Pak. J. Agri. Sci., Vol. 57(2), 621-625; 2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.1430
http://www.pakjas.com.pk

FATTY ACID PROFILE AND BIO-EFFICACY OF WHEAT GERM OIL IN HYPERLIPIDEMIC RABBITS

Hafeez-ur-Rehman[1], Umar Farooq[1,2]*, Kashif Akram[3], Zafar Hayat[4], Afshan Shafi[2], Farkhandah Sarfarz[1], Asim Sidhu[1] and Ayesha Hakim[5]

[1]Institute of Food Science and Nutrition, University of Sargodha, Sargodha (Pakistan); [2]Department of Food Science and Technology, MNS University of Agriculture, Multan (Pakistan); [3]Department of Food Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur (Pakistan); [4]Department of Animal Nutrition, University College of Veterinary and Animal Sciences, Jhang (Pakistan); [5]Department of Computer Science, MNS University of Agriculture, Multan (Pakistan)
***Corresponding author's e-mail: umar.farooq@mnsuam.edu.pk**

Wheat germ oil (WGO) is highly nutritious with significant biological value. In current research work, WGO was extracted through solvent extraction method by using hexane as solvent. The oil was analyzed for fatty acid profile and was later used for biological study in order to investigate hypocholesterolemia potential by using rabbits as animal model. The results revealed that the WGO contained 82.64 % unsaturated fatty acids with linoleic acid (82.64) as leading fatty acid and linolenic acid (6.81%). Other major fatty acid in the oil was oleic acid (16.12%). The biological studies showed that there was significant improvement in lipid profile of hyperlipidemic rabbits when fed with WGO as evident from reduction in cholesterol, triglycerides, LDL and VLDL whereas an increase in HDL level. It was concluded that the WGO possessed significant hypocholesterolemia potential.
Keyword: Wheat germ oil, WGO, biological study, hyperlipidemic rabbits.

INTRODUCTION

Cereals constitute an essential and dominant portion of our daily nutritional intake (Morris, 2016) wheat is mostly used as common ingredient around the world in preparation of different foods due to its good ability to be grounded into flour. China, India and Russia are the top wheat producing countries (Trademap, 2018) whereas Pakistan is 3rd largest producer of wheat in South Asia with 25.45 million tons of wheat production annually (PES, 2015-2016). During milling of wheat, 254.7-318.37 thousand tons of wheat germ (2.5% w/w of wheat) is obtained per annum as a byproduct. Wheat germ is very nutritious and contains high amount of protein and lipids (Dunford and Zhang, 2003; Kara, 2016). Wheat germ oil (WGO) has the highest amount of natural vitamin E (248-328 mg/100 g) and carotenoid contents ranging from 0.56 to 12.23 mg/100 g (Panfili *et al.*, 2003; Kumar and Krishna, 2015) and both are potent antioxidants (Hidalgo *et al.*, 2006; Olafisoye *et al.*, 2020). The wheat germ oil and other plant based essential oils also possess a number of biological effects like antimicrobial and anticancer (Sultan *et al.*, 2020; Sajid *et al.*, 2020). Further, phytosterols such as sitosterol and campesterol are also present which make about 64.64 % and 21.28 % of total unsaponifiable matter, respectively (Irmak *et al.*, 2006; Niu *et al.*, 2013).

According to previous studies WGO can protect from endotoxins and increase the activity of liver antioxidant enzymes like Glutathione S-transferase (GST), superoxide dismutase (SOD) and catalyase (CAT) and decrease inflammatory responses (Hussein *et al.*, 2014). The WGO may have the ability to protect from oxidative damage induced by benzene (Saleh *et al.*, 2010). Further, antioxidant behavior and cryoprotectant agent, WGO may also have potential to reduce LDL level in blood (Awadin *et al.*, 2015). Keeping in view the nutritional and biological advantages of wheat germ oil, present research was aimed to determine the effect of WGO on lipid profile of hyperlipidemic rabbits.

MATERIALS AND METHODS

All the chemicals used in this study for analysis were of analytical grade (Merck). Dried wheat germ was purchased from Sunny Flour Mill Pvt. Ltd, Lahore (Pakistan).

***Physicochemical Analysis of WGO*:** Physicochemical analysis including peroxide, saponification value, refractive index and free fatty acid (as of oleic acid) were determined by following the standard methods of American Oil Chemist Society (AOCS, 2004).

Fatty acid profile of WGO was determined by GC-MS (Eisenmenger and Dunford, 2008) after hydrolyzing with NaOH followed by methylation according to AOCS (2003).

Biological Study: Rabbits (New-Zealand breed) were selected as an experimental animal and twenty rabbits of weight 1-1.5 kg were obtained from Animal House,

Department of Pharmacy, University of Sargodha, Sargodha (Pakistan) and were kept in the same Animal House for biological study.

First of all, rabbits were divided into two major groups.

Normal Group: There were only 5 rabbits in this group and they were declared as positive control and were given "standard diet" (Shakirin *et al.*, 2010) during whole course of study.

Hyperlipidemic Group: Remaining 15 rabbits were placed in this group and hyperlipidemia was induced though feeding of the animals on "hyperlipidemic diet". The hyperlipidemia was confirmed from the blood analysis of the animals. This group was further segregated into 3 sub-groups each having 5 rabbits. These subgroups were then given experimental diet (standard diet supplemented with 0, 0.5 and 1.0% WGO) for 6 weeks. The formulation of diets is given in Table 1.

Table 1. Composition of standard and hyperlipidemic diet.

Ingredients (g/100 g)	Hyperlipidemic Diet	Standard Diet/U	T_1	T_2
Barley	35.0	35.0	35.0	35.0
Corn Starch	36.0	38.0	38.0	38.0
Sucrose	12.0	12.0	12.0	12.0
Salt (NaCl)	0.5	0.5	0.5	0.5
Vitamins	3.0	3.0	3.0	3.0
$NaHPO_4$	2.0	2.0	2.0	2.0
$CaCO_3$	2.5	2.5	2.5	2.5
Corn	7.0	7.0	6.5	6.0
Cholesterol powder	2.0	-	-	-
WGO	-	-	0.5	1.0

***Blood Analysis of Rabbits*:** Blood (3 ml) was drawn from their jugular veins on weekly bases with 5 ml hypodermal syringe and immediately transferred to blood sampling tubes containing Gel and Clot activator (REF: XLGA-GC4). Blood serum was separated and analyzed for lipid profile (Total cholesterol, Triglycerides (TG), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and Very Low-density Lipoproteins (VLDL) by using biochemical API kits.

Statistical Analysis: *The data collected was analyzed statistically to valida*te the results as recommended by Steel et al. (1997) using factorial design analyzed on Statistix 8.1 Software.

RESULTS

Physiochemical Analysis and Fatty acid profile of WGO: Physiochemical characteristics of WGO as given in Table 2 showed that the WGO had same typical yellow appearance like any other edible oil. Specific gravity of oil was found to be 0.912, refractive index was 1.473, free fatty acid was detected to be 3.32 % as of oleic acid while peroxide, saponification value and unsaponification matter was measured to be 2.74 m eq. O2/kg, 186.4 and 2.38 %, respectively.

The WGO was found to be rich in unsaturated fatty acids (82.14 %) with 64.41% polyunsaturated fatty acids and 17.73% monounsaturated fatty acids. It contained good ratios of unsaturated fatty acid (UFA)/saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA)/SFA with a mean ratio of 4.75 and 3.73, respectively.

Among SFAs palmitic (C16:0) was the dominant SFA while concentration of Stearic Acid (C18:0) and Arachidic acid (C20:0) was 0.61% and 0.2%, respectively. Among USFA, ω-3 Fatty acid (Linolenic Acid C18:3) was found to be 6.81% and Oleic acid (C18:1) was 16.12%. Linoleic acid (C18:2) was present in highest concentration as compared to other FAs with mean value of 57.6%.

The results were justified with the finding of some other researchers who also have reported that the wheat germ oil contained 81% unsaturated Fatty acid and the most prominent fatty acid was Linoleic acid which made up 56-60 % of oil (Megahad and El Kinawy, 2002; Dunford and Zhang, 2003; Arshad *et al.*, 2008; Kara, 2016). Results are also in line with the findings of Hidalgo *et al.* (2006), Niu *et al.* (2013) and Kara (2016) who have reported that in WGO the mono-unsaturated fatty acids make up 17.92-21.54% of total composition of oil and saturated fatty acids accounts for 18-18.87%. The presence of significant amount of ω-3 and ω-6 fatty acids in wheat germ oil indicates its Nutraceuticals value for management of intake of these important fatty acids in specific ration.

Table 2. Physiochemical analysis and fatty acid profile of WGO

Parameter	Values	
Physical Appearance	Yellow	
Specific Gravity	0.91 ± 0.02	
Refractive Index	1.47 ± 0.05	
Free Fatty Acid %	3.32 ± 0.07	
Peroxide Value (PV) (m eq. O_2/kg)	2.74 ± 0.05	
Saponification Value	186.4 ± 2.11	
Unsaponifiable Matter %	2.38 ± 0.08	
Fatty acid profile	**Mean values (%)**	**Category wise**
Palmitic (16:0)	16.47	17.28% (SFA)
Stearic (18:0)	0.61	
Arachidic (20:0)	0.20	
Palmitoleic (16:1)	0.21	17.73%
Oleic (18:1)	16.12	(MUFA)
Gadoleic (20:1)	1.40	
Linoleic (18:2)	57.60	64.41%
Linolenic (18:3)	6.81	(PUFA)

Biological Studies: Effect of wheat germ oil on lipid profile of rabbits is given in Table 3. It showed that the highest concentration of cholesterol (172.59 mg/dL) and triglycerides (TGs) (83.29 mg/dL) was observed in untreated

Table 3. Effect of WGO on blood lipid profile of the rabbits (mg/dL)

Parameter	Normal	Hyperlipidemic Rabbits		
		U	T_1	T_2
Cholesterol	78.96 ± 2.35^{C}	172.59 ± 6.27^{A}	151.58 ± 7.55^{B}	149.11 ± 6.29^{B}
Glycerides	83.29 ± 3.28^{C}	184.31 ± 8.22^{A}	166.31 ± 7.04^{B}	160.23 ± 7.25^{B}
HDL	22.58 ± 0.54^{A}	17.55 ± 0.70^{D}	19.63 ± 1.00^{C}	20.74 ± 1.14^{B}
LDL	29.19 ± 1.00^{D}	51.00 ± 1.98^{A}	42.79 ± 1.64^{B}	38.45 ± 1.52^{C}
VLDL	12.16 ± 0.31^{D}	26.53 ± 1.21^{A}	20.54 ± 0.82^{B}	19.32 ± 0.79^{C}

Values with different alphabets in rows are significantly different; Mean±SE; P<0.05

Table 4. Effect of treatment period on blood lipid profile of the rabbits (mg/dL)

Weeks	Cholesterol	Total Glycerides	HDL	LDL	VLDL
0	181.82 ± 7.00^{A}	200.8 ± 8.99^{A}	15.94 ± 0.79^{E}	53.23 ± 2.00^{A}	28.17 ± 1.12^{A}
1	173.96 ± 8.16^{AB}	182.28 ± 8.89^{B}	16.38 ± 0.89^{E}	50.91 ± 2.03^{B}	25.50 ± 0.77^{B}
2	165.42 ± 5.66^{B}	177.43 ± 7.91^{B}	18.45 ± 0.60^{D}	48.49 ± 1.56^{C}	23.93 ± 1.04^{C}
3	148.62 ± 6.44^{C}	164.94 ± 7.64^{C}	18.60 ± 0.92^{D}	43.41 ± 1.55^{D}	21.49 ± 1.21^{D}
4	147.45 ± 4.86^{C}	162.02 ± 7.02^{C}	20.55 ± 1.04^{C}	38.24 ± 1.96^{E}	19.89 ± 0.95^{E}
5	146.84 ± 7.7^{C}	155.47 ± 7.19^{CD}	21.87 ± 1.06^{B}	38.45 ± 1.39^{E}	18.38 ± 0.91^{F}
6	140.21 ± 7.11^{C}	149.03 ± 4.87^{D}	23.36 ± 1.30^{A}	35.83 ± 1.50^{F}	17.54 ± 0.58^{F}

Values with different alphabets in column are significantly different; Mean±SE; P<0.05

hyperlipidemic rabbits (U) rabbits. The hyperlipidemic rabbits fed on 0.5% (T1) and 1.0% (T2) WGO had low cholesterol level and triglyceride contents with mean values of 151.58 ± 7.55 mg/dL and 149.11 ± 6.29 mg/dL; 166.31±7.04 mg/dL and 160.23±7.25 mg/dL, respectively.

HDL contents in the blood of rabbits fed on control diet (normal group) was observed to be 22.58 mg/dL while it was 17.55 mg/dL in untreated hyperlipidemic rabbits (U). The hyperlipidemic rabbits fed with WGO (T_1 and T_2) showed higher concentration of HDL as compared to untreated hyperlipidemic rabbits.

Similarly, LDL content and VLDL content of Hyperlipidemic rabbits (U) was found to be 51 mg/dL and 26.53 mg/dL, respectively. The incorporation of WGO in the diet of rabbits showed reduction in LDL and VLDL content of blood with mean values of 38.45±1.52 mg/dL and 19.32±0.79 mg/dL, respectively at 6th week of treatment period.

The mean effect of treatment period on blood lipid profile of hyperlipidemic rabbits is shown in Table 4. There was reduction in mean cholesterol and total glyceride contents with the passage of time period. The mean decline in cholesterol levels was from 181.82±7.00 mg/dL to 140.21±7.11 mg/dL (22.65% mean reduction) during the treatment period of 6 weeks while Total glycerides were reduced from 200.8±8.99 mg/dL to 149.03±4.87 mg/dL during the treatment period.

Mean HDL level of rabbits increased with the passage of treatment duration. At start of the experiment, the mean HDL contents were measured to be 15.94±0.79 mg/dL and highest mean HDL contents (23.96±1.30 mg/dL) of treated rabbits were recorded at the end of treatment period (6-weeks). The LDL and VLDL contents were highest at zero week with mean value of 53.23 mg/dL and 28.17±1.12 mg/dL, respectively. LDL contents were recorded to be 35.83 mg/dL at 6th week whereas the mean VLDL level were found to be 17.54-18.38 mg/dL at 5th and 6th week of treatment period.

The results further elaborated that there were no significant changes in different parameters of blood lipid profile in the rabbits during entire period of six weeks.

To graphically elaborate the findings, seaborn heatmap (Eagle and Whalen *et al*. 2017) has been presented in Figure 1, based on correlation of all the parameters of the blood lipid profile. The heatmap clearly shows the negative correlation of HDL with all the other parameters. All other parameters have positive correlation with each other.

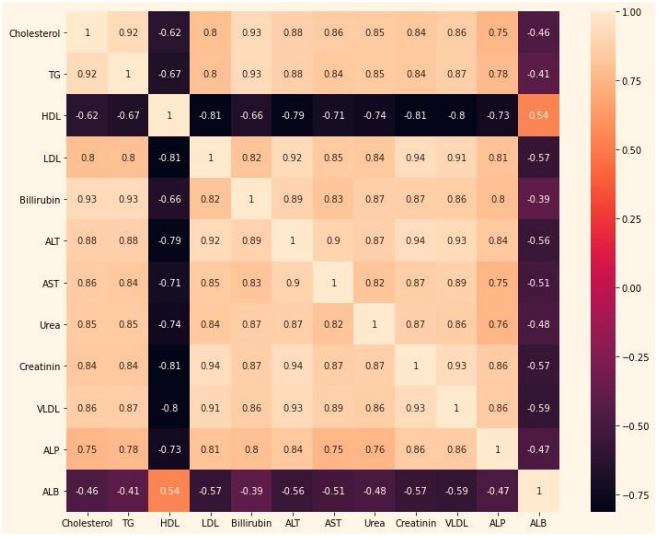

Figure 1: Seaborn Heatmap illustrating correlation of all the parameters of the blood lipid profile of wheat germ oil.

DISCUSSION

The significant decline in all the parameters of the blood lipid profile expect HDL, due to intake of WGO was clear indication of anti-hyperlipidemic potential of wheat germ oil. Similarly, increment in HDL contents due to intake of wheat germ oil was also a positive sign for hypolipidemic behavior of the oil. This hypolipidemic behavior of the WGO might be due to the presence of natural tocopherols (Vitamin E) or omega-3 Fatty acids or it may be due to inactivation of acyl transferase by polyunsaturated fatty acids present in the oil as also reported by Ostlund (2002), Wijendran and Hayes (2004), Na (2005) and Brinton and Mason (2017) who also reported the same mechanisms for anti-hyperlipidemia or it may be due to the reverse transport of cholesterol from blood to liver as reported by Pizzini *et al.* (2017). Moreover, the reduction in production of malondialdehyde (MDA) level in plasma as observed by Gorusupudi and Baskaran (2013) and excessive n-3 fatty acids also reduce the synthesis of Fibrinogen and triglycerides in liver (Haglund *et al.*, 1991). So, the reduction in production of malondialdehyde (MDA) and presence of Vitamin E might be the reason for Triglyceride lowering effect (Zakaria *et al.*, 2017) of wheat germ oil.

The results are also in accordance with the findings of Mehranjani *et al.* (2007), Fattah *et al.* (2011) and Said and Azab (2006) who observed an increase in HDL level and decrease in LDL level due to intake of wheat germ oil while working on different animal models. The hypolipidemic potential of wheat germ oil can also be linked with a specific ration of omega 3 fatty and omega 6 acids in the oil which causes increment in HDL and decrease in LDL and VLDL (Park and Harris, 2003; Ajayi and Ajayi, 2009; Awadin *et al.*, 2015; Brinton and Mason, 2017). Arshad *et al.* (2008) and Arshad *et al.* (2013) observed that there was reduction in HDL and increment in LDL due to intake of cookies fortified with wheat germ oil and they suggested that the presence of high amount of natural tocopherol might be the effective ingredient in this regard. Similarly, in an *in-vitro* study, Mason *et al.* (2016) observed that high chain Omega-3 fatty acid in wheat germ oil inhibited oxidation of ApoB (lipoproteins) and resultantly there was decline in LDL contents of the blood. These findings also support the outcomes of the current study.

Conclusion: It was concluded that the wheat germ oil containing 82.64 % unsaturated fatty acids possessed significant hypocholesterolemic potential. There was a significant decline in total cholesterol, triglycerides, LDL and VLDL contents in the blood of hyperlipidemic rabbits due to intake of wheat germ oil foe a period of 6 weeks. Moreover, the HDL contents of the blood of rabbits were significantly increased due to ingestion of wheat germ oil during the treatment period of 6 weeks revealing its hypocholesterolemic potential. This health promoting behavior of the oil might be due to the presence of polyunsaturated fatty acids and natural antioxidant in the oil.

Acknowledgement: I am thankful to Higher Education Commission (HEC) of Pakistan for providing financial assistance for research activities.

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[Received 23 Nov 2020; Accepted 3 Mar 2021; Published (online) 18 April 2021]