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RESEARCH ARTICLE

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

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Abstract

Background: *Pulicaria jaubertii E. Gamal-Eldin* is a traditional medicine and flavoring used in Yemen where a large number of people depend on herbal plants to treat their ailments. This study was conducted to determine and compare the effects of *Pulicaria jaubertii* leaves and flowers aqueous extract (PJAE) and *Pulicaria jaubertii* leaves and flowers in powder form mixed with diet (PJPD) on obesity induced rats by high-fat diet (HFD).
Methods: Obesity was experimentally induced in male Sprague Dawley rats by feeding them a high-fat diet for 6 weeks. The rats were divided into 3 groups, the HFD control group, whereas the rats fed HFD only and the PJAE group where the rats fed HFD + PJAE and PJPD where the rats fed HFD + PJPD. 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. Food intake and gain weight were taken 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 functions parameters.
Results: The results indicated a significant decrease in the percentage of weight gain and in the PJPD group at the fifth and sixth weeks of treatment compared to the HFD control rats. Also, there was a significant decrease in the level of blood triglycerides in the PJPD compared to the HFD group. Where no significant change was observed in the percentage of weight gain and triglyceride level in the PJAE group compared to the HFD control rats. Also, there was no significant changes 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: The results obtained from this study showed that the leaves and flowers of *Pulicaria jaubertii* mixed with HFD had an anti-obesity effect in rats fed with HFD, and the anti-obesity effects of PJ could be attributed to the phytochemicals present. Therefore, the current study scientifically confirms the traditional use of PJ as a potential candidate for body weight loss.
Keywords: Anti obesity, *Pulicaria jaubertii*, Yemeni traditional medicine, Yemen.

INTRODUCTION

Obesity is considered a health problem globally and it has been well addressed to be a risk factor for other diseases such as hyperlipidemia, type II diabetes, and cardiovascular complications. Nowadays, obesity is considered one of health concerns in both developing and developed country. Obesity and its complications such as insulin resistance are growing in Middle East countries[1,2]. That resulted in growing attention on the screening many traditional plants medicines in Middle Eastern to manage the obesity. Previous studies, reported the use of plant extracts and their isolated compounds in obesity prevention and treatment widely, and therefore, reduction in body weight and reduced the effect of high fat diet induced obesity[3,4]. Other natural products have demonstrated antioxidant, hypoglycemic, and hypolipidemic properties such as cinnamon[5], green tea[6] and oats[7].

Pulicaria genus species are spread widely in Asia and Middle East and they have been known as traditional medicines[8]. The Yemeni plant *Pulicaria jaubertii Gamal-Eldin* (PJ) is used as food flavor and in traditional medicines prevention of infections and fever[9]. Methanolic extract of PJ leaves showed cytotoxic effect against brest cancer cells MCF-7 and also shown to have 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]. Chemical characterization of PJM and its fractions hexane and dichloromethane fractions as long as water fraction was reported[12]. Many compounds have been identified in PJM and its fraction including carvotanacetone, fatty acids, sugars and catechin-like compounds[12]. Human and animal studies have reported the antiobesity effect of catechin[13,14]. The antiadipogenic effect of PJ methanolic extract (PJM) and its dichloromethane fraction has been reported in vitro by reducing the accumulation of TG and modulate the cellular anti-oxidant activity[12].Thus, PJ might be useful in the prevention and treatment of obesity by reducing the accumulation of fat in adipose tissue. The use of animal models in obesity research has strong contributions[15]. Where, feeding them with diet high in fat can induced obesity and its complications as observed in obese human [16,17]. The aqueous extract of PJ leaves and flowers was chosen because people in Yemen use the flower with the leaves to drink tea, and another form was mixed with the diet because PJ leaves and flowers are consumed by people in Yemen as food and flavor additives. Therefore, the aim of this study was to investigate the anti-obesity activities and potential body weight reduction of PJ leaves and flowers in the form of aqueous extract and as a powder mixed with a diet in an animal model fed with HFD.

MATERIALS AND METHODS

Plant material

P. jaubertii 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 three collected samples from different places were mixed and used for this study. Identification of the plant was done by Hassan Ibrahim, a professor of plant taxonomist, Department of Botany, Faculty of Agriculture, Sana'a University, Yemen. The voucher specimen was deposited in the Herbarium of the Department of Botany, Faculty of Agriculture, Sana'a University, Yemen with assigned number PJS 1500. Name of the plant was 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. Then 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 in current study on its active constituents and some of its biological activities.

Preparing of aqueous extract

The dried PJ was finely ground with an electric grinder (Panasonic, Model MX-GX1571, China). PJ was administrated to each rat was 1g/kg body weight. Briefly, calculated amount of PJ powder to each rat was added to 3 ml of boiling water and soaked for 1 hour. The infusion was cooled to room temperature and then filtered. Approximately 3 ml of a filtered aqueous extract containing 1 g of PJ powder was administered to the rat daily by gavage for 6 weeks. The aqueous extract was prepared fresh before gavage of the animals.

Preparation of high fat diet

High fat diet (HFD) was prepared at animal house unit in Faculty of Agriculture, Sana`a University, Yemen. The composition of the diet were based on study reported by Rahman *et al.*,[18], with some modification. The diet was adjusted to contain 20% fat as a total energy Table 1 is showing the HFD ingredients. HFD contains: Casein- 30%, starch 20% sucrose 20% cellulose- 8.5%, fat 20%, mineral mixture (1%), vitamins mixture (0.5%). All ingredients of the diet were homogeneous used and mixed with water manually and cut to small pieces and dried in an oven at 45–50^{o}C.

Experimental design

Male *Sprague-Dawley* rats were obtained from College of Science, Sana'a University, Yemen. The rats were breeding in Faculty of Agriculture, Sana`a University, Yemen. Eighteen *Sprague-Dawley* male rats, each was weighing 120-150 g, 4-5 weeks of age were used in this study. This study was conducted with the permission of ACUC (Animal Care and Use Committee), Faculty of Agriculture, Sana`a University, Yemen (ACUC No: FASU/0075). The rats were housed under 22±2^{o}C 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. Rats were randomly divided in to three groups: first group HFD control was fed with HFD 20-30 g/ day to be able to compare food intake between different groups, second group HFD+PJAE was fed HFD and administration of 1 g/kg body weight of aqueous 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%. Rats were fed HFD and treated with PJAE or PJPD for 6 weeks. All the experiment process was done in Animal house unit, Faculty of Agriculture, Sana`a University.

Measurement of body weight and food intake

Rat's weight and the food intake were taken once a week with non-fasting condition. Efficiency of food intake was calculated by observing the consummation of food (g) in each cage for each animal per day basis. After 6 weeks of high-fat diet feeding, rats were sacrificed using ethyl ether. Blood samples were obtained from the eye and centrifuged at 1500 rpm for 15 min to separate to serum and blood cells.

Biochemical analysis of serum samples

Serum glucose level, triglyceride (TG), total cholesterol (TC), high-density lipoprotein high-density lipoprotein cholesterol (HDLC) low-density lipoprotein cholesterol (LDLC) and serum aspartate aminotransferase (AST) alanine aminotransferase (ALT), urea and creatinine levels were analysed using assay kits in private laboratory in Sana`a Alawlaki laboratory.

Statistical analysis

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 weights 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 compare the difference in weight gain for the different groups.

Table 1: 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

As shown in Figure 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 (p=0.015) than the body weight of HFD+PJMAE group. As shown in Figure 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:

$$\text{Weight gain (\%)} = \frac{\text{New weight} - \text{Initial weight}}{\text{Initial weight}} \text{x100}$$

Several studies have reported the associated of many chronic diseases including cancer, atherosclerosis, type 2 diabetes and metabolic syndrome with the presence of obesity[18]. Previous studies have elucidated that feeding with HFD resulted in increases of body, and liver weight and also fat mass, as well as increased in lipid profile levels and glucose levels in the serum[19,20].

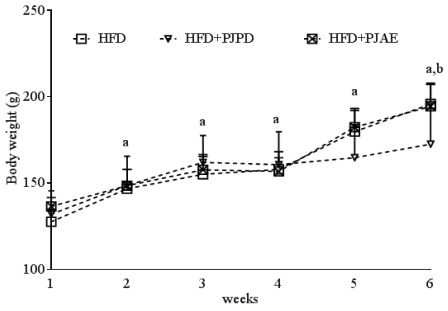

Figure 1: Effect of PJ treatment on body weight in rats fed a HFD for 6 weeks.

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).

The antiadipogenic properties and regulation of TG accumulation in 3T3-L1 adipocytes of Methanolic extract of PJ has been reported[12]. The present study shows the weight reduction effect of PJPD in HFD-induced fed rats. The results showed that PJPD reduced the induction of obesity by HFD- by management of body weight increases and reducing the serum triglycerides level.

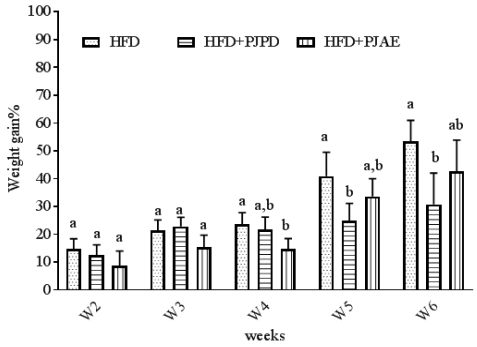

Figure 2: Percentage of weight gain of rats fed HFD for 6 weeks.

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).

In the current study model, a high-fat diet was used that contains 20% of the total energy from fat, and this can be explained by the absence of significant changes in TC, LDLC, and glucose level and a significant increase in kidney and liver indices compared to other studies that used fats with 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 have shown anti-obesity activities *in vivo*[22]. The consumption of catechins 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 current study, *P. jaubertii* leaves and flowers were used 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.

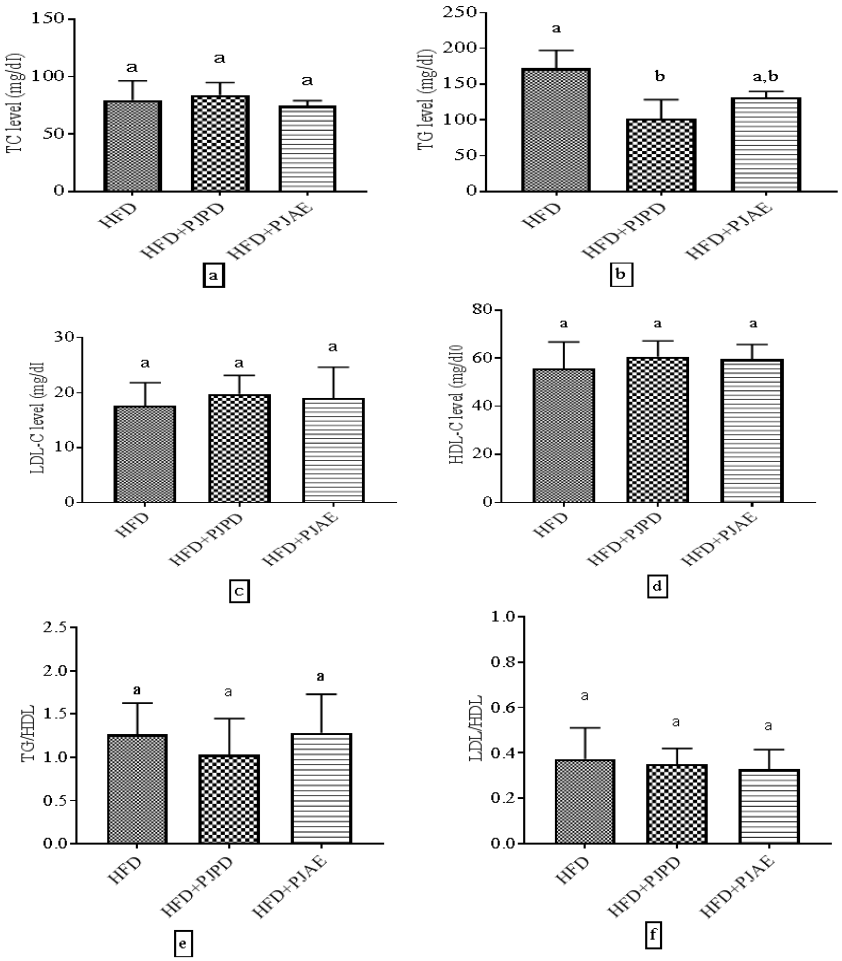

Figure 3: Effect of PJ treatment on lipid profile in rats fed a HFD for 6 weeks. Data represent the mean±STD of six rats. Different characters statistically significantly different using one-way ANOVA: post hoc test: Tukey using GraphPad Prism 7 software (p<0.01).

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

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 laboratory (data not shown).

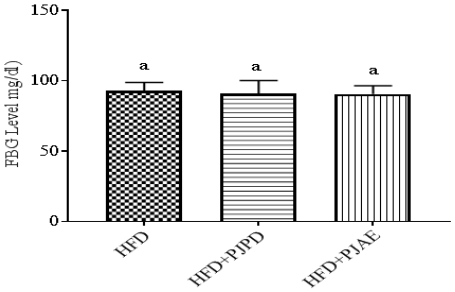

Figure 4: Effect of PJ treatment on serum glucose level in rats fed a HFD for 6 weeks. Data represent the mean±STD of six rats.

Different characters statistically significantly different using one-way ANOVA: using GraphPad Prism 7 software.

Table 2: Effect of PJ treatment 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.

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 *Pulicaria undulata*[1] 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 (Figure 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.

Serum Blood Glucose:

Furthermore, as shown in Figure 4, there were 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. In the study report by Al-Naqeb *et al*,.[12] the aqueous 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 anti radical 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 current study, the levels of lipid profile parameters measures 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 HFD ingredients where we have different mixed of fat sources animal source and plant source.

Effect of PJ Treatment on kidney and liver function parameters

As shown in Table 2 there were 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. 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 by Sung *et al*., [27]. In current 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 current study demonstrated that the leaves and flower of *P. jaubertii* E. Jamaluddin in powder form were effective in preventing increased body weight and serum TG levels compared to rats fed HFD. Renal and hepatic function test showed no signs of toxicity resulting from the 6-week treatment of either PJPD or PJAE on rats. The results of this study highlight the basis of future investigations of PJ as a source of natural products that could be developed as medicinal ingredients to prevent and treat obesity and other metabolic diseases in humans. Another study is required to address the cellular and molecular mechanism of anti-obesity effect of *P. jaubertii E. jamaluddin.*

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AUTHOR'S CONTRIBUTION

Kaokabah N: literature survey, data collection. **Al-Qubati A:** formal analysis, supervision. **Al-Naqeb G:**

writing original draft, statistical analysis. All authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

None to declare.

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