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Effect of the heart of date palm aqueous extract administration on antioxidant enzymes and obesity-related hormones levels in rats

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Summary. The aim of this study was to determine the levels of antioxidant enzymes and some hormones in rats samples after administration of aqueous extracts of heart of date palm (HDP). HDP extracts were prepared from three Saudi date palm cultivars (Sukkari, Naboat Saif and Solleg) and administered orally every day for eight weeks. Activities of glutathione peroxidase (GPx) superoxide dismutase (SOD) and Catalase (CAT) were determined in rat liver and plasma, while Leptin, Ghrelin, Insulin and glucose were determined only in plasma. Animals in all groups had normal growth and health through the experiment. The results showed significant increase in activities of GPx, SOD and CAT in both rat liver and plasma. Circulating levels of leptin and insulin decreased significantly after administration of the three HDP extracts to the animals. Only the level of ghrelin in rat plasma increased significantly ($p< 0.05$) in case of Sukkari HDP extract. Although HDP have significant effects on leptin, ghrelin and insulin in rats, relationship of HDP administration to obesity could not be confirmed, and further research is needed to investigate the effect of HDP on human body weight control.

Key words: Heart of date palm, antioxidant enzymes, leptin, ghrelin, insulin, obesity.

Introduction

Date palm (*Phoenix dactylifera L.*) is a well-known tree in Saudi Arabia, and mainly cultivated for its fruit (1), the heart of date palm (HDP) or the apical meristem of the date palm tree is edible, and produced for commercial purposes (2, 3). In Saudi Arabia, the heart of date palm (*Al-Guomar*) is extracted from date palm tree and consumed fresh by some Saudi people (4). HDP was considered as a rich source of protein, dietary fiber, minerals and antioxidants (2, 4, 5). Antioxidant enzymes activities in embryonic stages of date palm were studied by Zein El din and Ibrahim (6), and these enzymes included peroxidase and polyphenol oxidase. Endogenous antioxidants in the body deactivate free radicals and minimize the risk of oxidation and damage of cells in the body. Some natural antioxidants from plants (e.g. antioxidant enzymes from fruits and vegetables) were investigated as protective agents against free radical's damage (7). Glutathione peroxidase and superoxide dismutase activities were examined in the plasma of rats before and after date palm seed steeping treatment, and these enzymes activities were significantly increased after the treatment (8).

Camel thorn plant (*Alhagi maurorum*) was described by Sheweita et al. (9) as promising medicinal plant because it has high contents of flavonoids and phenolic compounds which are antioxidant phytochemicals, aqueous and methanolic extracts, of this plant when administered to induce diabetes in rats lead to decreased oxidative stress, also these treatments lead to changes in antioxidant enzymes activities in the rat's plasma (9).

Leptin and ghrelin in humans are hormones that seem to have roles in food intake, energy balance and body weight regulation (10). Leptin is produced mainly from adispose tissue (11). Leptin decreases appetite

and food intake (12), while ghrelin, a peptide produced mainly from the stomach, seems to stimulate appetite and induces food intake (13, 10). Ghrelin in rats has vital roles as stimulator of growth hormone secretion, energy balance and body weight regulation (14), while leptin in rats has different effects on body weight, appetite, liver and kidney functions, and plasmatic glucose level (15). Leptin has been suggested to play causative role in insulin resistance associated with obesity in humans (16). In their study of leptin levels in obese Nigerian women, Obsegbe et al. (17) found that in these women, leptin Serum levels were positively correlated with insulin resistance which is a characteristic of types 2 diabetes mellitus. Also, Leptin and its relation to insulin and obesity were investigated, in type 2 diabetes mellitus model rats, by Velasquez et al. (18). They reported that a significant positive correlation between plasma leptin and plasma insulin was observed in the entire groups of rats, plasma leptin as well was positively correlated with fasting plasma glucose (18).

The present study may be considered as a continuation of the previous study (4), and aims at assessing the levels of antioxidant enzymes in rat's liver and plasma, and Insulin, Leptin, Ghrelin in rat's plasma after administration of aqueous extracts of HDP. The results of the analyses of this work may highlight a possible role of HDP as a natural product, in applications of food science and human nutrition.

Material and methods

Preparation of heart of date palm aqueous extract: HDP samples from three Saudi date palm cultivars (Sukkari, Naboat Saif and Solleg) were prepared as previously described (4). Five grams of finely powdered freeze dried HDP samples flours were mixed with 50 ml distilled water, and the mixture was stirred using a magnetic stirrer for one hour at room temperature, and then centrifuged for 20 minutes at 4000g to obtain clear aqueous extract. The extract was prepared fresh daily and used immediately in the rat bioassays.

Animals: A total of twenty-four male rats (Wistar strain) weighing 180-200 grams were obtained from Experimental Animals Care Center, College of Pharmacy, King Saudi University, Saudi Arabia. Animals were maintained in a controlled environment at 25^{0}C and 12/12 light / dark cycle, and animals had free access to water and chow diet throughout the experimental period. Rat chow diet was obtained from the grains silos and flour mills, Riyadh, Saudi Arabia. All animals' experiments were carried on according to the ethical Guidelines of Experimental Animals Care Center, College of Pharmacy, King Saudi University, Riyadh, Saudi Arabia.

Rat bioassays: Animals were kept individually in single rat cages for an adaptation period of a week, and then divided randomly into four groups of six rats each, as follows:

a. Group 1: Animals consuming 3 ml Sukkari extract
b. Group 2: Animals consuming 3 ml Naboat Saif extract
c. Group 3: Animals consuming 3 ml Solleg extract
d. Group 4: (Control group) received 3 ml distilled water.

For all groups of rats the specified HDP extract was administered by oral gavage for each rat at fixed time of the day.

Preparation of plasma and liver samples: At the end of the eight weeks experimental period, rats were anesthetized with pentobarbital sodium (60 mg/kg body weight). Blood samples were collected from each rat from the heart into EDTA Tubes, and then centrifuged for 15 minutes. Plasma samples were stored at -80^{0}C and used for glucose, insulin, Leptin, Ghrelin and antioxidant enzymes analyses. Liver samples were removed and cleaned from other tissues, then stored at -80^{o}C, to make liver homogenates, portions of liver samples were homogenized with phosphate buffer (pH 7.4) and the homogenates centrifuged to obtain clear supernatants which were used for antioxidant enzymes analyses.

Biochemical analyses

Glutathione peroxidase: Glutathione Peroxidase was analyzed by using diagnostic kits from Bio-diagnostic (Egypt). The ultra violet (UV) method as described by Goldberg and Spooner (19) was followed.

Superoxide dismutase: Superoxide dismutase was analyzed using diagnostic kits from Bio-diagnostic (Egypt). Superoxide dismutase activity was determined by measuring the inhibition in photo reduction

of nitro blue tetrazolium by superoxide dismutase enzyme and as determined by Kumar, et al. (20).

Catalase: Catalase was analyzed by using diagnostic kits from Bio-diagnostic (Egypt). Catalase activity was measured spectrophotometrically according to the method of Aebi (21).

Leptin: Leptin ELISA kit was purchased from Abcom. (UK). For quantitative measurement of Leptin in the samples, enzyme Linked immure sorbent assay was followed according to the manufacturer's instructions.

Ghrelin: Ghrelin EA kit was obtained from sigma- Aldrich (USA). Enzyme immune assay was used for *in vitro* quantitative assay for detecting ghrelin peptide (22).

Insulin: Insulin was determined by diagnostic kits from Diasorin (Italy) *in vitro* quantitative determination of insulin in the samples was carried on by using chemiluminesence immune assay technology.

Glucose: Glucose diagnostic kit was abstained from Human (Germany). For quantitative determination of glucose in the samples, the enzymatic colorimetric test for glucose as described by Barham and Trinder (23) was followed.

Statistical Analysis: Statistical package for social sciences (SPSS) program version 16.0 was used to obtain all statistical parameters. All analyses were done in triplicate and the results expressed as mean ± standard deviation. Significant differences ($P<0.05$) between treatments and control groups, or rats, were determined by pair sample T-test, and correlations between insulin and Leptin were calculated using Pearson's Test.

Results and discussion

Aqueous extracts of the three hearts of date palm (HDP) cultivars were administered orally to the experimental groups of rats, for eight weeks which was the experimental period. No negative signs regarding animals' growth or health were observed.

Levels of antioxidant enzymes: Levels of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in liver and rat plasma was shown in figure 1 (A-D). Administration of Naboat Saif and Solleg HDP extracts lead to significant increase ($p<0.01$) in CAT level in the liver. The level of CAT for Sukkari HDP was 0.568 U/L/g tissues; a little lower than that of the control 0.614 U/L/g tissue. GPx levels in liver of rats received HDP extracts of Sukkari, Naboat Saif and Solleg were significantly higher ($p<0.01$) compared to the control (Figure 1 A). Rats in the control group have higher level of SOD compared to HDP groups of Sukkari and Naboat Saif ($p>0.05$), and also higher than that of Solleg ($p>0.01$) (Figure 1 B). Some studies have reported increase in antioxidant enzymes activities in rat liver, when these rats received different dietary treatments, e.g. *Hibiscus Sabdarrifa* extract (24), *Azdirachta indica* leaf extract (25) and Gum Arabic extract (26).

Administration of HDP extracts lead to significant increase in CAT values in rat plasma in case of Sukkari ($p<0.01$) and Solleg ($p<0.05$). CAT activity in case Naboat Saif was found to be 0.200 U/L almost similar to control (0.204 U/L). GPx activity increased significantly ($p<0.01$) when Sukkari and Naboat Saif HDP extract was administered to rats and also increased ($p<0.05$) in case of Solleg HDP extract (Figure 1 C). Significant increase in SOD level, in case of Sukkari and Solleg ($p<0.01$), and in case of Naboat Saif ($p<0.05$) has been observed after treatment with HDP extracts (Figure 1 D). It could summarize that the levels of CAT, GPx and SOD in rat plasma significantly increased after administration of

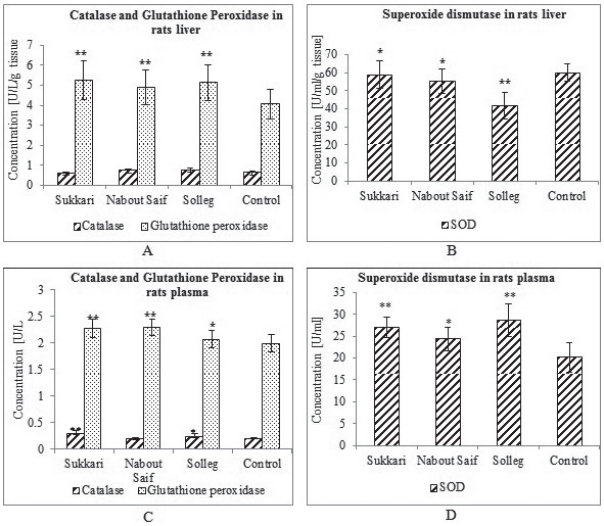

Figure 1. Effect of heart of date palm aqueous extract administration on Catalase, Glutathione peroxidase and Superoxide dismutase level in rat's liver (A-B) and plasma (C-D).

aqueous extracts of the three cultivars. This may due to the capacity of HDP itself as antioxidant, because of its, content of total phenols and flavonoids (4). Date palm seeds steeping treatment results in significant increase in GPx and SOD activities in rat plasma (8). Also another study (27) reported that soy isoflavones supplementation significantly enhances activities of SOD, CAT in liver tissue and serum in exercised rats. Increasing the body levels of antioxidant enzymes by increasing intake of foods rich in antioxidant enzymes is a therapeutic approach to protect the body from oxidative injury and its associated disorders (28).

Levels of hormones: Figure 2 showed the levels of Leptin and Ghrelin in Plasma of rats after administration of HDP extracts. These hormones are involved in regulating appetite and energy balance in human subjects, and rats (29). Treatment with HDP extracts resulted in significant decrease in levels of Leptin in rats receiving Sukkari and Solleg extracts ($p<0.01$) and Naboat Saif extract ($p<0.05$).

Levels of Ghrelin in rats receiving Sukkari extract, increased significantly ($p<0.05$). Contrary to this levels of Ghrelin in rat's plasma receiving Naboat Saif extract (0.661 pg/ml) and Solleg (0.660 pg/ml) were not significantly different compared to the control group (±0.667 pg/ml). It can be concluded that the levels of Leptin decreased and level of Ghrelin increased to some extend in the rats plasma due to HDP extracts treatments.

Type and composition of the diet have significant effects on circulating Leptin and Ghrelin levels in humans (10) and in rats (29). In the same context, Azizi (13) reported that Leptin level and body mass index (BMI) decreased, while Ghrelin level increased in female subjects after 8 weeks aerobic training program. Levels of glucose and insulin in rat's plasma after treatment with HDP extracts are shown in figure 3 (A-B). The levels of random Glucose in rat's plasma after treatment of HDP extracts were significantly increased compared to the control group figure 3A, this may be due to the presence of different amounts of fructose, glucose and sucrose in HDP extracts (4). Treatments of HDP extracts lead to significant decrease in Insulin levels in rats receiving Sukkari ($p>0.01$), Naboat Saif extracts ($p>0.01$), and Solleg extract ($p>0.05$) figure 3B. The decrease in insulin levels was reported after dietary treatment for diabetic rats (9) and decrease in Insulin levels was also observed by Azizi (13) after aerobic physical exercise in humans.

Correlations between insulin and Leptin, in rat's plasma have been reported in Table 1. Insulin and Leptin in levels were positively correlated in case of the three HDP samples, although to different correlation coefficient values. Leptin and Ghrelin are associated with body weight control in case of weight reduction or

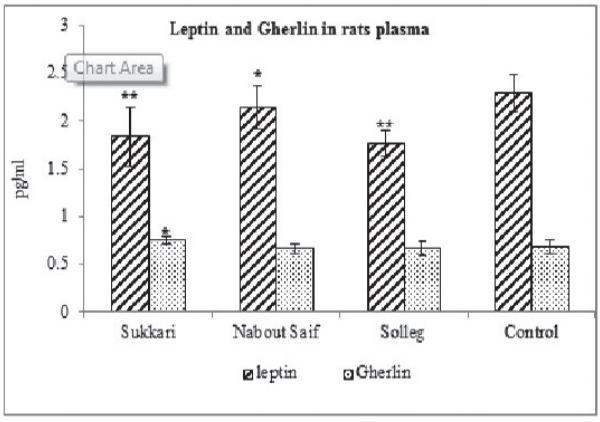

Figure 2. Effect of heart of date palm aqueous extract administration on Leptin and Gherlin level in rat's plasma

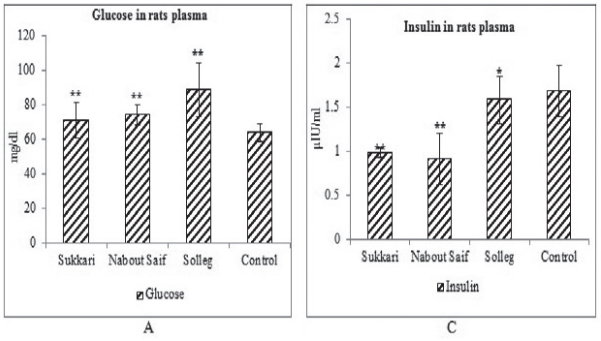

Figure 3. Effect of heart of date palm aqueous extract administration on glucose (A) and insulin (B) in rat's plasma

Table 1: Pearson correlations between insulin and leptin in plasma of the rat after treatment of heart of date palm aqueous extract

Sample (n = 18)	**Leptin x Insulin**
Sukkari	0.279
Naboat Saif	0.834**
Solleg	0.624**
** Correlation is significant at the 0.01 level.	

obesity, also there may be relationship between Insulin resistance and obesity, as study reported that high levels of circulating Leptin and Insulin are characteristics signs of obesity in humans and animals (30). Diet-induced obesity rats when fed on high-energy diet for 10 days gained more body and fat weights and had higher Leptin and Insulin levels (31). The correlation between leptin and insulin in rat plasma as observed in this study was confirmed by Rossetti et al. (32) for rodents and also Antuna-Puente et al. (33) reported that serum Leptin levels were significantly and highly correlated with body weight and Insulin levels in human subjects.

Conclusion

HDP is a secondary product of date palm tree, and is known to be a rich source of minerals and antioxidants. The results of the present study confirmed the significant effect of HDP in increasing of antioxidant enzymes levels in rats; also the results revealed that HDP treatments have significant effects on the levels of the hormones leptin, ghrelin, and insulin in rat plasma. However, the clinical interpretation of these findings to human diseases (e.g. obesity) cannot be made at this stage. Further investigation may be needed to test the potential of HDP as therapeutic agent against nutrition-related disorders in humans.

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