

Evidence from this study showed that cholesterol gall stones in hyperglycemic mice is correlated with reduced ability to gain weight; deranged hematological profile, liver function

Table 3 Blood inflammatory markers in hyperglycemic and cholesterol gall stone induced mice treated with seed extracts of *Moringa oleifera*

Groups	Neutrophil/lymphocyte ratio	Monocyte/lymphocyte ratio	Platelet/lymphocyte ratio	Platelet/nitric oxide ratio
Control	0.343 ± 0.033	0.0243 ± 0.0048	1190 ± 30.53	513.5 ± 8.482
CG	0.41 ± 0.015	0.055 ± 0.0087 ^{a,c,d}	1763 ± 68.76 ^{ac}	1995 ± 12.20 ^{a,c,d}
CG+whole seed	0.42 ± 0.038	0.0153 ± 0.0003	1362 ± 47.85	560.3 ± 7.496
CG+seed cake	0.487 ± 0.0285 ^a	0.028 ± 0.006	1695 ± 50.29 ^{ac}	995.9 ± 3.548 ^{ac}
CG+seed oil	0.347 ± 0.0186 ^d	0.023 ± 0.0052 ^b	2103 ± 26.12 ^{abcd}	2252 ± 135.3 ^{abcd}

Key: Intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled *M. oleifera* whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M. oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M. oleifera* seed oil (CG + seed oil). Significance: ^a compared with control, ^b compared with CG, ^c compared with CG + whole seed, ^d compared with CG + Seed cake, ^e compared with CG + seed oil

and redox status; as well as raised blood inflammatory markers and lipid content. We demonstrate that different *Moringa olifera* seed extracts have differing effects on cholesterol gall stone formation and progression in hyperglycemic Swiss mice. We showed for the first time, the efficacy of *Moringa olifera* seed cake in improving the pathophysiological manifestations of cholesterol gall stones during hyperglycemic state and used thin layer chromatography to analyze the amino acid, amine, and peptide content of the seed cake.

The high monounsaturated fatty acid (oleic acid) content of *Moringa olifera* seed oil makes it considerably healthy for human consumption (Ghazali and Mohammed 2011), but there are concerns for the correlation of weight change and adipose tissue volume with fatty acid intake (St-Onge et al. 2003). Medium chain triglycerides are usually used as part of weight loss diet regimens (St-Onge et al. 2008), but we found significant weight loss in the group treated with *Moringa olifera* seed oil (a long chain triglyceride (Vlahov et al. 2002)). Conversely, seed cake treatment significantly increased weight gain, signifying the need to further analyze the specific amino acid content of the seed cake.

Our present study highlights the need to extract necessary components of functional foods as opposed to using the whole plant part. Seed cake treatment significantly reduced blood glucose levels compared with whole seed and seed oil treatment, contrary to reports of enhanced glycemic control via high monounsaturated fatty acid diet (Vessby et al. 2001; Due et al. 2008). Since adjuvant therapies are not stand-alone treatments, the relative increase in blood glucose levels of seed cake and seed oil-treated mice compared with control animals should be made-up for by standard antihyperglycemic drugs.

In fact, the possible interaction of adjuvant therapies with standard drugs is a major concern in the management of diabetes complications (Olurishie et al. 2016). The further PCV, RBC, and Hb count increase in the seed cake treated group shows the potential of *Moringa olifera* seed cake to improve the blood transport of nutrients and gasses. However, seed oil treatment significantly increased inflammatory markers like platelet and white blood cell count, as well as platelet/lymphocyte ratio and platelet/nitric oxide ratio in consonance with studies reporting diet-related inflammation in humans (Arouca et al. 2018), though there are reports of reduced

Table 4 Blood lipid profile of hyperglycemic and cholesterol gall stone induced mice treated with seed extracts of *Moringa oleifera*

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Low density lipoprotein (mg/dl)	Very low density lipoprotein (mg/dl)	High density lipoprotein (mg/dl)
Control	36 ± 1	44 ± 0	14.73 ± 3.34	8.73 ± 0.29	23.33 ± 2.59
CG	67.33 ± 2.33 ^{a,d,e}	73.67 ± 4.8 ^a	22.73 ± 0.35	10.53 ± 1.16	18 ± 1.91 ^{a,c,e}
CG+Whole seed	53 ± 6.35	40.67 ± 2.19 ^b	7.47 ± 1.53 ^{b,c}	11.27 ± 1.22	31.33 ± 2.83
CG+Seed cake	34 ± 0.58 ^{b,c}	43 ± 1.16 ^b	12.13 ± 1.11 ^{b,c}	8.6 ± 0.23	16.67 ± 0.62 ^{a,c,d}
CG+Seed oil	40.25 ± 3.09 ^b	35.75 ± 0.75 ^b	32.35 ± 1.36 ^{a,b,c,d}	11.1 ± 0.23	34.33 ± 0.41

Low-density lipoprotein and very low-density lipoprotein level was calculated using the Friedewald's equation (Friedewald et al. 1972). Key: Intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled *M. oleifera* whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M. oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M. oleifera* seed oil (CG + seed oil). Significance: ^a compared with control, ^b compared with CG, ^c compared with CG + whole seed, ^d compared with CG + seed cake, ^e compared with CG + seed oil

Table 5 Liver and kidney function test of hyperglycemic and cholesterol gall stone induced mice treated with seed extracts of *Moringa oleifera*

Groups	Aspartate transaminase (AST) (IU/l)	Alanine transaminase (ALT) (IU/l)	Alkaline phosphatase (ALP) (IU/l)	Bilirubin ($\mu\text{Mol/l}$)	Creatinine ($\mu\text{Mol/l}$)
Control	165.3 $\pm$ 17.61	114.3 $\pm$ 1.33	185.3 $\pm$ 5.32	21 $\pm$ 0	0.63 $\pm$ 0.03
CG	189.3 $\pm$ 21.87 ^{acde}	171.7 $\pm$ 9.33 ^{acde}	242.7 $\pm$ 9.14 ^{a,c,d,e}	23.67 $\pm$ 0.33 ^a	0.57 $\pm$ 0.09
CG+whole seed	169.3 $\pm$ 5.36 ^b	114.3 $\pm$ 0.67 ^b	152.3 $\pm$ 6.73 ^b	20.33 $\pm$ 0.33 ^b	0.7 $\pm$ 0.06
CG+seed cake	160 $\pm$ 23.52 ^b	121.7 $\pm$ 2.91 ^b	191 $\pm$ 8.12	22.33 $\pm$ 0.33 ^c	0.53 $\pm$ 0.03
CG+seed oil	181 $\pm$ 4.16 ^a	154 $\pm$ 9.5 ^{a,c,d}	222 $\pm$ 9.58 ^{a,c,d}	24.33 $\pm$ 0.67 ^{a,c,d}	0.6 $\pm$ 0

Key: Intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled *M. oleifera* whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M. oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M. oleifera* seed oil (CG + seed oil). Significance: ^a compared with Control, ^b compared with CG, ^c compared with CG + whole seed, ^d compared with CG + seed cake, ^e compared with CG + seed oil

platelet aggregation in humans fed monounsaturated fatty acids (Smith et al. 2003).

Monounsaturated fatty acid rich diets have earlier been reported to have no effect on blood lipids (Hegsted et al. 1965), but emerging evidences show reduction in total cholesterol, low density lipoprotein and triglycerides, as well as increased high density lipoprotein (Lunn 2007; Keys et al. 2017), which are markers for atherosclerotic plaques and cardiovascular disease (Grundy et al. 2002). Our present study also shows improved blood lipid profile in mice exposed to whole seed, seed cake or seed oil, but further studies need to take the high-density lipoproteins to low density lipoprotein ratio into consideration. The cholesterol gall stone-induced disruption of liver function that we recorded in this study

was maintained by seed oil treatment but ameliorated by whole seed and seed cake treatment. This outcome correlates with reports of increased liver enzyme levels in the plasma of humans with gallstones (AL-Obiady et al. 2019), conversely, almond oil which majorly contains oleic acid like Moringa seed oil improves liver function after toxic exposure of rats to xenobiotics (Al-Attar 2020).

Hepatic pathologies are largely correlated with cholesterol gall stone disease and oxidative stress (Portincasa et al. 2006; Li et al. 2015), we therefore monitored liver antioxidants and found reduced activities of SOD and catalase, as well as level of GSH in hyperglycemic and cholesterol gall stone-induced mice, similar to reports of reduced antioxidant activity in gall bladder (Geetha 2002) and blood (Worthington et al. 2004) of

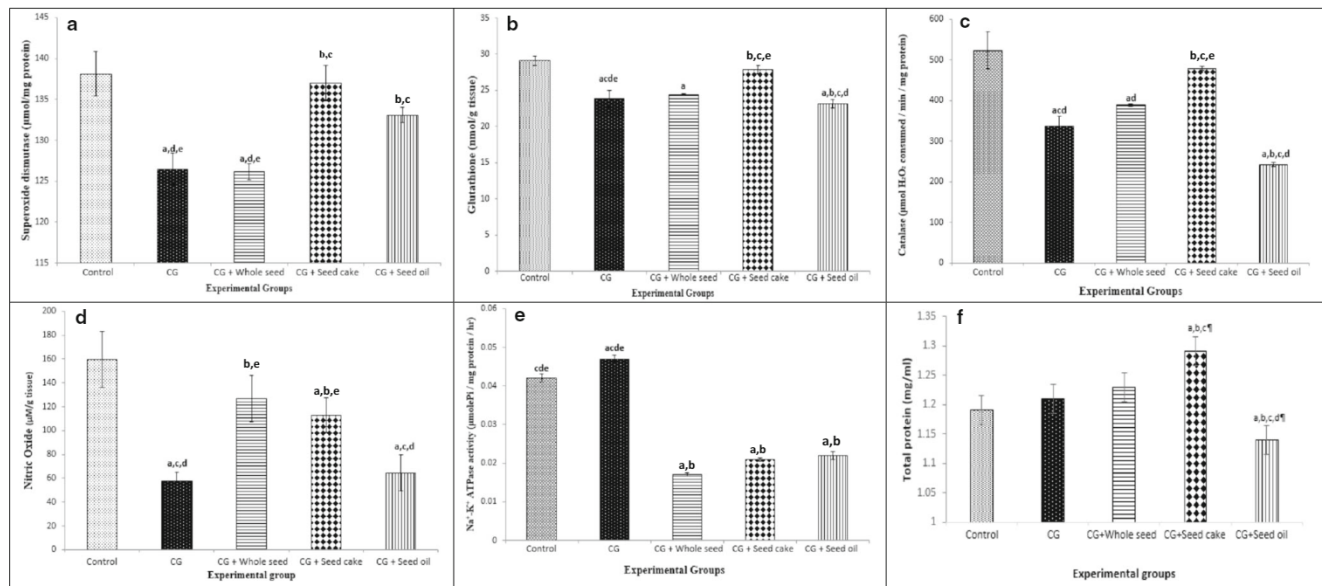


Fig. 4 Biochemical parameters in the liver of hyperglycaemic and cholesterol gall stone induced mice treated with seed extracts of *Moringa oleifera*. **a** Superoxide dismutase (SOD). **b** Glutathione (GSH). **c** Catalase. **d** Nitric oxide (NO). **e** Na⁺/K⁺-ATPase. **f** Total protein. Key: Intact control (control); hyperglycemia + cholesterol gallstone (CG); hyperglycemia + cholesterol gallstone + 30% milled

M. oleifera whole seed (CG + whole seed); hyperglycemia + cholesterol gallstone + 30% *M. oleifera* seed cake (CG + seed cake); hyperglycemia + cholesterol gallstone + 0.2 ml *M. oleifera* seed oil (CG + seed oil). Significance: ^a compared with control, ^b compared with CG, ^c compared with CG + whole seed, ^d compared with CG + seed cake, ^e compared with CG + seed oil

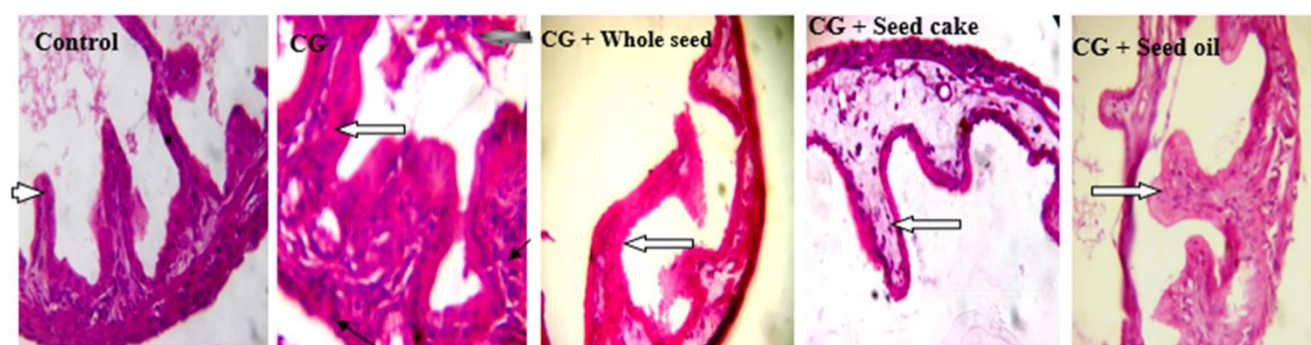


Fig. 5 Photomicrograph of gallbladder sections (H&E stain, mag $\times$ 400) showing: Control with normal architecture, single layer of normal tall columnar cells lining luminal folds of the gallbladder mucosal layer (white arrows), no mucus secretion present, no active mitotic division. CG with moderately normal architecture, proliferated layer of columnar cells lining luminal folds of the gallbladder mucosal layer which appear thickened, presence of mild gall stone, mild infiltration of inflammatory cells, active mitotic division. CG + whole seed with moderately normal

architecture, moderately thickened mucosal layer and folds of the gallbladder, no mucus secretion strands present, no active mitotic division. CG + seed cake with moderately normal architecture, single layer of tall columnar cells lining luminal folds of the gallbladder mucosal layer, no mucus secretion present, no active mitotic division noted. CG + seed oil moderately normal architecture, single layer of tall columnar cells lining luminal folds of the gallbladder mucosal layer, moderate mucus secretion strands, no active mitotic division

patients with cholesterol gall stones. There are records of restored liver antioxidant activity in mice (Das et al. 2015) and rats (Hamza 2010) fed with *Moringa oleifera* seed extracts, similar to our result of improved antioxidant activity in mice fed Moringa seed cake. The inability of seed oil and the whole seed to restore antioxidant levels in this study may be related to the hyperglycemic comorbidity.

Nitric oxide—the gastro enteric vasodilator and inhibitory neurotransmitter—affects cholesterol homeostasis via its effect on myoelectric activity in the enteric nervous system, intestinal peristalsis and transit time, as well as serum cholesterol levels (BROOKES 1993). Deviation in systemic cholesterol homeostasis, including liver uptake and biliary cholesterol secretion, predisposes humans to atherosclerosis and cholesterol gall stone formation (Di Ciaula et al. n.d.). The reduced nitric oxide levels that we recorded in the liver of the CG and CG + seed oil groups is in line with reports of increased serum cholesterol levels in mice mutated for the inducible nitric oxide synthase gene (Ihrig et al. 2001), but in contrast with reports of increased transcripts of endothelial nitric oxide synthase in immortalized human endothelial cells (Couloubaly et al. 2007).

The susceptibility of mice to gall stone formation has been linked with increased hepatic canalicular membrane expression of bile salt export pump (Hoda and Green 2003) which is a sodium-dependent pump (Stieger et al. 2007). This is similar to our findings of increased sodium potassium pump activity in the liver of hyperglycemic and cholesterol gall stone induced mice, which is ameliorated by *Moringa oleifera* seed extracts, in line with published evidences of the hepatoprotective effect of the functional food (Hamza 2010). Cholesterol gall stone formation typically disrupts the normal architecture of the gall bladder (Lavoie et al. 2012), we highlighted a thickened mucosal layer undergoing mitotic cell division with substantial infiltration of inflammatory cells in the gall bladder

of hyperglycemic and cholesterol gall stone induced mice. Interestingly, seed extracts of *Moringa oleifera* restored the normal gall bladder architecture, which indicates the cytoprotective property of *Moringa oleifera* seed extracts.

Conclusion

Moringa oleifera seed extracts probably restores deranged gall bladder architecture by reducing the infiltration of inflammatory cells. The seed cake is the most efficacious seed extract as a potentially adjuvant therapy for cholesterol gall stones in hyperglycemic state.

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Materials availability All materials used are available upon reasonable request made to the corresponding author.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethics approval Requisite approval (UI-ACUREC/19/005) was gotten from the Animal Care and Use Research Ethics Committee of the University of Ibadan.

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