Effect of Hydrolysable Tannin on Nutrient Intake Obesity and Other Associated Metabolic Risk Factors in Polycystic Rats

Faiza Manzoor (✉ drfaizaahmadmmt@gmail.com)

Institute of Home and Food Science, Faculty of Life Sciences <https://orcid.org/0000-0002-0905-5572>

Mahr Un Nisa

Institute of Home and Food Science, Faculty of Life Sciences

Hafiz Amjad Hussain

Faisalabad Medical University, Faisalabad, Pakistan, Department of Internal Medicine

Muhammad Kamran Khan

Government College University, Faisalabad, Pakistan

Rabia Shabir Ahmad

Government College University, Faisalabad, Pakistan

Nazir Ahmad

Institute of Home and Food Science, Faculty of Life Sciences

Muhammad Imran

Institute of Home and Food Science, Faculty of Life Sciences

Huma Umbreen

Institute of Home and Food Science, Faculty of Life Sciences

Research

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Abstract

Background: Food intake and eating behavior are two important risk factors that lead to obesity and other metabolic and reproductive disorders like polycystic ovarian syndrome (PCOS). Most of the phytonutrients like hydrolysable tannin (HT) have the ability to reduce the nutrient intake that might be a suitable remedy for weight management of females in their reproductive age. Therefore, the present research is aimed to find out the effect of HT on nutrient intake, weekly body weight, weight gain, serum lipids, minerals, immunoglobulins and satiety hormones in PCOS rats.

Methods: A total of forty five adult healthy female rats of 56 days old, weighed 135 ± 5 g with two consecutive estrous cycles were selected. In order to induce PCOS in rats, the intramuscular injection of 4mg/rat/kg Estradiol-Valerate was used. After induction, a Complete Randomized Design was used to divide the rats into five equal groups (n=9) named as Pc0, Pc0.5, Pc1, Pc1.5 and Pc2. The groups of rats were offered different doses of HT i.e. 0, 0.5, 1, 1.5 and 2% respectively per kg body weight in solution form through oral gavage once in a day for 60 days.

Results: After the intake of different levels of HT, the statistical results had shown a significant decrease ($p < 0.05$) in the weekly nutrient intake, body weight, water intake, weight gain, blood glucose in PCOS rats. A similar trend of decrease ($p < 0.05$) was noticed in serum iron, IgM, IgG, leptin, ghrelin, cholesterol, low density lipoprotein and triglycerides while a significant improvement ($p < 0.05$) was also observed in high density lipoprotein in the PCOS rats. However, a non-significant effect ($p > 0.05$) was observed on serum protein and calcium levels.

Conclusions: The study concluded that HT had a therapeutical potential to decrease the nutrient intake and its anti-nutritional property could be used as remedy for the management of body weight, hyperglycemia, dyslipidemia and cardiovascular risk factors of PCOS rats.

Background

Obesity is one of the leading metabolic diseases which mainly ascribe to sedentary life style and bad eating habits, including consumption of excessively junk food. In the recent era, it is also believed that the growing incidences of polycystic ovarian syndrome (PCOS) in the young females are due to the poor eating habits and obesity and these factors are considered as the main causes for the progression of reproductive and metabolic abnormalities. About 40–80% of females with these conditions are reported to be overweight or obese [1, 2]. The PCOS is a heterogeneous, metabolic and hormonal disorder and has shown hyper-androgenism, insulin resistance, diabetes and metabolic abnormalities in lipid profile and incidence of cardiovascular diseases (CVD) [3]. Since obesity plays a comprehensive role in the development and progression of PCOS induced hormonal complications, therefore, an attention to healthy nutritional remedies can be practiced for maintaining an ideal body weight and for the management of associated PCOS complications. Pharmaceutical products had shown deleterious side effects when used for the treatment of PCOS patients [3, 4]. Life style modification, balanced diet with richest source of bio-molecules (plant based) might be best weight loss treatment strategies for the obese patients [1, 5]. Hydrolysable tannin (HT) is one of the plants bio-molecules and has shown many health promoting properties like anti-diabetic, anti-obesity, improvement of serum lipids and reduced the CVD risk factors [6, 7]. The HT is a phenolic compound and exerts negative effects on nutrient feed intake and results in weight loss [8]. However, some previous studies had shown no effect of HT on feed intake, calcium and phosphorus absorption [9] but observed negative effects on iron absorption [10]. The present study is therefore, focused to find an alternative

therapy in order to reduce body weight in PCOS patients. Many medicinal plants and herbal therapies had been used for the treatment of PCOS. The goal of this study was to find the effect of healthy and anti-nutritional properties of HT on weight management, abnormal blood biochemical profile and other associated metabolic risk factors of induced PCOS rats.

Methods

Adult female Albino Wistar rats of about 56 days old were purchased from Riphah International University, Lahore. A total of 45 female rats were recruited with 4 to 5 days regular estrous cycle. They were kept at $25 \pm 1^{\circ}\text{C}$ with 45 to 55% relative humidity and (12 hours) dark/light cycle in the animal house of Pharmacology Department, Government College University Faisalabad (GCUF) Pakistan. The study was approved by the Directorate of Advanced Studies and the Animal Ethical Committee of GCUF had permitted for all the due animal procedures by following the instructions of Laboratory Animal Care (NIH Publications No. 8023, reviewed 1978). All the rats were offered *isocaloric* and *isonitrogenous* diet.

Induction of Polycystic (PCOS)

Before the start of the induction procedure of PCOS, the initial body weight (weighing Balance) and blood glucose (Accu-check Glucometer, Byer) levels of the female rats were taken. For PCOS induction (rats with 2 consecutive estrous cycle) estradiol valerate (Progynova, Bayer Pharmaceutical Co Ltd) tablets were crushed 4mg/rat/kg and then dissolved in distilled water using Vortex Mixture and were given through intramuscular injection in a single dose [11, 12]. The rats were then observed daily through visual method for the detection of irregular estrous cycles [13]. To confirm the PCOS, a vaginal smear test was performed at the 6th week of the estradiol injection [14] in which leukocytes, epithelial and cornified cells were observed. Other signs of PCOS were also observed on weekly basis which included body weight changes and blood glucose level $>200\text{mg/dl}$ [15, 16].

Experimental Design

All female rats were completely randomized into five equal groups with 9 rats in each on 45th day of PCOS confirmation. The groups were named according to the HT dose levels i.e. Pc0.5: group with 0.5% HT; Pc1: group with 1% HT; Pc1.5: group with 1.5% HT; Pc2: group with 2% HT; Pc0: group with 0% HT and was considered control. Identification marks were given on the tail of each rat using permanent ink markers of different colors. These four levels were calculated according to the method described by Erhirhie [17]. Then the calculated doses in mg were dissolved in warm distilled water to prepare their required concentrated solutions and then given through oral gavage/rat/kg body weight/day for two months [18].

Nutrient Feed Analysis

The daily feed intake of each rat was recorded in the last 7 days of the experiment after 60 days of treatment with HT. Split and left over feed samples were pooled and stored in a tight plastic jar at -20°C for analysis. To calculate the daily amount of water drank per experimental rat the following formula was used: Initial water (ml) – left over water (ml) [19]. The proximate analysis of feed was done according to the Official Method of Analysis. The feed samples were dried at 65°C for 48 hours in a hot air oven to determine the moisture contents and then by applying formula we measured dry matter: $\text{DM \%} = 100 - \text{Moisture \%}$. The feed samples were burnt in Muffle Furnace at 550°C for 4 hours for ash determination; crude fat was determined with petroleum ether extraction method (PEE);

for crude fiber determination, the feed samples were boiled in H₂SO₄ and then with NaOH [20]. Kjeldahl Method was used for determining the crude protein while carbohydrates were determined with deduction method by Adenike [21] % Carbohydrates (NFE) [100 - (% moisture + % crude fat + % crude fiber + % crude protein + % ash)].

Blood Sampling

The rats were sacrificed early morning at 8:00 o'clock using chloroform anesthesia after twelve hours overnight fast. The jugular vein of the rats was cut with sharp blade and the blood was collected directly into the labeled test tubes and allowed to clot. Serum had been collected after centrifugation at 5000 rpm for 20 min and stored at -20 °C for biochemical analysis.

Biochemical Analysis

Serum cholesterol and triglycerides were analyzed spectrometrically; Serum HDL was determined enzymatically; Serum iron was determined by photometric colorimetric test method by using Kits of Human Diagnostic worldwide, Netherland; serum LDL was measured by subtracting the average cholesterol and cholesterol in the supernatant (Dia-Sys Diagnostic System GmbH, Germany); Serum calcium was determined by Arsenazo III Colorimetric method [22]. Serum leptin and ghrelin were analyzed by enzyme-linked immune-sorbent assay ELIZA (Elabscience Biotechnology Inc. Corporate USA); Biuret Method was used to detect Serum protein [23] (Human Diagnostic worldwide, Netherland); Serum IgG and IgM were assessed by Bindarid Radial Immune-Diffusion (RID) kit method (The Binding Site Ltd., Birmingham, UK).

Statistical Analysis

Statistical presentation of data, as Mean ± SEM and differences of significance were calculated by using Analysis of Variance (ANOVA) *IBM SPSS statistics 21* (USA) at *p* value <0.05.

Results

Composition of Diet on Dry Matter Basis (%)

The rats were fed *ad libitum* diet as per AIN-93 guidelines [24, 25]. However, on the dry matter basis, the composition of the diet contained 88.9% dry matter, 8.3% ash, 5.5% crude fat, 6.5% crude fiber and 18.6% crude protein contents.

Feed Conversion Ratio and Weekly Nutrient Intake (%)

The results regarding feed conversion ratio (FCR) and weekly nutrient intake in different HT fed groups of PCOS rats are presented in Figures 1 to 6. A weekly significant decrease (*p*<0.05) in dry matter intake, crude fat, protein, fiber and ash intake of all treatment groups was noticed as compared to Pc0 as presented in Figures 1 to 5. The statistically maximum increase was observed in FCR of about 54.24% in Pc2, 42.39% in Pc1.5, 42.04% in Pc1 while Pc0.5 had shown 10.14% increase as compared with Pc0 as shown in Figure 6.

Weekly Body weight, Weight gain (g), Blood glucose (mg/dl) and Water intake (ml)

The weekly body weight (BW), weight gain (BWG), blood glucose and water intake of Pc0 and after 2 months intervention with HT in Pc0.5, Pc1, Pc1.5 and Pc2 are presented in Figures 7 to 10. A significant increase (*p*<0.05)

in weekly BW, BWG, blood glucose levels and water intake were noticed in Pc0. However, a significant reduction ($p < 0.05$) trend was observed throughout eight weeks in weekly BW, BWG and blood glucose as shown in Figure 7, 8 and 9. While in the last week of the trial, Pc2 had shown 5.87% reduction in weekly BW, 90% in BWG and 36.54% in blood glucose level. A significant decrease ($p < 0.05$) was observed in weekly water intake of Pc1.5 and Pc2 as compared to Pc1, Pc0.5 and Pc0 as shown in Figure 10.

Serum lipids (mg/dl)

The statistical results regarding serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) are shown in Table 1. A significant increase ($p < 0.05$) was observed in serum cholesterol, LDL and TG; while a highly significant decrease ($p < 0.05$) was observed in serum HDL of Pc0 as compared to healthy reference values (Un-published data). After dietary intervention with HT, the serum cholesterol levels were decreased significantly ($P < 0.05$) in Pc1 (145.37mg/dl), Pc1.5 (139.18mg/dl) and Pc2 (144.19mg/dl) as compared to Pc0.5 (150.22mg/dl) and Pc0 (152.77mg/dl) as shown in Table 1. The statistical results of serum HDL had shown 20.83% improvement in Pc2, 18.51% in Pc1.5, 14.18% in Pc1 and 7.60% in Pc0.5 as compared to Pc0. The results of Pc1.5 and Pc2 had shown maximum reduction in serum LDL levels which was about 18% and 15.8% respectively as compared to Pc0. The levels of serum TG had shown 12.29% reduction in Pc1 and 11.52% in Pc1.5 as compared to Pc0.5, Pc2 and Pc0.

Serum Minerals, Satiety Hormones and Immunoglobulin's

The statistical results regarding serum protein (g/dl) calcium (mg/dl), iron (ug/dl), leptin, ghrelin (ng/ml), IgG and IgM (g/l) in Pc0 and after the treatment with HT in Pc0.5, Pc1, Pc1.5 and Pc2 of PCOS rats are presented in Table 2. The serum protein, calcium and ghrelin levels had shown non-significant effects ($p > 0.05$) while a significant increase was observed in serum iron, leptin, IgG and IgM in Pc0 as compared to healthy reference value. After two months of treatment with HT, a non-significant effect ($P > 0.05$) was observed in the serum calcium levels in all the treatment groups as compared to Pc0. The statistical results of serum iron had shown 5.98% reduction in Pc1 and 4.79% in Pc2 as compared to Pc1.5, Pc0.5 and Pc0. The statistical results regarding serum leptin and ghrelin are shown in Table 2. Levels of serum leptin had shown significant reduction which was about 14% in Pc2, Pc1.5 and Pc1 as compared to Pc0.5 and Pc0. Level of serum ghrelin was reduced significantly ($p < 0.05$) in Pc1.5 (174.78ng/ml) as compared with Pc1 (174.89ng/ml), Pc2 (174.85ng/ml), Pc0.05 (174.97ng/ml) and Pc0 (174.98ng/ml) as shown in Table 2. A non significant effect ($p > 0.05$) was observed on serum protein while the serum IgG level had shown significant decrease, represented as 10% in Pc1.5 and 14% in Pc2 as compared to Pc0.5, Pc1 and Pc0. The statistical data of serum IgM levels had shown 19.1% reduction in Pc2 and 11.1% in Pc1.5 as compared to Pc1, Pc0.5 and Pc0.

Discussion

Obesity is a very serious health concern for the people of developed countries due to their life style and eating habits which further contribute many other metabolic disorders like hyperglycemia and CVD's. The phytonutrient hydrolysable tannin had shown anti-obesity and anti-diabetic activities [7]. So the present study was conducted to further explore its therapeutical effects on PCOS rat's model. Abnormal reproductive hormones and insulin resistance had found in PCOS patients and were responsible for the development of adiposity and imbalance in energy homeostasis and weight gain [26, 27]. The first finding of the present trial was the increase in nutrient intake, BWB and WBWG of PCOS control rats and the standard treatments involved changes in eating habits and

weight reduction [28]. So in the present study, HT helped to decrease BW and BWG and nutrient intake of the PCOS rats. Another finding of this study was the improvement in the percentage of FCR in 1.5% and 2% HT fed PCOS rats. The proposed mechanism of weight reduction and the improvement of FCR were due to the reason that HT had the anti-nutritional properties which were further responsible for poor macronutrients and utilization of feed which resulted in weight loss and increased FCR [29]. However, the decrease in nutrient intake might be due to the reason that HT had slowed down the process of digestion and resulted satiety signals generated as a feedback to the nerves that were involved in intake control center of the rats and decreased the feed intake and body weight. The present study had also shown the increased levels of serum leptin, but showed no effect on serum ghrelin levels in the control group of the PCOS rats and these results also supported the previous findings [30]. Leptin and ghrelin were the two important hormones which controlled the intake and maintained energy homeostasis of the body while increased level of serum leptin indicated the condition of leptin resistance in PCOS rats which was further associated with increased intake [31]; while the treatment with HT improved the leptin resistance and also decreased the serum ghrelin in the present trial which might be due to the decrease in BW (adipose mass) and nutrient intake. In the present trial, hyperglycemia was observed in the PCOS rats which was also confirmed previously [32] but the ingestion of 1%, 1.5% and 2% HT reduced the blood glucose levels in PCOS rats, and this possible effect might be due to the fact that HT helped to enhance the glucose transport through insulin mediated signaling pathways in adipocytes which as a result reduced the blood glucose level [33]. In the pathogenesis of PCOS, insulin resistance was considered the major initiative element of the oxidative stress (OS) which further contributed to hyperandrogenism [14]. Hyperglycemia and increased androgen levels were further responsible to decrease in the serum HDL level, increased serum LDL, hypercholesterolemia and hypertriglyceridemia, which were also reported previously [3] and the present study had also found the same results. But after the intake of HT, a decrease was observed in serum cholesterol, LDL, triglycerides and improvement in serum HDL in all treated groups. The improvement in the serum lipids in the PCOS rats might possibly be due to the increase in peripheral insulin sensitivity to rat's adipose tissues and inhibited the lipogenesis or by increasing the activity of lipo-protein lipase enzyme [34]. However, HT might also be contributed in the cholesterol biosynthesis inhibitory activities [35]. Many previous studies also reported that in PCOS patients, insulin resistance was one of the reasons of serum iron over load [36] which was another finding of the present research as a high level of serum iron was observed; while no effect on serum calcium levels had observed in PCOS control rats. A study on phenolic compounds showed that they had the metal chelating activities when consumed in large quantities and might affect the iron status and as a result inhibited the absorption of non-heme iron [37]. In the line of previous findings, our results showed that the intake of HT in the PCOS treated rats induced the formation of stable iron/HT complex in the gut and thus decreased the plasma iron concentration [38]. The present study had also shown the increased serum IgG and IgM levels as also reported previously [39]; while no effect on serum total protein was observed in PCOS control group. Serum immunoglobulins were the important parts of the humoral immune system which was involved in pathogen killing activities but high levels of these immunoglobulins (IgG and IgM) were also involved for the development of cardiovascular diseases in PCOS patients [39]. In present study the dietary intervention with HT helped to decrease the serum IgG and IgM levels which might positively be associated with digestibility of protein in rats [40]. However, the lack of experimental effect of HT was observed on the serum total protein which was in agreement with the previous study [41] and showed that HT had shown non toxic effects on protein metabolism. In this study the increased water intake was also observed in PCOS rats which were also observed in various previous research trials [6]; but the experimental effect of HT was observed to decrease the water intake in rats [42]. However, this effect might be due to the role of HT to maintain the water homeostasis which helped in the prevention of water loss from body [43].

Conclusions

The hydrolysable tannin had shown a considerable reduction in the satiety hormones (leptin and ghrelin) and immunoglobulins (IgG and IgM), nutrient intake and blood glucose levels. The study concluded that HT can be used as an alternate herbal medicine for the management of obesity, diabetes, dyslipidemia and cardiovascular risk factors associated with PCOS.

Abbreviations

PCOS
Polycystic ovarian syndrome; HT:Hydrolysable tannin; EV:Estradiol valerate; HDL:High Density Lipoprotein; LDL:Low density Lipoprotein; TG:Triglycerides; IgG:Immunoglobulin G; IgM:Immunoglobulin M; FCR:Feed conversion ratio; BW:Body weight; WBW:Weekly Body weight; WBWG:Weekly body weight gain; CVD:Cardiovascular disease; ELIZA:Enzyme-linked immune-sorbent assay; AOAC:Official Method of Analysis; H₂SO₄:Sulfuric Acid; NaOH:Sodium Hydro-Oxide

Declarations

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Ethics approval and consent to participate

Animal Ethical Committee of Government College University, Faisalabad, Pakistan had proved this research by following Procedures of Laboratory Animal Care.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study is included within the article

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Author Contributions

FM Contributed in conduction and execution of experimental work; MUN planned and supervised; HAH provided Lab assistance and supervised experimental analysis; MKK and RSA contributed to experimental measurements, discussion and interpretation of the results; NA and MI supervised and supported the data processing, edited manuscript; HU supervised and edited manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Authors Information

¹**Faiza Manzoor** PhD Scholar Food and Nutrition (**Corresponding Author**), Email: drfaizaahmadmmt@gmail.com; ¹**Dr. Mahr UN Nisa** Associate Professor; Email: Linknisa@gmail.com; ²**Dr. Hafiz Amjad Hussain** Associate Professor; Email: dramjadpulmo@gmail.com; ¹**Dr. Muhammad Kamran Khan** Assistant Professor Email: mk.khan@gcuf.edu.pk; ¹**Dr. Rabia Shabir Ahmad** Associate Professor Email: rabiaahmad@gcuf.edu.pk; ¹**Dr. Nazir Ahmad**, Assistant Professor Email: drnazirahmad@gcuf.edu.pk; ¹**Dr. Muhammad Imran** Assistant Professor Email: Imran@gcuf.edu.pk; ¹**Huma Umbreen** Assistant Professor Email: humaumbreen@gcuf.edu.pk

1: Institute of Home and Food Science, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan

2: Department of Internal Medicine, Faisalabad Medical University, Faisalabad, Pakistan

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Tables

Table 1: Serum Lipid Profile of PCOS Rats

Groups	Healthy Reference Value	Pc0	Pc0.5	Pc1	Pc1.5	Pc2
Cholesterol (mg/dl)	108.19	152.77±0.84 ^a	150.22±2.38 ^a	145.37±2.52 ^b	139.18±1.27 ^c	144.19±0.64 ^b
HDL (mg/dl)	36.62	29.76±1.43 ^c	32.21±1.22 ^{abc}	34.68±1.03 ^{bc}	36.52±1.71 ^{ab}	37.59±0.62 ^a
LDL (mg/dl)	51.07	94.44±1.05 ^a	90.37±0.61 ^b	85.63±1.43 ^c	77.38±0.38 ^{bc}	79.49±0.68 ^b
TG (mg/dl)	102.16	142.86±0.35 ^a	138.19±1.63 ^{ab}	125.30±0.61 ^c	126.40±1.05 ^c	135.56±0.52 ^b

Healthy Reference values: Un-published Data; LDL: High Density Lipoprotein; HDL: Low Density Lipoprotein; TG: Triglycerides; Pc0: Polycystic group with 0% HT; Pc0.5: group with 0.5% HT; Pc1: group with 1% HT; Pc1.5: group with 1.5% HT; Pc2: group with 2% HT

Table 2: Serum Minerals, Satiety Hormones and Immunoglobulin's of PCOS rats

Groups	Healthy Reference Value	Pc0	Pc0.5	Pc1	Pc1.5	Pc2
Calcium (mg/dl)	11.15	11.13±0.020 ^a	11.14±0.023 ^a	11.15±0.019 ^a	11.15±0.023 ^a	11.12±0.024 ^a
Iron (ug/dl)	234.51	287.20±0.22 ^a	286.16±0.25 ^b	270.03±0.27 ^d	278.66±0.24 ^{cd}	273.56±0.25 ^c
Leptin (ng/ml)	6.50	8.70±0.1 ^a	8.10±0.09 ^b	7.45±0.27 ^c	7.43±0.24 ^c	7.41±0.19 ^{bc}
Ghrelin (ng/ml)	174.95	174.98±0.26 ^a	175.97±0.06 ^a	174.89±0.02 ^b	174.78±0.02 ^c	174.85±0.12 ^b
Protein (g/dl)	5.66	5.74±0.03 ^a	5.75±0.03 ^a	5.73±0.02 ^a	5.75±0.05 ^a	5.74±0.02 ^a
IgG (g/l)	3.27	5.40±0.10 ^a	5.27±0.12 ^b	5.15±0.12 ^{ab}	4.86±0.17 ^c	4.62±0.09 ^d
IgM (g/l)	0.74	0.99±0.10 ^a	0.97±0.26 ^a	0.91±0.18 ^b	0.88±0.13 ^{bc}	0.80±0.23 ^c

Healthy Reference values: Un-published Data; IgG: Immunoglobulin G; IgM: Immunoglobulin M; Pc0: Polycystic group with 0% HT; Pc0.5: group with 0.5% HT; Pc1: group with 1% HT; Pc1.5: group with 1.5% HT; Pc2: group with 2% HT

Figures

Figure 1

Weekly dry matter intake (%) of PCOS rats

Figure 2

Weekly crude fat intake (%) of PCOS rats

Figure 3

Weekly crude protein intake (%) of PCOS rats

Figure 4

Weekly crude fiber intake (%) of PCOS rats

Figure 5

Weekly ash intake (%) of PCOS rats

Figure 6

Feed conversion ratio (%) of PCOS rats

Figure 7

Weekly body weight (g) of PCOS rats

Figure 8

Weekly body weight gain (g) of PCOS rats

Figure 9

Weekly blood glucose (mg/dl) of PCOS rats

Figure 10

Weekly water intake (ml) of PCOS rats