Effect of dry and germinated oat on hypercholesterolemic rats

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ABSTRACT

The main target of this study was evaluating the effect of two concentration of dry and germinated oat consumption 15% and 30% for 6 weeks on hypercholesterolemic rats. Thirty six male albino rats weighing 200±20 g were used in this study and divided into six groups (6 rats in each group). First group fed on basal diet and used as a (negative control group). The second group fed on hypercholesterolemic diet (HCD) (diet containing 1% cholesterol,16% saturated fat and 0.25% colic acid) and used as a positive control group. Groups (3 and 4) rats fed on HCD containing 15 and 30% whole dry oat. Groups (5 and 6) rats fed on HCD containing 15% and 30% germinated oat. At the end of the experimental period body weight gain and organs weight was estimated. On the other hand lipid fractions, liver enzymes including aspartate aminotransferees (AST), alanine aminotransaminase (ALT), kidney function including creatinine, uric acid, urea and antioxidant enzymes including glutathione reduced (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxides (GPx) were determined in the serum The results demonstrated that germinated oat contained highest fiber while, dry oat contained lowest levels of fiber. HCD caused a significantly increase in TC, TG, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), Atherogenic index (AI), LDL/HDL ratio, AST, ALT and kidney function, Furthermore, it caused a significantly decrease in HDL, HTR, GSH, CAT, SOD and GPx .The rats received dry oat with high concentration (30%) was the most effective to reduction of TC, TG, LDL-C and VLDL-C than dry 15% and germinated 15%. While, germinated oat 30% revealed non- significant effect on TC and LDL-C levels, although it has more effective to raise antioxidant enzymes a activity. All treatments revealed a significantly decrement of AI, AST, creatinine, uric acid and urea. Conclusion: addition of whole dry and germinated oat to HCD for 6 weeks has potential effects to reduced serum cholesterol levels, LDL-C and VLDL-C. The high concentration of dry oat and low concentration of germinated oat had more effective to reduced lipid profile.

Keywords: Hypercholesterolemia, oat, β-glucan, germination, antioxidant enzymes, lipid profile.

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INTRODUCTION

Hypercholesterolemia is a health problem characterized by elevation of the blood cholesterol level (WHO, 2018). The cholesterol is an essential component for healthy cell membranes like brain, skin, nervous tissue, and intestine. It is precursor synthesis of steroid hormones, bile acids that assist in the fat absorption process and a reserve energy source for the body and precursor of vitamin D syntheses (Mazroatul et al., 2016 and Huang et al., 2022). However, the higher cholesterol is linked with heart disease (Byrne et al., 2019). Hypercholesterolemia is suspected to be a factor in the development of coronary heart disease (Jempormase et al., 2016). Furthermore, high low-density lipoprotein (LDL-C) cholesterol levels in patients can accelerate the occurrence of plaque which causes by narrowing and hardening of the arteries (Ibrahim et al., 2020).

Hypercholesterolemia, or high cholesterol, can be caused by a person's lifestyle, hereditary factors, or, less frequently, secondary health issues including kidney disease. Poor diet, inactivity, smoking, some drugs, and pathological disorders such as diabetes, obesity, chronic renal disease, hypothyroidism, and polycystic ovarian syndrome are all contributors to the rise in cholesterol (Kanter et al., 2012 and Hu et al., 2020). Genetics, age, and gender are a few of the non-modifiable risk factors for hypercholesterolemia, as they are for other kinds of CVD. One of the main factors leading to the development of cardiovascular disease (CVD) is hypercholesterolemia, which, when treated well, significantly lowers the risk of CVD-related morbidity and mortality. (Duggan et al., 2022 and Vahid et al., 2022)

Hypercholesterolemia is associated with increased risk for atherosclerotic cardiovascular disease (Carmena and Betteridge, 2019). Cardiovascular disease accounted 46.2% of overall mortality in Egypt (Hassanin et al., 2019). The prevalence of hypercholesterolemia among young age group in Saudi Arabia was 23.7% (Rosada et al., 2020). While, in the United Kingdom (UK) increased trend of crude prevalence of primary hypercholesterolemia and mixed dyslipidemia from 13.5% in 2009 to almost double at 23.5% by 2019 (Bilitou et al., 2022). 37% of Egyptian population has blood cholesterol levels with an overall target accomplishment of only 34.4% (Reda et al., 2014). To treat hypercholesterolemia, numerous cholesterol lowering medicines are used (Choi et al., 2022). Many times, medication like enzetimibe and statins fail to reduce LDL-C levels to the recommended level, having residual CVD risk (Bovet and Paccaual, 2011). Myotoxicity and statin related muscle side effects. Another negative effect of statins is the disruption of mitochondrial activity (Hussain et al., 2023). So, the dietary intervention strategies used to prevention and treatment of cardiovascular disease because effective compared with the lipid lowering drugs and they have less adverse effects (Backes et al., 2017 and Zhao et al., 2017).

The beneficial effects of foods have been linked to the presence of bioactive compounds and other nutrients, for example phenolic compound, catechin and chlorogenic acids have antioxidant activity (Zanotti et al., 2015). Presence β-glucan has a lipid lowering effect and polysaccharides have hypolipidemic effect (Korolenko et al., 2020). Lifestyle changes (such as an altered, healthy fat diet, moderate exercise, together with weight loss) have the potential to lower cholesterol to within normal ranges without the addition of cholesterol lowering drugs (Walter et al., 2019). Increasing whole grains intake are among various dietary adjustment strategies which widely investigated of blood lipid control (Chen et al., 2016 and Zong et al., 2016). The consumption of whole grain products is decrease risk of some diseases including cancers, diabetes, gastrointestinal and cardiovascular (Barrett et al., 2020) because of their effective of lipid lowering compared with drugs with less adverse effects (Backes et al., 2017 and Zhao et al., 2017).
Oat (*Avena sativa* L.) is a member of the *Poaceae* or *Gramineae* family; it is healthy whole grains that primarily provide carbohydrates in the form of starch. They also have a somewhat high fat content and include various micronutrients, including vitamin B1, B6, folate, Mn, Mg, Se, Fe, Zn, and Cu (Stewart and McDougall, 2014). Based on the dry weight of whole oats, the dietary fiber content is 0.2% resistant starch, 0.1% fructans, 0.6% cellulose, 3.8% β-glucan, 2.1% arabinoxylan, 2% lignin, and 1% other (Bach Kundsen et al., 2017 and Mao et al., 2022).

Numerous clinical and epidemiological research have suggested that germination of whole grains may have positive effects on a variety of health conditions, including diabetes, hypertension, cancer, and an improvement in the gut flora (Gong et al., 2018 and Yao et al., 2020). So, people have become increasingly interested in the creation of germinated whole grains in recent years, largely because these grains have more beneficial nutritional and functional qualities such as γ-oryzanol, phenolic acids, and aminobutyric acid (Sharma et al., 2016 and Dhillon et al., 2020). Germination, a complex process that involves physical, chemical, and structural changes in grains, has been discovered to be a low-cost, high-impact method of improving cereal quality. The development of the grain embryo, as shown by the growth of rootlets and the change of the endosperm contents, characterizes the germination process (Guine and Dos Reis Correia, 2013).

The germination process modifies grain structure and produces new, highly bioactive chemicals that can improve grain nutritional value and stability (Nonogaki et al., 2010). Numerous clinical and epidemiological researches have shown that germinated whole grains may help prevent diabetes, hypertension, cancer, and improve gut microbiota (Hsu et al., 2008; Ho et al., 2012 and Yao et al., 2020). So, the aim of this study was evaluation hypcholesterolemic effect of dry and germinated oat

**MATERIALS AND METHODS**

Whole dry oats were obtained from Agricultural Research Center, Giza, Egypt. Cholesterol was obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical Instruments: Casein, vitamins, minerals. Choline chloride, L-cystine and cellulose were obtained from Honest Company, 6 October, Giza, Egypt. Lamb fat and starch was purchased from the local market. Kits used to determine serum cholesterol, triglycerides, HDL, uric acid, urea. Creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and antioxidant enzymes supplied by Biodiagnostic Company, Dokki Giza, Egypt

Germination preparation of oat

Germination was prepared according to (Capanzana and Buckle, 1997) as follows: Whole dry oat was cleaned, washed and soaked in water at 35 - 40 C° for about 20-24 h, draining water and keeping in moist condition for 20-24 h, and during soaking period, changing the water every 3-4 h to prevent fermentation which usually produces undesirable odor and to maintain consistent water temperature. The long sprout of barley and oat ranged from 0.5 to 1mm. After that the germinated grains were then dried in oven at 45 C° for 24 hours and then stored separately in plastic bags. After that germinated and dried grains were grounded.

Chemical analysis of dry and germinated Oat

-Moisture content, total protein, ash content was determined according the method described by A.O.A.C. (2000). Total oil content was determined according to the method described by A.O.A.C. (1995). Total carbohydrate was determined by Phenol–Sulfuric acid method according to the method described by DuBois et al., (1956).

Biological experimental
Thirty six male Albino rats weighing about 200 -220 g used to this investigation, and divided into six groups (6 rats in each group) as follows:
Group (1): normal control group was fed on basal diet preserved as negative control. The basal diet was prepared according to Reeves et al., (1993).
Group2: (positive control): rats fed on hypercholesterolemic diet it contained 1% cholesterol + 16% staturated fat and 0.25% colic acid according to Harnafi et al., (2009).
Group (3): rats fed on hypercholesterolemic diet containing 15% dry oat.
Group (4): rats fed on hypercholesterolemic diet containing 30% dry oat.
Group (5): rats fed on hypercholesterolemic diet containing 15% germinated oat.
Group (6): rats fed on hypercholesterolemic diet containing 30% germinated oat.

Biochemical analysis

Rats were starved overnight before being sacrificed at the end of the study (6 weeks), and their blood was then taken and centrifuged separated serum was kept at -20°C until analysis.

Determination of lipids profile for hypercholesterolemic rat fed on dry and garmented oats

The triglycerides were determined according to the enzymatic method described by Bucolo and David, (1973). The Serum total cholesterol (TC) was determined according to the enzymatic method described by Allian, (1974). Serum HDL-Cholesterol was determined according to Warnick and Wood, (1995). LDL cholesterol was calculated by Fried Wald equation according to the method described by Wieland and Seidel, (1983) as follows:

\[ \text{LDL-C (mg/dl)} = \text{Total cholesterol} - [(\text{TG/5}) + \text{HDL}] \]

Very low density lipoprotein (VLDL) was calculated by Friedwald et al (1972).

Atherogenic index (AI) was calculated according to the method described by (Dobiasova and Frohlich, 2001) by the following equation:

\[ \text{Atherogenic index (AI)} = \log \left( \frac{\text{TG}}{\text{HDL}} \right) \]

\[ \text{LDL-C/HDL-C ratio= LDL-C /HDL-C x 100} \]

\[ \text{HTR=TC/HDL-C x100} \]

Determination of liver function for hypercholesterolemic rat fed on dry and garmented oat:

Alanine aminotransferase ALT and aspartate aminotransferase AST were determined according to the method described by Sherwin and Sacca, (1984).

Determination of kidney function for hypercholesterolemic rat fed on dry and garmented oat

Serum urea, uric acid and, creatinine were determined according to the enzymatic method that described by Tiffany et al., (1972); Barham and Trinder, (1972) and Bowers and Wong, (1980).

Determination of antioxidant enzymes for hypercholesterolemic rat fed on dry and garmented oat

- Superoxide dismutase activity (SOD) was determined according to the enzymatic method described by Nishikimi et al., (1972).
- Catalase activity (CAT) was determined according to the enzymatic method described by Fossati et al., (1980).
- Glutathione Peroxidase (GPx) was determined according to the enzymatic method described by Paglia and Valentine, (1967).

Determination of glutathione of hypercholesterolemic rats fed on dry and germinated oats
- Glutathione (GSH) activity was determined according to the enzymatic method described by Beutler et al., (1963).

Histopathological examination

The organs (kidney and liver) were washed by saline and fixed in buffered formalin with concentration 10% then dehydrated in ascending series of ethyl alcohol. The sections of liver and kidney were cut to 5 μm thick and stained with hematoxylin and eosin. The sections were scrutinize by light microscope (Banchroft and Ganble, 2008).

Histopathology technique

The tissues of liver and kidney were fixed immediately after dissection in 10% neutral formalin for 24 h, then dehydrated in sending concentration of alcohol, cleaned in xylene and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 micron and stained with hematoxylin and fusing stains (Banchroft and Ganble, 2008). All tissues were examined by the light microscope for detection of any histopathological alteration.

Statistical Analysis

The statistical analysis was conducted by using SPSS software. The data were analyzed using one way analysis of variance technique (ANOVA) and flowed by Duncan multiple range test, the results were expressed as means ±SD. according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Chemical composition of dry and germinated of oat:

Data in Table (1) showed that the higher moisture content (11.4±0.55, g/100g) and total carbohydrate (59.23±3.78 g/100g) was recorded to dry oat. Lower moisture content and carbohydrate was found in germinated oat (10.6±0.41 and 52.45±0.25 g/100g) respectively. Meanwhile, germinated oat have highest content in protein, oil, fiber and ash (12.8±0.19, 5.80±0.35, 14.55±2.03 and 3.8±0.42 g/100g) respectively. The germination process increased protein from 12.5±0.19 to 12.8±0.19 g/100g) and fiber from (9.53±1.33 to 14.55±2.03 g/100g) and ash from 3.3±0.27 to 3.8±0.42 g/100g) respectively. These results are on line with results by Khadar, (1983), which discovered that the amount of total protein rises following germination from (12.69 to 14.27). Also, Farooqui et al., (2018) and Sharma et al., (2016), they claim that germination causes the protein content of seeds to rise.

Germination process decreased total carbohydrate of dry oat from 59.23±3.75 to 52.45±0.25g/100g. The decrease in carbohydrate may be due to increase in alpha – amylose activity. Recent study by Huang et al., (2022), showed that an increase in the activity of the enzyme alpha-amylose, which converts complex carbohydrates into more easily absorbed sugars, may be responsible for the drop in carbohydrates found in germinated grains.
Effect of dry and germinated oat on hypercholesterolemic rats

Table (1): Chemical composition of dry and germinated oat

<table>
<thead>
<tr>
<th>Treatments g/100g</th>
<th>Dry oat</th>
<th>Germinated oat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>11.4±0.55</td>
<td>10.6±0.41</td>
</tr>
<tr>
<td>Protein</td>
<td>12.5±0.19</td>
<td>12.8±0.19</td>
</tr>
<tr>
<td>Oil</td>
<td>4.04±0.01</td>
<td>5.80±0.35</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>59.23±3.78</td>
<td>52.45±0.25</td>
</tr>
<tr>
<td>Fiber</td>
<td>9.53±1.33</td>
<td>14.55±2.03</td>
</tr>
<tr>
<td>Ash</td>
<td>3.3±0.27</td>
<td>3.8±0.42</td>
</tr>
</tbody>
</table>

Results are presented as mean ±SD

Effect of dry and germinated oat on body weight gain and weight of organs of hypercholesterolemic rats:

The results in Table (2) revealed that the body weight gain in HC group show significantly increase P≤0.05 (59.39±4.14 g) when compared to normal control group (26.51±4.58 g). The rise in body weight gain may be caused by eating more calories from fat and cholesterol which causes the body to store the extra calories as fat in the fat cells over time and results in weight gain. Previous studies by Lecumberri et al., (2007); Baraket and Lamiaa, (2011) and Shehata and Soltan, (2013), they reported that the rats fed on hypercholesterolemic diet for 6 weeks caused body weight gain raised.

Meanwhile, the body weight gains of all treated groups fed on dry and germinated oat showed significantly decrease P≤0.05 compared to HC group. Meanwhile, HC group fed on dry oat 15% and 30% showed significantly decrease in body weight gain which recorded (4.82±0.81g) and (0.65±0.79 g) respectively.

On the other hand rats fed on 15% and 30% germinated oat showed considerably decrease in BWG. These treatments showed more effective to reduce of body weight gain. The decrease in weight may be due to the content of oat rich in phenolic compounds, dietary fiber especially β-glucan, this bioactive component have reduction of body weight gain. Brockman et al., (2013) and Shehzad et al., (2023), reported that the oat is a rich in β-glucan, free phenolic compounds and dietary fibers.

Also, Saltzman et al., (2001) mentioned that the diet containing oat caused loss body weight and improved lipid profile

Table (2): Effect of dry and germinated oat on body weight gain and weight of Organs of hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight gain (g)</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.51±4.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.77±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>59.39±4.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.05±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.57±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry Oat 15%</td>
<td>4.82±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.12±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.32±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry Oat 30%</td>
<td>0.65±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.86±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germinated Oat 15%</td>
<td>-3.53±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.69±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.47±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germinated Oat 30%</td>
<td>-17.66±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.28±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.48±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents the means ±SD. Mean with the different superscript letters in the same column were different significant at P≤ 0.05.

The data presented in Table (2) demonstrated that liver weight increased significantly in rats fed on HC diet from (6.77±0.03 g) to (10.05±0.02g). The increase in liver weight may be
due to the diet containing 16% saturated fat and 1% cholesterol caused accumulation of fat in hepatic tissue which leads to liver weight rises. In general animal liver contains about 5% lipids; this percentage could be increased up to 30% under the influence of diet or physiological disturbance (El-Sayed et al., 2019). Previous research by (Milagro et al., 2006) reported that the increase in liver weight may be due to the accumulation of fat in liver tissues. HC group fed on oat revealed reduction in liver weight of all treatments at two concentrations.

Results obtained in the same table showed kidney weight increased significantly in rats fed on HC diet. HC rats received oat decreases kidney weight. The best results of kidney weight have of dry oat 15% and 30% followed by germinated oat 15%, and 30% responsivity Also, dry oat 15% and 30% showed non-significant difference between that results and negative control group. According to our knowledge, the present study is the first one determine effect dry and germinated oat on kidney weight.

Effect of dry and germinated oat on total cholesterol and triglycerides levels of hypercholesterolemic rats:

Results from Table (3) revealed that hypercholesterolemic diet (positive control) lead to significantly increase p≤0.05 in total cholesterol (102.18±5.89 mg/dl) compared with group fed on standard diet (negative control) (62.78±5.20 mg/dl). These results are consistent with finding by (Shehata and Soltan (2013); Haranfi et al., (2009) and Kumar et al., (2013), they reported that hypercholesterolemic group have higher total cholesterol than negative control.

The results indicated that the consumption of dry oat at 15%, 30% and germinated oat 15% revealed that significantly decrease in total cholesterol which recorded (92.64±4.00, 64.59±5.89 and 83.44±4.00 mg/dl) respectively when compared to positive control (102.18±5.89 mg/dl). 30% dry oat was more effective for the reduction total cholesterol to hypercholesterolemic rats. The soluble dietary fiber β-glucan found in oats, which lowers cholesterol, may be responsible for the drop in total cholesterol. β-Glucan can function as a physical barrier by preventing the absorption of bile acids, which are connected to the synthesis and dissociation of cholesterol (Bashir and Cho, 2017).

The reduction of total cholesterol was higher for oat seed may be due to the high molecular weight of beta-glucan in oat Larzaridou and Biliaderis, (2007), found that the molecular weights of beta-glucan for oat and barley were 31-2700x103 and 21-1100x103, respectively. Increase molecular weight of beta glucan increased the viscosity (Kim and White, 2013), increase viscosity decrease the absorption of cholesterol. High molecular weight beta glucan inhibiting fat absorption and reducing abdominal deposit fat (Aoe et al., 2020).

The viscosity of beta glucan increase is even greater at higher concentrations, which could be specific molecular associated or the presence of aggregated particles modifying the molecular entanglements (Wood, 2002).

By preventing the absorption of bile acids, which are linked to the synthesis and dissociation of cholesterol, β-glucan can function as a physical barrier (Bashir and Cho, 2017).

Treated rats with 15% germinated oat showed significant decrease for total cholesterol, while 30% germinated oat showed that non-significant improve in total cholesterol.

These results may be due to germination process caused breakdown of beta glucan. In general the feeding rats on high concentration germinated seeds had no effect on total cholesterol. These results may be due to decrease β-glucan content during germination process. These results are in accordance with (Amisi Kapepa et al., 2022) noted that germination process significantly increased the concentration of bioactive component but significantly decreased the content of β-glucan.
Table (3): Effect of dry and germinated oat on total cholesterol and triglycerides levels of hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol mg/dl</th>
<th>Triglyceride mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.78 ± 5.20</td>
<td>46.00 ± 5.08</td>
</tr>
<tr>
<td>Positive</td>
<td>102.18 ± 5.89</td>
<td>74.50 ± 13.18</td>
</tr>
<tr>
<td>Dry Oat 15%</td>
<td>92.64 ± 4.00</td>
<td>49.25 ± 5.23</td>
</tr>
<tr>
<td>Dry Oat 30%</td>
<td>64.59 ± 5.89</td>
<td>48.50 ± 3.38</td>
</tr>
<tr>
<td>Germinated Oat 15%</td>
<td>83.44 ± 4.00</td>
<td>53.75 ± 10.89</td>
</tr>
<tr>
<td>Germinated Oat 30%</td>
<td>100.88 ± 3.91</td>
<td>54.50 ± 3.66</td>
</tr>
</tbody>
</table>

Each value represents the means ± SD. Mean with the different superscript letters in the same column were different significant at P≤ 0.05.

Increased dietary intake of saturated fats may be the cause of the rise in TG. The increase in physiological requirements causes their conversion into TG in the liver, where they are bundled into VLDL-C and discharged into the bloodstream to be various tissues for storage or the creation of energy through oxidation (Balcavage et al., 1996).

Rats treatment with oat either dry or germinated caused a significantly decrease in TG. Table (3) demonstrated there was no discernible difference between the hypercholesterolemic groups fed on dry and germinated oat with the normal control group. Ibrügger et al., (2013) demonstrated that there was no significant difference in TG between the hypercholesterolemic groups receiving both barley and oat extract and the normal control.

Effect of dry and germinated oat on lipoproteins - cholesterol of hypercholesterolemic rats:

Data in Table (4) showed that HDL cholesterol of HC group decreased significantly (29.13±0.37 mg/dl) compared to normal group (35.83±0.50 mg/dl). All treatments at all concentration showed significantly P≤0.05 increase in HDL cholesterol.

The HC group fed on dry oat 30% has the highest improve in HDL cholesterol with percentage (14.143%).

Results of LDL cholesterol are presented in the same table HC diet increased LDL cholesterol from (17.75±5.73) to (58.16 ±7.82 mg/dl). The HC group fed on dry oat 30% was the highest decrease of LDL cholesterol which recorded ratio (21.64±6.91). While, rats treated with germinated oat 30% showed no significant deference between that treatment and positive control group. These results may be due to the germination process decrease β-glucan content which responsible to LDL –C lowering effect.
Table (4): Effect of dry and germinated oat on HDL-C, LDL-C, VLDL-C and atherogenic index of hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>HDL-c mg/dl</th>
<th>LDL-c mg/dl</th>
<th>VLDL-c mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35.83±0.50d</td>
<td>17.75±5.73a</td>
<td>9.20±1.16a</td>
</tr>
<tr>
<td>Positive</td>
<td>29.13±0.37a</td>
<td>58.16±7.82d</td>
<td>14.90±2.64b</td>
</tr>
<tr>
<td>Dry Oat 15%</td>
<td>31.50±1.84b</td>
<td>51.29±2.45c</td>
<td>9.85±1.05a</td>
</tr>
<tr>
<td>Dry Oat 30%</td>
<td>33.25±2.22c</td>
<td>21.64±6.91a</td>
<td>9.70±0.68d</td>
</tr>
<tr>
<td>Germinated Oat 15%</td>
<td>32.50±1.34b</td>
<td>40.19±3.83b</td>
<td>10.75±2.18a</td>
</tr>
<tr>
<td>Germinated Oat 30%</td>
<td>32.50±0.45b</td>
<td>57.48±3.91cd</td>
<td>10.90±0.73a</td>
</tr>
</tbody>
</table>

Each value represents the means ±SD. Mean with the different superscript letters in the same column were different significant at P≤ 0.05.

Oat phenolic has been shown to increase the resistance of human LDL to oxidation in a dose dependent fashion (can synergistically increase with vitamin C in diet (Meydani, 2009). Also, Whitehead et al., (2014) reported that consuming oat β-glucan with a molecular weight (MW) reduced serum total LDL cholesterol in humans.

The results of VLDL cholesterol which presented in the same table clarify the HC group had a significant higher in VLDL-C value P≤0.05 (14.9±2.64) compared to healthy control group (9.20±1.16).

All treatments showed improved in VLDL-c values. In addition no significant difference was observed between groups fed on dry and germinated oat that with normal control group.

The decrease in VLDL-C may be due to soluble dietary fiber content in oat especially β-glucan. Previous research by Martinez et al., (2013) reported that β-glucan in whole grain decrease lipid blood.

Effect of dry and germination of oat on AI, HTR and LDL/HDL Ratio of hypercholesterolemic rats:

Data in Table (5) demonstrated that HC diet caused a significantly increase p≤0.05 of AI and LDL/HDL ratio from 0.11±0.06 to 0.40±0.09 and from 0.50±0.10 to 2.00±0.28 respectively.

Consumption of oat by HC group results lower AI levels than positive control.

Table (5): Effect of dry and germinated oat on AI, HTR and LDL/HDL ratio of hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>AI mg/dl</th>
<th>HTR mg/dl</th>
<th>LDL/HDL Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.11±0.06a</td>
<td>57.39±4.79e</td>
<td>0.50±0.16a</td>
</tr>
<tr>
<td>Positive</td>
<td>0.40±0.09d</td>
<td>28.59±1.84a</td>
<td>2.00±0.28d</td>
</tr>
<tr>
<td>Dry Oat 15%</td>
<td>0.19±0.05ab</td>
<td>34.01±1.48ab</td>
<td>1.63±0.09c</td>
</tr>
<tr>
<td>Dry Oat 30%</td>
<td>0.16±0.06ab</td>
<td>51.95±6.97d</td>
<td>0.67±0.24a</td>
</tr>
<tr>
<td>Germinated Oat 15%</td>
<td>0.21±0.11b</td>
<td>39.04±2.76c</td>
<td>1.24±0.14b</td>
</tr>
<tr>
<td>Germinated Oat 30%</td>
<td>0.22±0.04b</td>
<td>32.25±0.94ab</td>
<td>1.77±0.10c</td>
</tr>
</tbody>
</table>

Each value represents the means ±SD. Mean with the different superscript letters in the same column were different significant at P≤ 0.05.
HC group fed on dry oat 30% was the lowest level of AI and LDL/HDL ratio which recorded (0.16±0.06 and 0.67±0.02). The decrease in LDL-C/HDL-C ratio and AI may be due to the oat is good source of dietary fiber, and an antioxidant compound has potential antiatherogenic effect. These results are in agreement with Li et al., (2007), reported that the phenolic antioxidant like Avenathrami in oat have antiatherogenic effect.

On the other hand, results of HTR presented in the same table showed HC group consumed dry oat 30% and germinated oat 15% revealed increased significantly in HTR. The rats received dry oat 30%, was the best to improve HTR more than other treatments. These results are on line with Makni et al., (2008) who reported that the increase in HDL or HTR ratio is one of the most important criteria of anti-hypercholesterolemia.

**Effect of dry and germinated oat on liver function of hypercholesterolemic rats:**

Results of liver function are shown in Table (6) Groups consumed germinated oat 30% and dry oat 30% have the most effective to decrease AST activity and non-significant difference was observed between that treatments and normal control group. Meanwhile, the rats received germinated oat 30% was the most impact to decrease AST activity (134.00±7.10 IU/L) compared to other treatments.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST IU/l</th>
<th>ALT IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>139.67 ± 28a</td>
<td>71.33±2.88a</td>
</tr>
<tr>
<td>Positive</td>
<td>183.00 ± 2.19c</td>
<td>85.67±15.8b</td>
</tr>
<tr>
<td>Dry Oat 15%</td>
<td>149.00 ± 22.84ab</td>
<td>79.33±0.52ab</td>
</tr>
<tr>
<td>Dry Oat 30%</td>
<td>144.00 ± 4.98ab</td>
<td>77.33±0.52a</td>
</tr>
<tr>
<td>Germinated Oat 15%</td>
<td>160.28 ±1.48b</td>
<td>79.00±0.00ab</td>
</tr>
<tr>
<td>Germinated Oat 30%</td>
<td>134.00± 7.10a</td>
<td>76.33±0.52a</td>
</tr>
</tbody>
</table>

Each value represents the means ±SD. Mean with the different superscript letters in the same column were different significant at P≤ 0.05

On the other hand, ALT activity increased significantly P≤ 0.05 in hypercholesterolemic groups (85.67±15.08 IU/L) compared to normal control (71.33±2.88 IU/L). Previous studies by Shehata and Soltan (2013) and El-Rabey et al., (2017) found that the hypercholesterolemic diet led to significantly increase in AST and ALT enzymes. Groups consumed germinated oat 30% and dry oat 30% have most effective to decrease AST and ALT activities, and no a significant difference was observed between that groups and negative control group.

Previous results by El Rabey et al., 2013 found that rats treated with 10% oat bran for 8 weeks caused lower AST and ALT compared to positive control.
Effect of dry and germinated oat on kidney function of hypercholesterolemic rats:

Data in Table (7) revealed that induced hypercholesterolemia caused a significant increase in kidneys enzymes $P \leq 0.05$ compared with normal control group. These results are agreement with Tomizawa et al., (2011) reported that mice fed on the HC diet had greater levels of renal enzymes than mice fed on a regular diet.

All HC group treated with dry and germinated oat at low and high concentration showed a significant decrease $P \leq 0.05$ in serum uric acid. The serum uric acid of group received 30% dry oat and 30% germinated oat showed the highest decrease $(2.03 \pm 0.05$ and $2.07 \pm 0.05$ mg/dl) respectively, compared to other treatments. These treatments were the most effective to lower uric acid, and non-significant difference was observed between that groups and negative control group. The decrease in uric acid may be due to the oat have high content total phenolic and flavonoids compounds especially after germination process. Xu et al., (2009) mentioned that the germination of oat increased total phenolic acid such as avenanthramides. Boz, (2015) reported that different phenolic component alterations and modifications to antioxidant activity are brought about by the germination process. Numerous studies have demonstrated that the polyphenols may stop the formation of uric acid from rising by blocking the uric acid-producing enzyme (Tresserra- Rimbau et al., 2014.).

Table (7): Effect of dry and germinated of oat on kidney enzymes level hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uric acid mg/dl</th>
<th>Creatinine mg/dl</th>
<th>Urea mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.07±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.33±1.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>2.50±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.84±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.83±1.47&lt;sup)c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry Oat 15%</td>
<td>2.13±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.67±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.00±0.89&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry Oat 30%</td>
<td>2.03±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.63±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.33±4.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germinated Oat 15%</td>
<td>2.20±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.67±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.33±1.86&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germinated Oat 30%</td>
<td>2.07±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.33±3.61&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents the means ±SD. Mean with the different superscript letters in the same column were different significant at $P \leq 0.05$.

Results of creatinine are shown in the same table (7) Serum creatinine of HC group increased significantly $P \leq 0.05$ $(1.84±0.02$ mg/dl) as compared to the normal control group $(1.48±0.08$ mg/dl). Increased TG and LDL cholesterol increased creatinine and decrease kidney function. Honzumi et al., (2018) discovered that there is a connection between the decreased megalin and the increased cholesterol loading on the kidney, and that the excess cholesterol is absorbed into the renal tubule epithelial cells where it inhibits cell proliferation, possibly contributing to kidney damage. All treated rats which were fed on dry and germinated oat showed a significant decrease of creatinine as compared to the positive control group. The best results of creatinine were to dry oat 30%.

Data presented in Table (7) revealed that rats fed on HC diet increased significantly serum urea $(56.83±1.47$ mg/dl) compared to healthy rats $(26.33±1.37$ mg/dl). Rats received 30% dry oat decreased urea from $(56.83±1.47$ mg/dl) to $(37.33±4.41$ mg/dl).

Also, rats treated with 30% germinated oat decreased significantly $P \leq 0.05$ serum urea from $(56.83±1.47$ mg/dl) to $(43.33±3.61$ mg/dl). Wang et al (2022) reported that the oat β-glucan improving renal function.
Effect of dry and germinated oat on hypercholesterolemic rats

Effect of dry and germinated oat on antioxidant enzymes of hypercholesterolemic rats:

Data on in Table (8), illustrated that a significant decrease p≤0.05 in serum GSH activity of hypercholesterolemic rats (1.45±0.14 IU/L) compared to normal control rats (3.83±0.38 IU/L).

Previous study by Nagib, (2017) showed that the rats fed on hypercholesterolemic diet showed reduction of GSH activity.

Serum GSH activity of hypercholesterolemic rats fed on dry and germinated oat revealed significantly increases p≤0.05 of all treatments at all concentration in comparison with the positive control rats (1.25±0.14 IU/L). The highest increase of GSH activity was observed of rats consumed dry oat 15% (2.77 ± 0.31IU/L) and germinated oat 30% (3.07±0.15 IU/L) respectively.

The increase in GSH activity may be due to oat is considered a good source of bioactive component such as phenolic component, flavonoids and carotenoids which potential antioxidant activity.

Previous studies by Singh and Belkheir (2013); Sang and Chu, (2017) reported that phenolic acid, flavonoids, and carotenoids found in oat grains are good sources and have strong antioxidant potential.

Rats consumed 30% germinated grains revealed higher GSH activity than dry grains. Germination process increases phenolic compound and flavonoids which potential effect as antioxidant activity. In general germinated oat 30% was the highest increase in GSH activity.

Table (8): Effect of dry and germinated oat on antioxidant enzymes (GSH, CAT GPx, SOD) levels of hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH IU/L</th>
<th>CAT IU/L</th>
<th>GPx IU/L</th>
<th>SOD IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.83 ± 0.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2152.6±110.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>216.18± 12.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>151.16±2.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>1.45 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1405.0±58.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>188.66±2.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.50±3.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry Oat 15%</td>
<td>2.77 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1560.0±1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>201.83±0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.66±1.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry Oat 30%</td>
<td>2.65±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1597.33±20.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>203.00±1.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>106.83±6.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germinated Oat</td>
<td>2.77 ±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1613.0±111.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>206.58±12.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>110.64±25.70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germinated Oat</td>
<td>3.07±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1737.33±14.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>220.89±2.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>148.50±0.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents the means ±SD. Mean with the different superscript letters in the same column were different significant at P≤ 0.05.

Serum CAT activity of rats fed on germinated seed revealed that significantly increases P≤0.05 at low and high concentration compared to hypercholesterolemic rats. The group received 30% germinated oat was the highest increase in serum CAT activity (1737.33±14.89 IU/L).

Results of GPx and SOD activities are shown in the same table and The rats fed on hypercholesterolemic diet caused significant declined in GPx and SOD (188.66±2.42 and 61.50±3.08 IU/L) receptivity when compared to the normal control group (216.18 ± 12.16 and 151.16±2.31 IU/L) respectively.

The rat’s treated with dry and germinated oat show significantly increase in GPx and SOD. The best results of GPx and SOD were observed of germinated oat 30%m (148.50±0.45 and 220.89±2.16 IU/L)
Oats are a rich source of antioxidants, including phenolic compounds, physic acid, sterols, vitamin E, flavonoids, and avenanthranides (AVAs), which may explain why their SOD levels have increased. Avenanthranides are regarded as an antioxidant defense because they increase in vivo antioxidant enzymes and trap reactive oxygen species (Skoglund et al., 2008; Chen et al., 2004 and Yang et al., 2014).

**Histopathological examination of liver:**

Microscopically, liver of rats from normal control revealed the normal histological structure of hepatic lobule (Photo 1). On the other hand, liver of rats from positive control revealed steatosis of hepatocytes (Photo 2). Moreover, examined sections from group's dry oat 15% and dry oat 30% showed steatosis of hepatocytes and focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (Photo. 3 ad 4). However, liver of rats from germinated oat 15% revealed small vacuolaes in the cytoplasm of some hepatocytes and slight activation of Kupffer cells as well as slight vacuolation of hepatocytes (Photo.5). Meanwhile, liver of rats from germinated oat 30% showed small vacuolaes in the cytoplasm of some hepatocytes and slight activation of Kupffer cells (Photo. 6).

Photo (1): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H and E X 400). (G1)

Photo (2): Liver of rat from group 2 showing steatosis of hepatocytes (H and E X 400). (G2)
Effect of dry and germinated oat on hypercholesterolemic rats

Photo (3): Liver of rat from group 3 showing steatosis of hepatocytes and focal hepatocellular necrosis associated with mononuclear inflammatory cells in filtration (H and E X 400). (G3)

Photo (4): Liver of rat from group 4 showing steatosis of hepatocytes and focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (H and E X 400). (G4)

Photo (5): Liver of rat from group 5 showing small vacuolaes in the cytoplasm of some hepatocytes and slight activation of Kupffer cells (H and E X 400). (G5)
Photo (6): Liver of rat from group 6 showing small vacuoles in the cytoplasm of some hepatocytes (H and E X 400). (G6)

-Histopathological examination of kidneys:

Microscopically, kidneys of rats from normal control revealed the normal histological structure of renal tissue (Photo. 7). On the other hand the kidneys of rats from positive control group showed vacuolar degeneration of epithelial lining renal tubules and congestion of glomerular tuft (Photo. 8). Meanwhile, kidneys of rats from dry oat 15%, dry oat 30%, germinated oat 15% and germinated oat 30% revealed no histopathological alterations (Photo.9, 10, 11and12).

Photo (7): Kidney of rat from group 1 showing the normal histological structure of renal tissue (H and E X 400).

Photo (8): Kidney of rat from group 2 showing vacuolar degeneration of epithelial lining renal tubules and congestion of glomerular tuft (H and E X400)
Photo (9): Kidney of rat from group 3 showing no histopathological alterations (H and E X 400).

Photo (10): Kidney of rat from group 4 showing no histopathological alterations (H and E X 400).

Photo (11): Kidney of rat from group 5 showing no histopathological alterations (H and E X 400).
CONCLUSION

The results of this study indicated that germination process increases protein, fiber and ash of oat. Oat consumption for six weeks had hypocholesterolemic effect appearing by decrease TC levels, LDL, VLDL, AI and resaid HDL and antioxidant enzymes Oat can lower TC concentration in rats fed on HC diet. High concentration of dry oat is more effective to the reduction of TC, LDL-C and VLDL-C more than low concentration. Also, low concentration (15%) of germinated oat has more impact as hypocholesterolemic effect than higher concentration (30%).

On the other hand, high concentration of dry oat was the most impact of HDL-C increase. While germinated oat at 15% and 30% had the same effect to increase HDL and decrease AI. All treatment at two concentrations decreases kidney function. But germinated oat on high concentration is the most effective to decreases AST. Furthermore, germinated oat 30% is the most effective to raise GSH, CAT, GPx and SOD activities.

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