Original Research Article

ANTI-OBESITY EFFECTS OF PULICARIA JAUBERTII E. GAMAL-ELDIN IN HIGH FAT DIET-INDUCED RATS

ABSTRACT

Background: *Pulicariajaubertii E. Gamal-Eldin* is a food additive and traditionalmedicine used in Yemen where a large number of people are depending on herbal plants to treat their diseases. The aim of this study was to investigate and compare the effects of *Pulicariajaubertii*leaves and flowers aqueous extract (PJAE) and *Pulicariajaubertii* leaves and flowers in powder form mixed with diet (PJPD) on obesity induced by high-fat diet (HFD) in rats.

Methods: Obesity was induced experimentally in Male Sprague Dawley rats by feeding them with prepared high-fat diet for a period of 6 weeks. Rats were divided into 3 main groups, HFD control group, where rats fed HFD only and PJAE group where rats fed HFD+PJAE and PJPD groups where rats fed HFD+PJPD treatment. The PJAE was administrated by gavage at 1g/kg body weight and the activity of PJPD was determined as oral administration at 10% of the diet. Body weight, water and food intake were calculated once a week and blood samples were obtained at the end of the experiment for lipid profile analysis, glucose level, and toxicity parameter including liver and kidney function parameters.

Results:Results indicated a significantly reduction in % of weight gain and in PJPD group at week 5 and 6 of treatment compared to the HFD control rats. Also, there was a significant reduction in serum Triglyceride level in PJPD compared to HFD group. Whereas, no significant change was observed in % of weight gain and Triglyceride level in PJAE group when compared to HFD control rats. Also, no significant different in all the tested parameters including, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol, liver enzymes and creatinine and urea levels.

Conclusion: Results obtained from this study showed that the *Pulicariajaubertii E. Gamal-Eldin* leaves and flowers mixed with HFD possess anti-obesity in HFD fed rats.The anti-obesity effects of PJ maybe attributed to the phytochemicals present. The present study, therefore, scientifically validates the traditional use of PJ as a potential candidate for body weight reduction.

Keywords: antiobesity, *Pulicariajaubertii*, Yemeni traditional medicine,

INTRODUCTION

Obesity is a global health problem and it has been well addressed to be associated with the development of serious chronic diseases such as hyperlipidemia, type II diabetes, and cardiovascular complications. Obesity has now been considered as a major health concern both in developed and developing countries. In the Middle levels of obesity are growing with the predicted sequelae like insulin resistance[1.2]. An increasing attention towards the identification of the anti- traditional Middle Eastern herbal medicines for obesity management has been growing nowadays. A variety of natural products have been reported in the previous studies, including crude extracts and isolated compounds from plants, that can induce body weightreduction and prevent diet-induced obesity. Therefore, they havebeen widely used in treating obesity[3.4].Other natural products have demonstrated antioxidant, hypoglycemic, and hypolipidemic propertiessuch as cinnamon[5], green tea[6] and oats[7].

Species of the genus *Pulicaria* are widespread throughout the Mediterranean, Middle East, and Asia, and their extracts are used as traditional medicines[8] (Liu*et al*., 2010). *Pulicariajaubertii Gamal-Eldin (*PJ) is a Yemeni plant used as food flavor and in traditional medicines[9].Extracts PJ showed cytotoxic effects towards MCF-7 cancer cells and moderate antimicrobial activities[10].Recent study indicated that PJ extract by Supercritical fluids extraction is a good source of total phenolic contents, antioxidant activity and antibacterial compounds[11].The in vitro evidence of antiadipogenic properties of Pulicariajaubertii E. Gamal-Eldin methanolic extract (PJM) was reported by Al-Naqeb *et al*., [12] their findings showed that the methanol PJM and its subsequent DCM fraction, likely through the presence of catechin-like compounds, possessed bioactivity towards inhibiting TG accumulation in adipocytes and their ability to modify cellular anti-oxidant properties. Chemical characterization of PJM and its fractions hexane and dichloromethane fractions as long as water fraction was reported by Al-Naqeb *et al*., [12]. The presence of catechin-like compounds, carvotanacetone. with several other compounds, including carvotanacetone, fatty acids and sugars in PJ is well documented [12]. Catechin compounds are known to take significant part in regulation of obesity in animal and human studies [13,14]. Results from our previous study reported the*in vitro* evidence of antiadipogenic properties of *Pulicariajaubertii E. Gamal-Eldin* methanolic extract and its subsequent DCM fraction, likely through the presence of catechin-like compounds which possessed bioactivity towards inhibiting TG accumulation in adipocytes and their ability to modify cellular anti-oxidant properties.Thus, suggesting that PJ might be useful in the prevention and treatment of obesity by limiting accumulation in adipose tissue. Animal models have provided major contributions to the investigations of various complex diseases including obesity[15].For the investigations of various complex diseases including obesity, the use of animal models has provided major contributions 15. So far, the animals model widely used in obesity research because they showed gain weight with high fat diet feeding. Also using animal models can allow strict control of all factors, which is very crucial in safety and efficacy. In this study, male *Sprague Dawley*rats were used as a model with its physiological properties replicates many of the features observed in obese human as reported by [16,17]. We have chosen the aqueous extract of the PJ leaves and flowers because people in Yemen are using the flower with leaves as drinking tea thee another form was mixed with diet because PJ leaves and flowers is consumed by peole in Yemen as a food additive and a flavoring.Therefore, the aim of the present study was to investigate the potential antiobesity and bodyweight reducing activities of PJ leaves and flowers in aqueous extract form and as powder form mixed with diet in an animal model fed with HFD.

MATERIALS AND METHODS

Plant material

Pulicariajaubertii plant was collected from Khawlan farms in the southeast of Sana'a, as well as from Hodeidah, and from the local markets in Al- Qaa and Bab Al Salam markets, Sana`a in September 2017. The plant was identified and authenticated by Dr. Hassan Ibrahim a plant taxonomist at the Department of Botany, Faculty of Agriculture, Sana'a University, Yemen. The plant name has been checked with http://www.theplantlist.org as accepted name of a species in the genus *Pulicaria* (family Compositae). Leaves and flowers were separated from the stems. washed with water to remove the adherent impurities several times. Leaves and flowers were dried naturally in dry air at room temperature (25-30 °C) for 24-72 hours and protected from light. And they were spread on a clean cloth with continuous stirring daily to prevent rotting.

After drying, they were kept in clean, airtight plastic bags away from moisture at room temperature. Investigated samples were aqueous extracts of PJ and PJ in powder form mixed with prepared diet. This natural herb used after some investigation that has been reported by our group on its active constituents and some of its biological activities.

Preparing of aqueous extract

Dried PJ was finely ground using an electric grinder (Panasonic, Model MX-GX1571, China). Briefly, 1g powdered PJ was added to 3 ml of boiling water and was steeped for 1 h. The infusion was cooled to room temperature and then filtered. About 3 ml of the filtered aqueous extract contain 1g of powdered PJ was given to the rat daily by gavage for 6 weeks. Aqueous extract was prepared freshly before gavage the animals.

Prepartion of high fat diet

High fat diet (HFD) was prepared at animal house unit in Faculty of Agriculture, Sana`a University,Yemen. The diet was adjusted to contain 20% fat as a total energy Table 1 is showing the HFD ingreidents. HFD contains : Casein-30% g, starch 20)% sucrose 20% cellulose-8.5%, fat 20 %, mineral mixture (1%), vitamins mixture (0.5%). All ingeridents of the diet were homogeneoused and mixed with water manually and cut to small pices and dried in an oven at 45–5^{0}C.

Table 1. Experimental diet compositions

Composition of High Fat Diet (HFD)		
Ingredient		Percentage
Protien	Casein	30
Carbohydrat	sucrose	20
	Starch	20
Fiber	Cellulose	8.5%
Fat	Vegetable oil (sunflower + palm oil)	%5
	Ghee (Animal butter)	10%
	Vegetable margarine	5%
Minerals	Mineral mix[a]	1%
Vitamins	Vitamin mix[b]	0.5
Total		100%

The vitamin premix provided (mg kg1 feed): thiamin 60, riboflavin 22.5, niacinamide 152, calcium pantothenate 56, choline chloride 2000, inositol 1000, folic acid 8.5, biotin 1, pyridoxine-hydrochloride 22.5, p-aminobenzoic acid 500, Vitamin B12 0.015, DL-tocopheryl acetate 50, menadione 4, retinyl acetate and retinyl palmitate 30 (15 000 IU), cholecalciferol 30 (3000 IU), Vitamin C 400.The mineral premix provided (mg /kg feed): sodium citrate dihydrate, FeSO4.7H2O 900, MnO2 140, KAl(SO4)2.12H2O 200, ZnSO4.H2O 125, KBr 20, NiSO4.6H2O 8.5, CuSO4.5H2O 100, CoSO4.7H2O 5, Na2MoO4.2H2O 5, KI 5, As2O3 0.2, NaF 8.5, Na2B4O7.10H2O 5, Na2SeO3.5H2O.

Experimental design

Male *Sprague-Dawley* rats were obtained from College of Science و Sana'a University, Yemen . The rats werebreeding in Faculty of Agriculture, Sana`a University, Yemen. Eightteen *Sprague-Dawley* male rats, each was weighing 120-150 g were used in this study. The rats were housed

under 22±2ºC temperature, 40-60% humidity and 12-12±1 h light-dark cycle. All rats were fed normal control diet which consisted of casein (14.8%), sucrose (20%), starch (44.2%) palm oil (10%), vitamin mixture (1%) (cellulose (5%), Mineral mixture (0.5%), for 2 weeks before the beginning of the experiment for adaptation. Normal diet was prepared in animal house unit, Faculty of Agriculture, Sana`a University Yemen.Rtas were randmly divided in to three groups: first group HFD control was fed with HFD 20-30 g/ day , second group HFD +PJAE was fed HFD and adminestration of 1g/kg body weight of auques extract of PJ in 3 ml water by gavage and the third group HFD+PJPD were fed with HFD with PJ powdered form mixed with diet at 10%. The concentration of the PJ was adjusted to be equal in the diet and in aques extract to compare the activity of the two different forms of PJ treatment. Rats were fed HFD and tretaed with PJAE or PJPD for 6 weeks. Fed were presented to rats in special non-scattering feeding cups to avoid loss of food and contamination. Tap water was provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. All the experiment process was done in Animal house unit, Faculty of Agriculture, Sana`a University.

Measurement of body weight and food intake

The body weights and food intake were measured under the condition of non-fasting once a week. The food intake efficiency was evaluated by monitoring the food consumption (g) in each cage and was calculated for animal per day basis. After 6 weeks of high-fat diet feeding, rats were sacrificed by cervical dislocation. Blood samples were obtained from the eye and centrifuged at 1500 rpm for 15 min to separate to surm and blood cells.

Biochemical analysis of surm samples

Surm glucose level, Surm levels of triglyceride (TG), total cholesterol (TC) and high-density lipoprotein high-density lipoprotein cholesterol (HDLC) low-density lipoprotein cholesterol (LDLC) were analysed using assay kits in privite laboratory in Sana`a Alawlaki laboratory. Surm aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrationsurea and creatinine levels were measured using reagent kits (Instrumentation Laboratory by ILab Chemistry Analyzer 300 PLUS in Alawlaki laboratory

Statistical analysis

Number of independent experiments and details on statistical comparisons and levels of significance can be found in the captions of figures/tables. If not indicated otherwise, statistical tests were performed using GraphPad Prism 7. Statistical difference data groups were analyzed by ANOVA with comparison of the means by Tukey.

RESULTS AND DISCUSSION

The effect of PJ treatment on food intake and body weight

To investigate the antiobesity effect of PJ at powder form and as an aqueous extract, rats were fed HFD in the presence or absence of PJ treatment. Food intake and body wights were measured once a week. For the food intake all groups approximately consumed the same amount of food intake 34±3.5 g/day that give us good impact to compared the different in weight gain for the different groups. As shown in Fig. 1, no significant different was noticed in the body weight of all groups from beginning of the experiment week1 until week 5 of the experimental time. At the end of the experiment, at week 6 the body weight of the HFD group(196 ± 12 g) was significantly higher($p < 0.01$) than the HFD grout treated with PJPD (173 ±23 g), whereas no significant different was observed between HFD rats and HFD treated with PJAE (194±13 g), the body weight of rats feed HFD+PJPD was significantly lower than the body weight of HFD+PJMAE group.

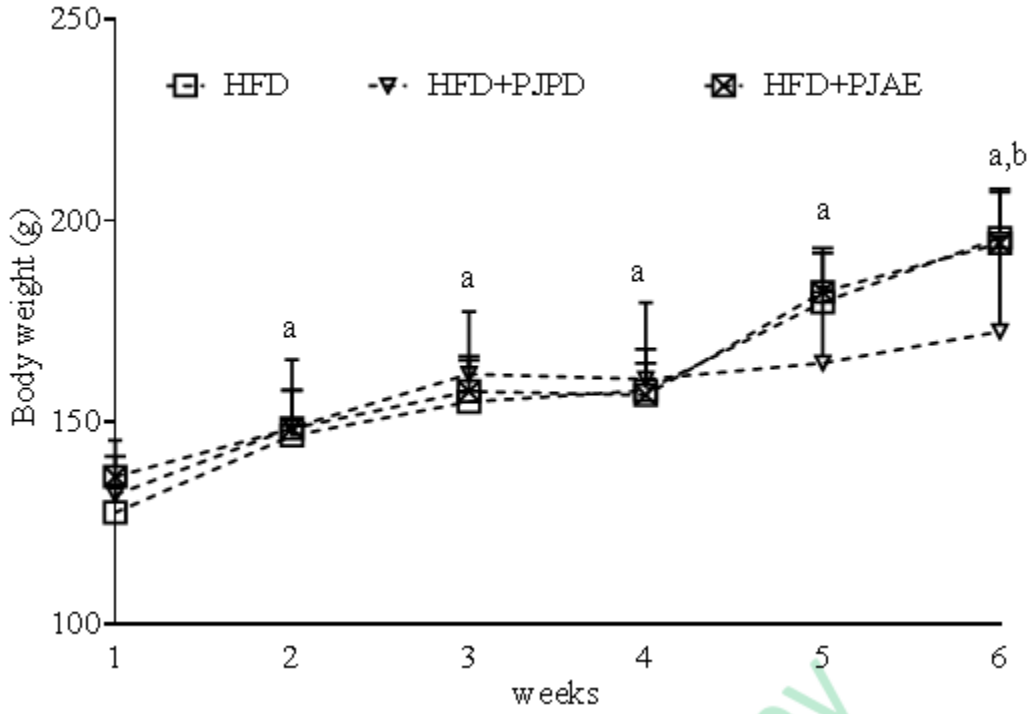


Fig 1. Effect of PJ treatment on body weight in rats fed a HFD for 6 weeks. Data represent the mean ± STD of six rats. Within the week, different characters statistically significantly different using two-way ANOVA: post hoc test: Tukey using GraphPad Prism 7 software ($p < 0.01$).

As shown in Fig. 2, % of weight gain was significantly decreased in PJPD administration at doses of 1g/kg mixed with the HFD at weeks 5 and 6 of the treatment compared to HFD group, without significantly altering the food intake. The % of weight gain was significantly decreased by HFD+PJAE only at week 4 of treatment.There was no significant increase in the % of gain weight after 2 and 3 weeks of in PJDP or PJAE treatments compared to HFD groups. the % of weight gain was calculated as following:

Weight gain (%) = New weight -Initial weight /Initial weight *100

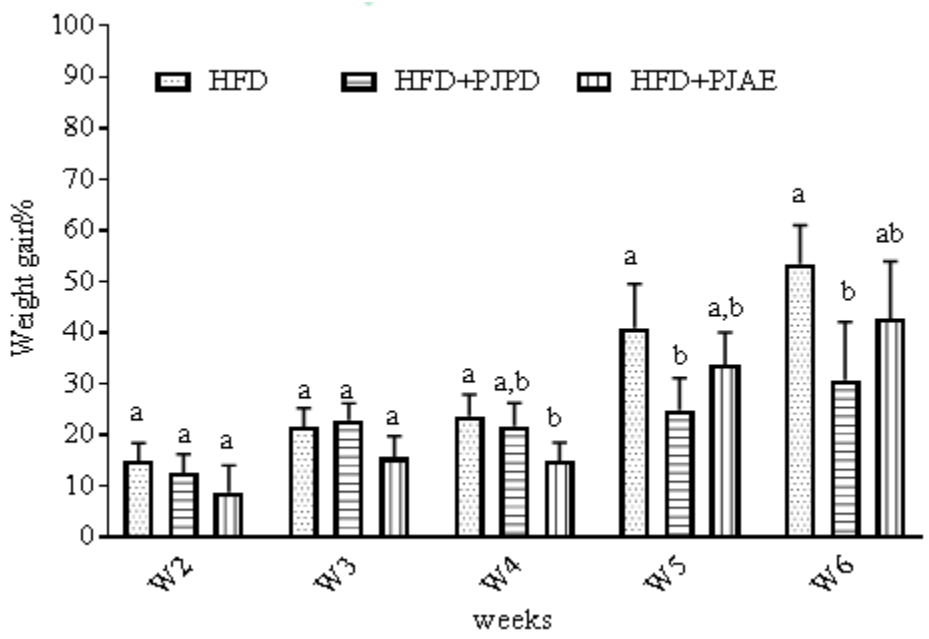


Fig 2. % of weight gain of ratsfed HFD for 6 weeks. Data represent the mean ± STD of six rats. Among each week, different characters statistically significantly different using two-way ANOVA: post hoc test: Tukey using GraphPad Prism 7 software ($p < 0.01$).

Numerous diseases, including atherosclerosis, cancer, type 2 diabetes, dyslipidemia and metabolic syndrome have been associated with the presence of Obesity [18]. Several studies have demonstrated that HFD causes elevation of body weight, liver weight and fat mass, as well as increased TG, TC, HDL-C, LDL-C and glucose levels in the serum [19, 20]. Previous studies by Al-Naqeb *et al*., [12], has reported that methanolic extract of *PJ* has shown antiadipogenic properties and regulated TG accumulation in 3T3-L1 adipocytes, indicating that *PJ* was able to inhibit adipocyte differentiation. The present study demonstrated the weight reduction effect of PJPD in HFD-induced fed rats. The results indicated that PJPD inhibited HFD-induced obesity by preventing the increase of body weight and reducing the serum triglycerides level. In our model of study, we use high fat diet with 20% of total energy from fat that might be explained why we did not find significant changes in TC, LDLC, glucose level and significant increase of kidney and liver parameters in compared with other studies that use fat as 45% of total energy as reported by Kim *et al*., [21].

Identification of active ingredient of PJ methanolic extract and its fractions was carried out by Al-Naqeb *et al*.,[12], they have reported the presence of catechin-like compounds, mainly in the methanolic extract and its dichloromethane fraction and both have shown bioactivity towards inhibiting TG accumulation *in vitro*. Catechins has shown anti-obesity activities *in vivo*[22]. The consumption of catechins in In controlled intervention trials resulted in reduction of body weight and body fat and serum TG levels with different mechanisms including with inhibiting intestinal lipases, decreasing fat absorption, increasing fat excretion, and decreasing lipogenic enzymes[23].

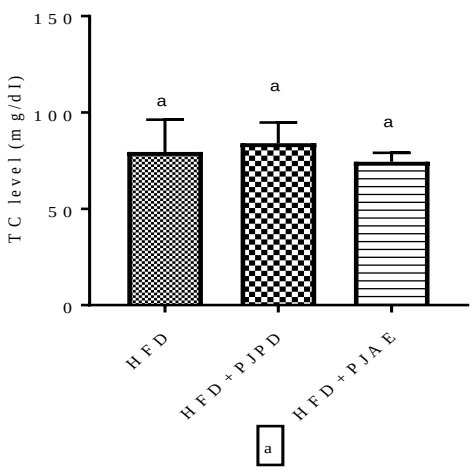
In this study, we have use the *PulicariaJaubertii* leaves and flowers in two different form, as an aqueous extract and as the whole powdered form of the PJ leaves and flower mixed with the diet, because in Yemen people are using this plant as drinking tea or as a food additive as a whole flower and leaves. We wanted to see if the applied form of this plant will have an effect in reducing body weight and triglyceride levels. The concentration of the given *PJ* was adjusted to

be approximately equal in the two different form of administration either in powder form mixed with the diet or as in aqueous extract.

For the body weight gain measurement at 5 and 6 weeks of the experiment there was a significant reduction in the % of body weight gain in HFD fed rats treated with PJPD at 10% diet. The reduction was about 40% relatively compared to HFD control whereas, the reduction on weight gain in the PJAE group was 15% relatively to HFD groups. In parallel the triglycerides level was also significant reduced in the rats fed HFD and treated with PJPD groups compared to the HFD groups that may be explained that the PJ in powdered form has many ingredients contribute to the gain weight reeducation, it might be due to the present of high amount of fiber content in the leaves and flowers (22±2.3) according to another study has conducted in our laboratory (data not shown). The high amount of the fiber content in another *Pulicaria*genuos leaves and flowers has been reported previously. The content of crude fibers was 24.56 % in Pulicariaundulata (Fahmi et al, 2019) and in the Pulicaria incisa subsp. Incisa the fiber content was reported to be 20.5±0.2 in the leaves and 14.4±0.17 in the flower [24].

Effect of PJ treatment on serum lipid profile

The effects of PJ on serum lipid levels of experimental rats were investigated at the end of the experimental period (Fig. 3 a-e). Serum TG, levels in the HFD group were significantly higher compared with group that fed HFD and treated with PJPD group ($P<0.01$). However, no significant different in TG level in HFD group and group that fed HFD and treated with PJAE. There was no significant difference in serum level of TC, LDL-C, LDL-C, TG/HDL and LDL/HDL in HFD group when compared to the PJ treated groups.



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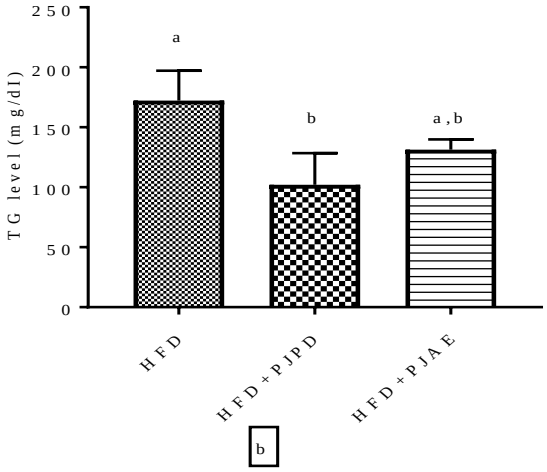

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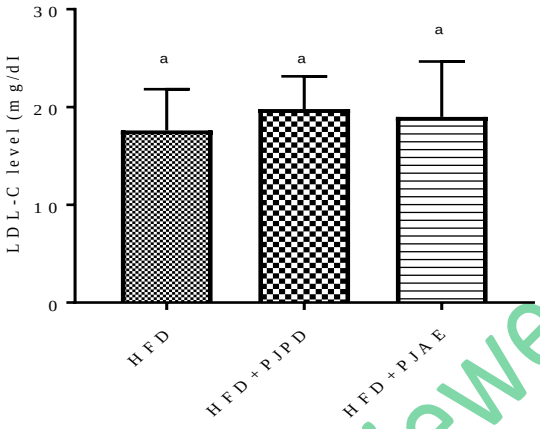

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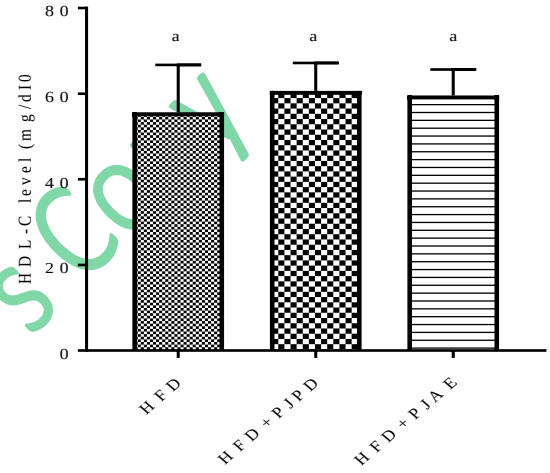

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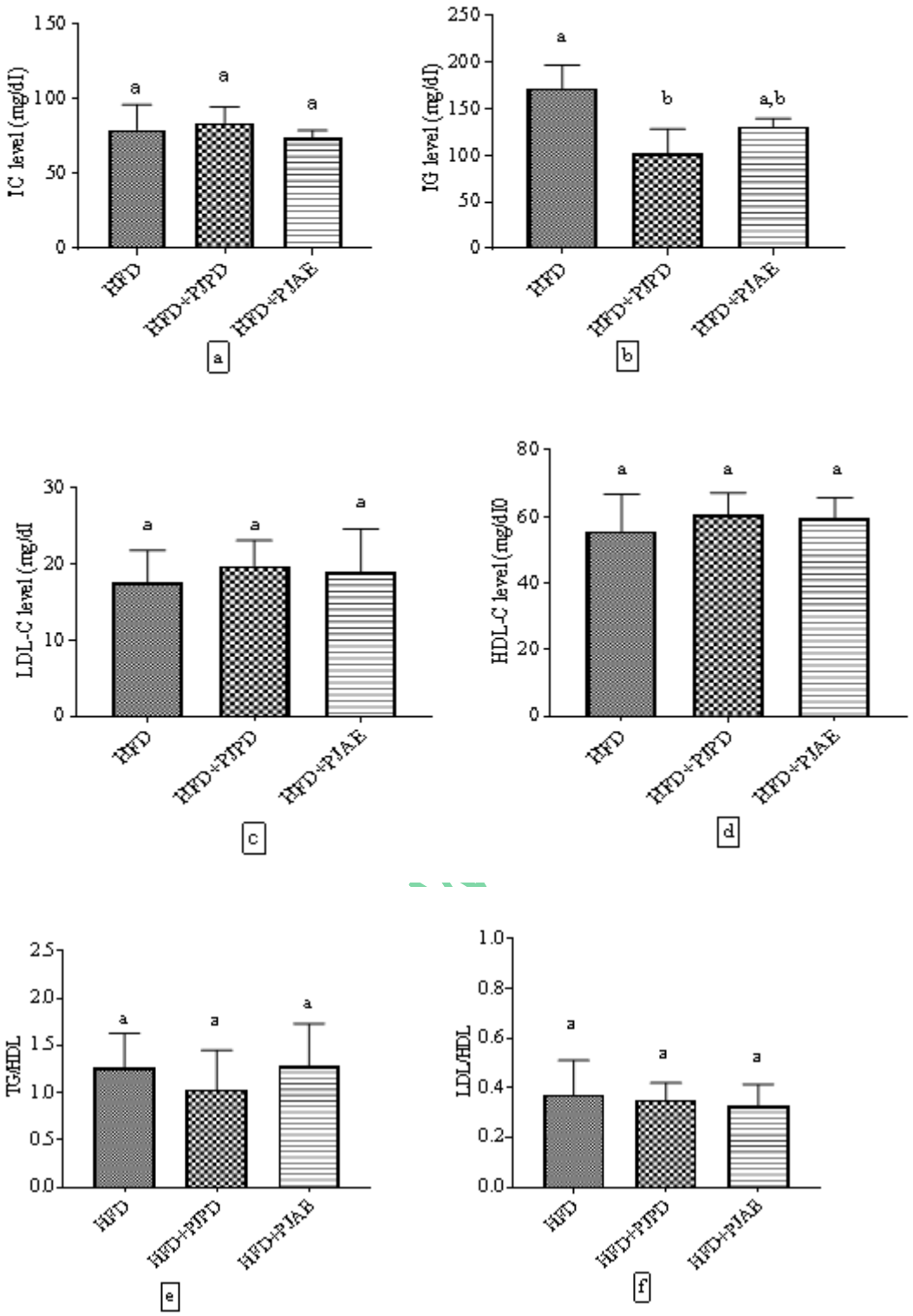

Fig 3. Effect of PJ treatment on lipid profile in rats fed a HFD for 6 weeks. Data represent the mean ± STD of six rats. Different characters statistically significantly different using one-way ANOVA: post hoc test: Tukey using GraphPad Prism 7 software ($p < 0.01$).

a= Total cholesterol level, b= Triglyceride level, c= LDL-C level. d= HDL-C level, e=the ratio of Triglyceride level to HDL-C and f= the ration of LDLCT to HDL-C

3.3 Serum Blood Glucose:

Furthermore, as shown in Fig. 4, there was no significant changes in serum glucose levels in the HFD group compared with the treated groups with PJ either as powder form mixed with diet or with PJ aqueous extract.

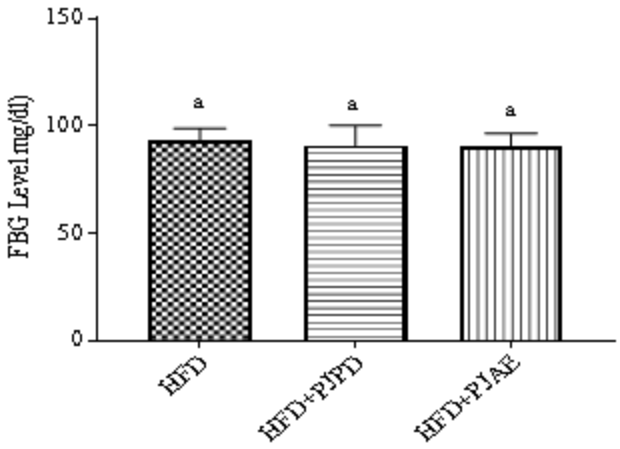

Fig 4. Effect of PJ treatment on serum glucose level in rats fed a HFD for 6 weeks. Data represent the mean ± STD of six rats. Different characters statistically significantly different using one-way ANOVA: using GraphPad Prism 7 software.

In the study report by Al-Naqeb *et al*,.[12] theaqueous fraction of the methanolic extract of PJ leaves was not effective in reducing the TG accumulation in 3T3-L cells when compared to the other fractions or the methanolic extract and that was due to the of absence of catechins like compounds in the water fraction compared to dichloromethane fraction or the crude methanolic extract, that might explained why the PJAE was not effective in reducing TG level significantly compared to HDF group. The reeducation of weight gain and TG level in the rats fed with HFD and treated with PJPD might be due to the presence of antioxidant in the whole leaves and flowers in PJ plant, The results of the free radical scavenging activity PJ leaves methanolic extract showed that the extract exhibited high antiradical activity towards DPPH radical and was close to the antiradical inhibition activity of L- ascorbic acid [25]. Recent study has reported the PJ extract obtained by supercritical fluid extraction method showed the high antioxidant inhibition activity[11.]

In our study, the levels of lipid profile parametersmeasures including TC, LDLC, HDL-C and the LDLD/HDL ration in HFD groups and treated groups with PJPD or PJAE all remains within the normal range that is may be due to the short period of study 6 weeks or it might be due to our HFD ingredients where we have different mixed of fat sources animal source and plant source or it might be because we use a rats and they not the good model for obesity study.

Effect of PJ Treatment on Kidney and Liver Function Parameters

As shown in Table 2 there was no significant changes in Kidney function parameters including creatinine and urea levels in HFD group compared to the treated groups with PJAE and PJPD. Also, no significant changes in liver enzymes level including ALT and AST in HFD group compared to the treated groups with PJAE and PJPD.

Table 2: Effect of PJ treatment at on Kidney and liver function parameters

Measured Pramater	HFD	HFD+PJPD	HFD+PJAE
Creatinine (mol/l)	0.73 ± 0.05[a]	0.72 ± 0.04[a]	0.74 ± 0.05[a]
Urea (mol/l)	32.67 ± 5.28[a]	31.67 ± 8.50[a]	30.6 ± 6.99[a]
ALT(U/L)	39.02 ± 12.62[a]	35.50 ±13.32[a]	38.20 ± 10.45[a]
SGT (U/L)	146.7 ± 61.96[a]	138.2 ± 24.81[a]	150 ± 46.09[a]

Data represent the mean ± STD of six rats. Different characters statistically significantly different using one-way ANOVA: using GraphPad Prism 7 software. Within each row, Same characters statistically not significantly different using one-way ANOVA: using GraphPad Prism 7 software.

To evaluate any potential toxic effects of PJ treatment, liver and kidney functions parameters were evaluated at the end of the 6-week experimental period. Administration of high-fat diet caused changes in parameters of hepatotoxicity (AST and ALT) as well as lipid metabolism (HDLC and TG) in animal model. The activities of AST and ALT were significantly enhanced in high-fat diet-fed mice, suggesting a hepatotoxic tendency[26]. The HFD-induced increase in ALT and AST was only in mice as reported bySung *et al*. [27].In our study, the liver and kidney tested parameters including ALT, AST, Urea and creatinine remains all in the normal range and did not change significantly in HFD groups and treated groups with PJPD OR PJAE, that is also show the selected doses of PJPD and PJAE were safe and nob toxic

CONCLUSIONS

The present study showed that *Pulicariajaubertii E. Gamal-Eldin* leaves and flower in powder form was effective in preventing the increase in body weight gain and serum TG levels compared to rats fed with HFD. Kidney and liver function test showed no signs of toxicity induced by 6 weeks treatment either PJPD or *Pulicariajaubertii E. Gamal-Eldin* aqueous extract on rats. Results of this study highlights the basis for future investigations of PJ plant as a source of natural product that has the potential to be developed as medicinal ingredients for prevention and treatment of obesity and other metabolic diseases in human. Another study to address the cellular and molecular mechanism of antiobesity effect of *Pulicariajaubertii E. Gamal-Eldin* is required.

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AUTHOR'S CONTRIBUTION

Noor Kaokabah is the researcher who carried out the research experiment and collected the data, Adana Al-Kobati is the supervisor who reviewed the research and Ghanya Al-Naqeb is the supervisor who did the writing partand statisticalanalysis and send the submitted the paper for publications.

CONFLICT OF INTEREST

There is no conflict of interest related to this work

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