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RESEARCH ARTICLE

Impact of Watermelon Seed Fortified Crackers on Hyperlipidemia in Rats

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ABSTRACT

Hyperlipidemia, commonly referred to as a high cholesterol condition, is a significant risk factor for cardiovascular diseases (CVDs), obesity, and metabolic syndrome. This condition involves elevated blood levels of lipids, which can be due to genetic or acquired causes. Researchers are increasingly turning to alternative medicine to tackle this disease. The prime objective of this study was to bring forth an innovative food product with significant anti-hyperlipidaemic potential. Many natural foods contain certain medicinal properties which may exert hypolipidaemic potential when taken on a daily basis. Watermelon (*Citrullus lanatus*) seeds (WMS) are rich in nutrients and nutraceuticals that may have potent effects against this disease. This research was designed to explore the hypolipidaemic impact of WMS in the form of fortified crackers (WMS-C) given to hyperlipidemic rats. A 21-day therapeutic trial was conducted on 12 male Wister rats divided into three groups: G1: negative control normal rats, G2: positive control high-fat diet fed hyperlipidemic rats, G3: 15% WMS-C fed group with hyperlipidaemic rats. The results demonstrated that incorporating WMS-C into the diet of hyperlipidaemic rats of G3 brought significant improvements in almost all biomarkers when compared to the results of G2. These significantly lowered body weight gain, total cholesterol, triglycerides, and LDL cholesterol in WMS-C group has strengthened its nutraceutical significance against hyperlipidemia and associated metabolic diseases. Therefore, the regular dietary intake of WMS could lower the negative health outcomes of CVDs and its associated health issues.

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INTRODUCTION

Hyperlipidemia, commonly described as a high cholesterol condition, is among the major risk factors for cardiovascular diseases (CVDs). Hyperlipidemia or elevated blood levels of lipids that result from either genetic causes or acquired disorders that disrupt the lipid transport system of the body (Mainieri *et al.*, 2023). Hyperlipidemia is mostly silent, and the person is discovered during the regular screening programs for the management of atherosclerosis. Discomfort in the heart due to angina or a heart attack may surface as a result of high levels of cholesterol (Gour *et al.*, 2023). The global burden of hyperlipidemia has been increasing in the past few decades. In 2008, the World Health Organization (WHO) estimated the global prevalence of elevated plasma total cholesterol levels among adults was 39%. Now, there are more than three million adults in the United States and Europe being diagnosed with hyperlipidemia in 2023 (Wei *et al.*, 2024). So, CVDs will become the leading cause of death and disability globally. The average of diagnosed cases of hyperlipidemia is 72.20% in the US, and the result of the study is a disappointment with 72.20% of the participants being diagnosed with this disease. Hyperlipidemia is the main determinant for CVD which contributes to the existing health crisis emerging from CVDs (Han *et al.*, 2023). Across Pakistan, the overall prevalence of Hypercholesterolemia has ranked highest in Punjab and lowest in Balochistan with 41.6 percent of the population affected followed by 22.7 percent (Zeeshan *et al*., 2023).

Hyperlipidemia can be classified based on the lipid and lipoprotein metabolism imbalances in relationship with atherosclerotic disease susceptibility. There is a

grouping of the lipoprotein fractions such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and TGs, which have been visualized and confirmed in chemical quantitation studies of plasma (Mainieri *et al.,* 2023). Hyperlipidemia may pose health-related serious outcomes, like one's vision could also be obstructed by conjunctivitis, which is mainly caused by an accumulation of fat beneath the eyes. Obstructions may appear as clots in arteries or veins from the brain and heart region hence interrupting or blocking blood flow. There is a widespread occurrence of metabolic abnormalities including obesity and impaired glucose levels in the respective individual suffering from the disease (Gour *et al.,* 2023).

Human and animal health are negatively impacted by diets high in cholesterol. Researchers are increasingly focusing on the use of alternative medicine to prevent CVDs (Bitrus *et al.,* 2018). Moreover, an anti-hyperlipidemia drug team persists in refusing to initiate statins and fibrates (the medications) as effective treatments for the elevated concentrations of plasma TC and TGs largely due to adverse effects on muscles and liver (Mainieri *et al.,* 2023).

Many natural foods contain certain medicinal properties e.g., hypolipidaemic, hypotensive, antidiabetic, anticancer, etc. (Akhtar *et al.,* 2022; Almundarij and Tharwat, 2023) Watermelon (*Citrullus lanatus*) is a tropical fruit in most parts of Africa, Asia, the United States, and Russia. It is an important horticultural crop in warm regions. It is mainly cultivated for its fruit part, but its seeds are also gaining popularity for health benefits (Seidu and Otutu, 2016). It is familiar all over the world for its taste and huge content of water. As now the by-products from watermelon including rind and seeds have been given less attention. Watermelon seeds have a variety of nutrients as well as a wide range of phytochemical molecules with potent activities against disease that promote human health during their clinical studies and previous dissections (Benmeziane and Derradji, 2023).

As various edible components of watermelon have been studied previously for various nutritional and nutraceutical benefits, its seeds are gaining significance for hypolipidemic potential. According to an earlier study, the phytochemicals in watermelon have the potential to exert a hypolipidemic and cardioprotective effect because they can significantly raise HDL and lower LDL, triglycerides, and total cholesterol while also improving the structural integrity of the heart muscles. An oral intake of 120 mg/kg/day of WMSs extracts for 3 weeks resulted in decreased serum cholesterol, C-reactive proteins, glutathione and catalase level in adult male mice which might be due to the presence of citrulline and arginine in WMSs (Zia *et al.,* 2021). Similarly, another study examined the nutritional value of WMS and oil on the biochemical parameters in rats during a 28-day oral administration period. In rats, a maximum dosage of 50 mL/Kg of WMS oil did not cause any harm. Without compromising the integrity of the liver, WMS oil dramatically reduced blood levels of TGs, TC, LDL, and alanine transaminase (ALT) while increasing those of HDL, VLDL, sodium oxide dismutase (SOD), and catalase (CAT) (Eke *et al.,* 2021).

Owing to this significant hypolipidemic potential of water seeds, this research was therefore designed to explore their hypolipidaemic impact in cooked ready to eat crackers form. The watermelon seed-fortified crackers (WMS-C) were given as a novel functional food to hyperlipidemic rats in order to evaluate the nutraceutical potential to control hyperlipidemia and related health complications.

MATERIALS AND METHODS

Procurement of materials: All the raw materials including watermelon seeds (WMSs) of a Crimson Sweet cultivar, wheat flour, ghee, etc. were bought from the local market in Faisalabad, Pakistan. WMSs were washed thoroughly and then these were sun-dried. The fully dried seeds were then peeled and ground in an ordinary kitchen grinder till a smooth homogeneous powder was obtained. The sample collection kits were purchased from Life Care pharmaceutical store in Faisalabad, Pakistan.

Chemicals and reagents: All the chemicals and reagents of analytical grade including ether, choloroform, sulfuric acid (H_2SO_4), potassium sulfate, copper sulfate, sodium hydroxide (NaOH) solution, boric acid solution, hydrochloric acid (HCl), bromocresol green and methyl red as indicators, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, ethanol, methanol, aluminum chloride ($AlCl_3$), potassium acetate (CH_3COOK), quercetin, catechin, ascorbic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent, and distilled water were purchased in a good quality from SAQI Chemicals in Faisalabad district of Pakistan.

Preparation of watermelon seed-fortified crackers (WMS-C): WMS were cleaned, processed into a fine powder, and added to 100g of wheat flour to replace 15g of wheat flour, for a 15% fortification level. To make a dough for WMS-C, 100g of WMS-fortified flour, 2g of salt, 4g of sugar, 30g of ghee, and 40mL of cold water were combined in a bowl mixer and stirred for approximately five minutes. Using a moulder, the dough was sheeted to a thickness of about 3mm, and the crackers were formed. The finished crackers were baked at 180°C for around 15min in a baking oven. After that, the baked crackers were cooled at room temperature for 1 hour and then packed in appropriately labelled zip lock bags.

Proximate composition of WMS-C: The chemical characteristics of the WMS-C were assessed following the procedures outlined in the AOAC methods (AOAC, 2019; Rabail *et al.,* 2022). Moisture content was determined by drying the 10g of crackers' samples in a hot-air oven at 105°C for 24 hours (Method No. 925.08), and total ash was quantified by burning the 5g of sample at 600°C for 6 hours in a muffle furnace (Method No. 923.03). Crude protein was assessed using the Kjeldahl method to find out the % Nitrogen (N) and this %N was multiplied with the factor of 6.25 to obtain the amount of protein (technique No. 979.09), while crude fat was extracted using Soxhlet's ether extraction method (Method No. 920.39). Additionally, ether-extracted fat-free samples were analyzed for crude fiber content after acid and alkali

treatments (Method No. 962.09). The total carbohydrate content in all samples was calculated as the difference using the formula given below:

Total carbohydrates (%) = 100 – [moisture (%) + ash (%) + protein (%) + fat (%) + fiber (%)]

Animal housing: With permission from the university's ethical review committee, a total of twelve male adult Wistar rats, weighing between 150–200g and aged 6–8 weeks, were well-housed in standard square foot cages with four rats per cage in the animal room in National Institute of Food Science and Nutrition, University of Agriculture, Faisalabad, Pakistan. The rats were raised in a continuous setting with a 25±5°C ambient temperature, an equal duration of light-dark cycle, and unlimited access to food and water. Rats were first acclimatized to the environment for a while then subjected to trial diets. Rats were fed a commercially accessible diet consisting of 25% proteins, 7% fats, 3% vitamins, and 35% carbs in rat pellet form.

Experimental design, drug, and diet allocation: For the induction of hyperlipidemia, a 45% high-fat diet (HFD) in the form of 45% Vanaspati ghee mixed in 55% control diet and was given for 28 days to the groups G2 and G3. A 21-day therapeutic trial was followed by the 28-day hyperlipidemia induction period on 12 male Wister rats divided into three groups. The first group, i.e., G1 is the negative control group with no induction of hyperlipidemia and was fed a normal diet throughout the trial. The second group G2 was the positive control group with hyperlipidemic rats, given a normal diet throughout the trial. The last group G3 was the treatment group with hyperlipidemic rats, given 100% WMS-C in replacement to the normal diet.

Physical and biochemical analysis: The body weight change in terms of increase or decrease in the total body weight was recorded on a weekly basis (Rabail *et al.*, 2022). For biochemical analysis, the rats were anesthetized with ether in an anesthesia chamber. As the rats have shown their first sign of unconsciousness, they were taken out immediately and the blood samples were taken by cardiac punctures of rats in serum separating yellow vials. After being put into the vials, the blood samples were centrifuged for 20 minutes at room temperature at 3700rpm. Following serum separation, blood glucose was measured immediately with 10µL and other parameters were measured with the remaining serum that was stored at -20°C (Mahmoud *et al.*, 2017; Albegali *et al.*, 2022)**.** Using the procedure suggested by Gao *et al.* (2021) blood samples were taken from the rats, serum was separated. The biochemical parameters related to hyperlipidemia including (TC, TG, HDL, LDL, VLDL), liver function tests (LFT) including (Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP)), and renal function tests (RFT) including (Urea, Creatinine, Bilirubin) (Yang *et al.*, 2022; Batool *et al.*, 2024).

Oxidative stress biomarkers and antioxidant enzymes: The collected sera of experimental subjects were further analyzed for oxidative stress biomarkers including total antioxidant capacity (TAC), total oxidative stress (TOS), and antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (GSH). The sera were centrifuged for 20 minutes at 5000rpm and refrigerated at a 4°C. After centrifugation, the collected supernatant in Eppendorf tubes was stored at -20°C for analysis (Verma *et al.*, 2022). To determine the activity of SOD, 250µL of 50mM phosphate buffer (pH=7.8) was added with nitro blue tetrazolium (50µL), EDTA (0.3mM), triton X (100µL), L-methionine (100µL), and distilled water (400 µL). The supernatant (50µL) and riboflavin (50 µL) were then mixed in a reaction mixture and tubes were illuminated for 20min in ultraviolet light. Finally, the absorbance of samples was measured at 560nm and compared with the standard (Kim *et al.*, 2016).

Statistical analysis: Three runs of the analyses were done to get the mean ± S.D. for each analysis. One-way analysis of variance (ANOVA), Two-way analysis of variance (ANOVA), independent sample t-test, and Tukey HSD test were used with IBM® SPSS® Modeller 16.0 to investigate treatment and mean differences at a 95% ($P<0.05$) confidence interval.

RESULTS

Proximate composition of WMS-C: As described in Table 1, increasing the amount of WMS flour significantly ($P<0.05$) enhances the moisture, ash, protein, fiber, and fat while reducing the carbohydrate content in crackers. This significant and gradual increase in moisture, ash, protein, and fat along with the increase in WMS fortification level has made gradual and significant decline in the carbohydrates content of crackers.

Body weights: The results of body weight listed in Table 2, indicated highly significant variations ($P<0.001$) in the initial and final body weights of rats in all three groups. While looking into the percentage body weight gain in this trial there was the least body weight gain in the WMS-C group and this reduction is also highly significant ($P\leq0.01$) when compared to other groups.

LFTs: The results of LFTs have been tabulated in Table 2. The results of LFTs have revealed a highly significant ($P<0.001$) incline in all three biomarkers of ALT ($F = 74.5$; $P<0.001$), AST ($F = 185.7$; $P<0.001$), and ALP ($F = 254.3$; $P<0.001$) in positive control G2 and compared to the negative control group G1. But in G3 these biomarkers have shown a significant decline which indicates the normalization of these biomarkers and hence indicates a hepatoprotective property of WMS.

RFTs: The results of RFTs in Table 2 also indicated the same trend as were noted for LFTs. There was a highly significant ($P\leq0.01$) increase in RFTs of positive control hyperlipidemic rats, when compared to negative control normal rats. Here too the administration of WMS in diet has helped balancing back these raised RFTs near to normal levels significantly. An increase in creatinine levels indicates impaired kidney function since the kidneys are unable to eliminate creatinine from the blood. Comparing the treatment with the aqueous extract of

Table 1: Results for the proximate composition of WMS-C

WMS Fortification	Moisture	Ash	Crude protein	Carbohydrate	Crude fat	Crude fiber
0% WMS	0.26±0.03d	1.05±0.02d	3.67±0.02d	56.36±0.03a	23.19±0.03d	1.36±0.01c
5% WMS	0.34±0.02c	1.16±0.02c	5.44±0.02c	53.22±0.04b	23.57±0.02c	1.39±0.03bc
10% WMS	0.41±0.01b	1.37±0.02b	8.16±0.04b	48.55±0.03c	24.26±0.02b	1.55±0.02a
15% WMS	0.47±0.01a	1.45±0.02a	10.94±0.03a	44.73±0.03d	24.92±0.02a	1.45±0.04b

Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at $p < 0.05$.

Table 2: Results of the body weight, LFTs, RFTs, oxidative stress biomarkers and enzymes

Groups	1	2	3	F-Value
Body Weight D-0	76.7±2.06c	89.3±6.60b	107.3±6.07a	33.28***
Body Weight D-49	110.5±3.87b	126.5±6.60a	131.3±8.02a	11.53***
Body Weight Gain	33.7±4.92a	37.3±0.500a	24.0±5.47b	10.38***
% Weight Gain	44.08±7.31a	41.9±3.06a	22.43±5.14b	19.08***
ALT	28.3±2.9c	49.5±2.0a	38.7±2.2b	74.5***
AST	20.5±1.3c	46.5±2.0a	33.7±2.2b	185.7***
ALP	297.3±5.3c	380.7±7.1a	318.0±3.1b	254.3***
Urea	22.7±2.5c	54.0±1.8a	38.7±2.2b	202.0***
Creatinine	0.85±0.13c	1.53±0.09a	1.07±0.09b	40.5***
T-Bilirubin	0.43±0.09c	1.33±0.13a	0.80±0.08b	77.4***
SOD	247.8±4.82b	189.5±5.73c	263.01±3.51a	263.995***
GSH	176.9±3.73a	118.3±5.76c	148.04±3.63b	170.319***
CAT	2.37±0.047b	1.86±0.072c	2.68±0.090a	131.230***
TAC	443.0±3.5a	295.7±3.7c	363.4±13.5b	307.7***
TOS	1.37±0.47c	7.25±1.25a	4.0±0.81b	41.9***

Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at *** = Very highly significant at P<0.001; ** = highly significant at P<0.01; *= significant at P<0.05; ns = non-significant at P>0.05.

WMS to the positive control, the concentrations of urea and creatinine were significantly lower. The potency of WMS therapy to cause a decrease in proteolysis may account for the notable drop in urea content following treatment.

Oxidative stress biomarkers: The results for SOD in Table 2 were very highly significant (P<0.001) among the three groups. The results of SOD were a maximum of 263.01±3.51 and a minimum of 189.5±5.73 for G2. The results for GSH in Table 2 were very highly significant (P<0.001) among the three groups. The results of GSH were a maximum of 2.68±0.090 for G3: WMS-C group and was minimum for G2 respectively, while GSH was a maximum of 176.9±3.73 for G1 in the control group.

Lipid Profile: The results of TC in Table 3 indicate that there was a highly significant variations (P≤0.001) in TC values for both group-wise and day-wise. The initial TC values for all groups were almost the same and non-significant (P≥0.05), these made significant changes (P≤0.05) in G2 and G3 for the last day values. The highly significant decline (P≤0.001) of TC from 217 to 196.7 in G3 indicates its hypolipidemic potential. Similarly, the results of TG in Table 4 have indicated highly significant variations (P≤0.01) among the groups and among the days. TG levels increased significantly (P≤0.05) upon hyperlipidemia induction in groups G2 and G3. Later, the TG in G3 has made significant decline which again here justifies the hypolipidemic potential of WMS. The results of HDL have been presented in Table 5. These reveal a highly significant increase in the HDL content of G3 rats in the last days-49. This increase in HDL justifies the health-protective and lipid profile ameliorating the potential of WMS. Likewise, the results of LDL have been presented in Table 6. These results again have shown the same incline after the hyperlipidemic inductions, whereas the levels of LDL have marked highly significant declines in G3 which is a positive outcome and hypolipidemic potential of WMS.

Table 3: Results of TC

Group	TC			F-Value
	D-0	D-28	D-49	
1	171.9±5.6c	175.1±7.6c	172.6±3.0c	101.25***
2	173.4±6.9c	217.8±4.4a	209.3±2.9a	
3	173.7±5.9c	217.0±2.8a	196.7±1.7b	
F-Value	114.76***			25.43***

Note: Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at *** = Very highly significant at P<0.001; ** = highly significant at P<0.01; *= significant at P<0.05; ns = non-significant at P>0.05.

Table 4: Results of TG

Group	TG			F-Value
	D-0	D-28	D-49	
1	100.7±2.9d	101.7±1.8d	101.7±1.5d	557.53***
2	103.3±3.8d	163.0±3.5a	151.3±2.7b	
3	103.7±3.5d	163.0±3.3a	126.3±1.5c	
F-Value	572.24***			160.70***

Note: Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at *** = Very highly significant at P<0.001; ** = highly significant at P<0.01; *= significant at P<0.05; ns = non-significant at P>0.05.

Table 5: Results of HDL

Group	HDL			F-Value
	D-0	D-28	D-49	
1	48±2.5b	49±1.8ab	50±1.8ab	136.04***
2	49±2.5ab	28.5±1.3c	30.7±0.95c	
3	49±2.1ab	29±1.8c	53.2±1.7a	
F-Value	146.9***			85.10***

Note: Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at *** = Very highly significant at P<0.001; ** = highly significant at P<0.01; *= significant at P<0.05; ns = non-significant at P>0.05.

Table 6: Results of LDL

Group	LDL			F-Value
	D-0	D-28	D-49	
1	103.7±4.5d	103.3±4.3d	102.2±1.7d	272.97***
2	103.7±4.0d	156.7±3.5a	148.3±2.2b	
3	104.0±4.5d	156.7±3.8a	118.3±1.7c	
F-Value	290.57***			98.89***

Note: Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at *** = Very highly significant at P<0.001; ** = highly significant at P<0.01; *= significant at P<0.05; ns = non-significant at P>0.05.

DISCUSSION

The variation in proximate composition of WMS-C can be attributed to the inherent properties of WMS. When mixed into the cracker's dough, WMS contains water, contributing to the overall moisture level. Additionally, the hydrophilic nature of the fibers in the seeds helps to retain more water in the cracker (Gómez-García *et al.*, 2020). Adding WMS flour to cracker's dough increases the total protein content because WMS are rich in protein, reflected in the increased protein levels observed in the final product (Zhang *et al.*, 2023). WMS is a good source of essential calcium, magnesium, and potassium minerals. Therefore, incorporating muskmelon seed flour into crackers increases their mineral content, as the higher ash levels indicate. It also significantly improves the fiber content, promoting digestive health and contributing to a feeling of fullness. The increase in fiber content is directly linked to the high fiber composition of the WMS (Gómez-García *et al.*, 2020). Also, WMS contains beneficial fats, including unsaturated fatty acids, which ultimately raise the fat content of biscuits, altering the texture and flavor to make them richer and potentially more tender. In contrast, the carbohydrate content in crackers was reduced because WMS have a lower carbohydrate content than standard wheat flour (Zia *et al.*, 2021). Similar findings have been reported by Kaur *et al*. (2019) in which the cookies were prepared by adding flax seed flour in a common recipe for the cookies. The incorporation of WM protein isolates into the cookies, resulting in higher values of moisture, ash, protein, fiber, and fat contents and lower values of carbohydrate contents (Wani *et al.*, 2012). Likewise, Laczkowski *et al.* (2021) prepared cookies by incorporating chia seed flour and reported the same findings regarding proximate composition.

On the other hand, the results of efficacy trial in hyperlipidemic rats indicated reduction in body weight in WMS-C group. As long as the body weights are concerned, a previous study reported the same remarkable decrease in the final body weights of rats when given WMS. This decrease in body weight gain indicates that WMS may be able to inhibit the activity of the enzyme HMG-CoA reductase, which is in charge of the manufacture of cholesterol. Moreover, it has been claimed that linoleic acid, or 9,12-octadecadienoic acid, is an important fatty acid that can prevent body weight growth in those who are prone to obesity (Eke *et al.*, 2021). As increased body weight and obesity is directly linked with prevalence of hyperlipidemia and CVDs, therefore, the incorporation of WMS in diet of hyperlipidemic patients could have a significant potential to prevent or reduce obesity and other related metabolic disorders.

Similarly, while discussing the outcomes of serum chemistry there were similar significant hepatoprotective ameliorations in LFTs as previously have been reported in the works of Rabiu *et al.* (2020). Furthermore, it is widely accepted that raised AST activity is a sign of damage to the liver's mitochondria and that increased ALT activity indicates the extent of damage to the liver's cell membrane. The blood concentrations of the hepatic enzyme markers ALT and AST were markedly lowered by WMS extract in the previous study. Thus, the most significant and useful indicator for assessing liver cell damage is AST. The findings of the experiment demonstrated that the administration of CCl4 to mice immediately increased the levels of ALT and AST in their blood. Serum ALT and AST enzyme activity dropped following WMS extract pretreatment, indicating that WMS extract may be an efficient hepatocyte protector (Zhan *et al.*, 2016). The lowering of LFTs by administration of WMS is a good indicator of its hepatoprotective potential against hepatic stress induced by elevated blood lipid biomarkers. Likewise, the WMS extract's ability to ameliorate the kidneys and increase their rate of filtration may account for the treatment's notable drop in creatinine content (Ogbeifun *et al.*, 2020). A rise in creatinine levels implies poor kidney function or renal disease. This disorder produces an increase in creatinine levels in the blood due to renal inability to remove it. When compared to the diabetic control group, the WMS extract administration significantly reduced urea and creatinine levels. The significant decline in urea concentration after treatment might be due to the extract's capacity to lower glucose levels and increase insulin concentration, resulting in less proteolysis. The significant decline in creatinine concentration after therapy may be attributed to the extract's ability to ameliorate the kidneys, hence increasing the rate of filtration by the kidney (Ogbeifun *et al.*, 2020).

While discussing the oxidative stress biomarkers, SOD catalyzes the conversion of superoxide free radicals to less harmful hydrogen peroxide, while GSH breaks down hydrogen peroxide into water and oxygen, and it can directly detoxify lipid peroxides. The study found that WMS extract successfully prevented the decrease of antioxidant activity. GSH is also essential for cellular defense against oxidative stress. GSH can directly react with reactive electrophiles to remove oxygen species. It protects cells from free radicals, peroxides, and other harmful substances. WMS extract might greatly boost GSH activity. WMS extract may decrease lipid peroxidation, which stabilizes the liver cell membrane and reduces cell damage (Zhan *et al.*, 2016).

Among the results for oxidative stress biomarkers, there was a sharp and highly significant decline in TAC values of hyperlipidemic rats in G2, which significantly improved values on WMS-C administration in diet. Whereas the results of TOS have increased after hyperlipidemia induction, which was again significantly reduced in the WMS group. These results are good indicator of the very high antioxidative potential of WMS. The presence of various phytochemicals like saponins, lycopene, and glycosides in WMS makes it a potent source for anti-oxidation (Seidu and Otutu, 2016). Some other studies also report the antioxidative potential of WMS by confirming the presence of phytochemicals and antioxidant properties in its peels and seeds (Mehra *et al*., 2015; Neglo *et al.*, 2021).

The results of the lipid profile are in line with the results of a similar study, which reported that WMS considerably boosted the concentrations of HDL and considerably decreased TC, TG, and LDL. It was discovered that this decrease was dosage-dependent (Francis *et al.*, 2019). In this study the TC decreased from 217.0 to 196.7 and this decrease is more than double as

we recorded in G2 with no WMS. Similarly in a study the levels of TC, TG, and LDL were reduced by the doses of 2 and 5 mL/kg, VLDL and HDL were dose-dependently increased. The substantial drop in cholesterol levels indicates that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol, may be decreased by repeatedly consuming WMSO for 28 days. Linoleic acid lowers total and LDL cholesterol, as demonstrated by randomized clinical studies, when saturated fat is substituted. Thus, the high content of linoleic acid in WMSO may help to maintain a balanced lipid profile (Eke *et al.*, 2021). The reductions in TG (163.0 to 126.3) are achieved in the similar way as that of TC. TG are produced by liver and are not soluble in water and bind to lipoproteins in the bloodstream. A study reported that WMS extract at a dosage of 100 mg/kg body weight effectively reduced serum TG levels. Research suggests that WMS extract can reduce TG levels and protect the liver (Zhan *et al.*, 2016). In another study, the aqueous extract of WMS reduced TC, TG, and LDL, while increasing HDL in all treatment groups after 7-21 days. WMS aqueous extract may have anti-hyperlipidemia and hyperglycaemia benefits due to its high content of flavonoids, alkaloids, and tannins (Ogbeifun, Peters, and Monanu, 2020). Similar improved lipid biomarkers revealed in a previous study upon feeding WMS has justified the cardiac protectiveness of these seeds. The test animals had an elevated HDL/LDL ratio because of the combined effects of increased HDL and lower LDL. Given that a high HDL/LDL ratio has been demonstrated to be advantageous and is suggestive of a decreased risk of coronary heart disease, it is possible that adding to one's diet might lessen the chance of developing CVD (Ayo-Lawal *et al.*, 2015).

Conclusions: One of the main risk factors for obesity, metabolic syndrome, and CVDs is hyperlipidemia, often known as high cholesterol. In essence, hyperlipidemia is the presence of high blood lipid levels due to either acquired or inherited factors. Globally, researchers are concentrating more and more on using complementary and alternative medicine to treat this illness. When regularly consumed, the therapeutic qualities of many natural foods may have the potential to cause hypolipidemia. WMS includes a range of minerals and nutraceuticals that have strong anti-disease properties. This research was therefore designed to explore the hypolipidaemic impact of WMS in the form of fortified crackers (WMS-C) given to hyperlipidemic rats. The study's findings showed that adding WMS-C to the food of hyperlipidaemic rats had health-improving effects considerably more quickly and significantly. In comparison to the G2 group, the G3 group's findings for practically every biomarker, such as body weight, lipid profile, liver, and renal function tests, were quite near to the normal levels of the G1 group. The relevance of the fortification of WMSs in the development of crackers or biscuits as a functional food against obesity, hyperlipidemia, and CVDs has been reinforced by the results of our study. Biscuits or crackers are usually consumed on a daily basis, so they are the best vehicles for the fortification of functional ingredients in it like WMSs to control the widely spreading health complications of CVDs, obesity, fatty liver and diabetes. The small sample size (number of rats in each group) is the limitation of our research. The results may be clearer and more accurate if research is carried out on more rats. So as a future perspective, it is recommended to carry out research on large sample size. Moreover, it is recommended to perform proper sensory evaluation of the WMS-C as a new bakery product by engaging trained panelists to determine its overall acceptability for carrying out human trials.

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Authors contribution: Najla AlMasoud: Conceptualization, Investigation, Data curation, writing original draft, Writing review & editing, Supervision. Seemal Munir: Data collection, Experimentation, Software, Writing review & editing. Taghrid S. Alomar: Conceptualization, Investigation, Writing review & editing. Roshina Rabail: Investigation, Formal analysis, Writing original draft. Syed Ali Hassan: Conceptualization, Methodology, Writing review & editing. Rana Muhammad Aadil: Conceptualization, Investigation, Formal analysis, Data curation, writing original draft, writing review and editing, Supervision.

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