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Reproductive hormonal status of rats treated with date pits

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Abstract

In this study normal and acid-treated powdered date pits were added to the feed of male rats at concentrations of 7 and 14% for 28 consecutive days. The body weight of rats, their feed and water intake, and urine and faecal output were monitored daily. At the end of the trial period, blood plasma was analysed for testosterone, oestradiol and luteinizing hormone (LH) concentrations, and for some indicators of hepatic and renal functions. Sections from some vital organs (liver, kidney, heart, lung, spleen and testes) were histologically examined. The results indicated that treatment with normal date pits (14%) significantly increased the body weight of rats ($p < 0.05$). The other treatments did not significantly affect the body weight. At concentrations of 7 and 14%, the normal date pits significantly increased the concentration of testosterone in plasma ($p < 0.05$), while the acid-treated date pits (14%) slightly but significantly increased the concentration of LH. Plasma oestradiol concentration and the other measured variables were not affected by any of the treatments. Treatment of female rats for 10 days with a lyophilized extract of date pits, or with a polar or non-polar fraction prepared from it, at oral and intraperitoneal doses of 500 mg kg^{-1}, did not significantly affect the body or uterine weights, nor did it affect the degree of vaginal orifice opening. Oestradiol (2 mg kg^{-1}, subcutaneously) increased uterine weight and degree of vaginal orifice opening. Compared to the control, the lyophilized extract and the polar fraction significantly reduced plasma oestradiol concentration by about 25 and 36%, respectively. The non-polar fraction, however, increased the hormone level by about 12%, but this was not significant.

1. Introduction

Inclusion of date flesh and pits in the diet of farm animals (e.g. sheep, chickens and fish) has been attempted by several workers (e.g. Jumah, Al-Azzawi & Al-Hashimi, 1973; Al-Kinani & Alawash, 1975; Al-Hiti & Rous, 1978; Kamel, Diab, Ilian & Salman, 1981; Yousif, Osman & Alhadrami, 1996), but no agreement on its nutritional usefulness emerged. The inclusion of date products has been claimed to increase body weight gain, improve feed efficiency and enhance meat palatability (Rashid & Alawash, 1976). Elgasim, Alyousef, and Humeida (1995) found that date flesh and pits were effective in increasing body weight gain and deposition of back fat of sheep. Furthermore, it was found that the aqueous extract of the date pits induced uterus contraction *in vitro* and increased uterine weight in immature rats, in a fashion similar to that of oestrogens. The presumed beneficial effect of the dates was therefore explained on the basis of the presence of oestrogens in the date flesh and pits.

In the study by Elgasim et al. (1995) no direct hormonal measurements were conducted. In this study, therefore, we measured the concentrations of oestradiol, lutenizing hormone (LH) and testosterone in male rats treated subchronically with two types of powdered date pits (normal and treated with acid to remove fibre coat), and also determined the uterine weights and plasma oestradiol concentration in immature female rats treated with various preparations of date pits and, for comparative purposes, with oestradiol.

2. Materials and methods

2.1. Date preparation

Fresh date pits were obtained locally from the United Arab Emirates University farm. They were crushed, powdered and used immediately in the feeding trials. In some experiments, powdered normal (intact) date pits were used; in others the fibrous coat of the pits was removed by soaking them in 70% H_2SO_4 for 30 min, followed by washing with a continuous jet of tap water

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for 10 min, during which the dates were hand-rubbed against each other to remove the softened coats. Finally the resulting endosperms were finely powdered.

2.2. Chemical composition of date pits

The chemical composition of the date pits was determined (AOAC, 1984) as previously described (Yousif et al., 1996).

2.3. Preparation of date pits extracts

2.3.1. Lyophilized extract

Date pits were coarsely powdered and 200 g was macerated with distilled water (1500 ml) for 6 h with occasional shaking. The extract was filtered and the volume was adjusted to 2000 ml with distilled water. The extract was freeze-dried to give a lyophilized extract (yield 7.0%).

2.3.2. Non-polar fraction

Coarsely powdered date pits (500 g) were extracted with hexane using a soxhlet extractor. The hexane extract was evaporated under vacuum to give an oily yellowish-brown residue (yield 6.55%).

2.3.3. Polar fraction

The marc left after hexane extraction was dried and macerated with distilled water (4000 ml) for 6 h with occasional shaking. The extract was filtered and lyphilized to give a dark-brown extract (yield 4.2%).

2.4. Animals and treatments

Male Wistar rats (220–250 g) and female immature Wistar rats (about 35 g) were obtained from the animal facility of the United Arab Emirates University. The male rats were housed individually in metabolic cages to facilitate measurement of feed and water intake, and urinary and faecal output. Female immature rats were grouped in plastic cages (6 rats/cage). All animals were provided with food (Abu Dhabi Animal Feed Factory) and water *ad libitum*. The feed provided to the male rats was powdered and thoroughly mixed with the date-pit powder at concentrations of 7 and 14% w/w. This feed was given to the rats for 28 consecutive days and on the 29th day the rats were weighed, killed by stunning and decapitation, and their blood collected in heparinized tubes. The blood was centrifuged at 900 ***g*** for 15 min at 5°C. Part of the plasma obtained was stored at −70°C, pending hormonal analysis, and part was analysed immediately for the common clinical parameters. Certain vital organs (liver, kidney, heart, lung, spleen and testes) were inspected visually for any macroscopic changes and portions placed in formol–saline for subsequent histological examination. Female rats were divided into five groups (12 rats/group) and treated with normal saline (2 ml kg^{-1}/6 rats orally and 6 rats intraperitoneally) or with the lyophilized date extract or the polar or non-polar fractions (all at a dose of 500 mg kg^{-1} daily for 10 days). For comparison, a group of rats was injected with oestradiol benzoate (2 mg kg^{-1} subcutaneously) for 10 days. All rats were examined daily for opening of the vagina. Rats were stunned and decapitated 24 h after the last dose. Blood and plasma were obtained as before. The uteri of the rats were excised and weighed. Uteri were then dried to a constant weight in an oven at 100°C and their dry weight determined.

2.5. Hormonal analysis

The concentrations of testosterone, oestradiol and LH in male rats, and that of oestradial in female rats, measured by validated radioimmunoassay (RIA) techniques (kindly carried out by Dr John Pickup, Guy's Hospital, London, UK) The LH RIA kit, which is specific for rats, was obtained from Amersham Life Sciences, U.K. The minimum detectable concentration was 0.8 ng ml^{-1} with an intra-assay coefficient of variation (cv) of 6.5% at 4.7 ng ml^{-1}. The oestradiol and the testosterone RIA kits specific for rats were obtained from Euro DCP. In the oestradiol kit, oestrone has a 10% cross-reactivity. The minimum detectable concentration was 29 pmol per litre with an intra-assay cv of 7.5% at 183 pmol per litre.

2.6. Clinical chemistry

The concentrations of glucose, creatinine, urea, cholesterol, protein and the activities of aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transferase were measured in a clinical analyser (Cobas, Fara, Basel, Switzerland) in plasma.

2.7. Statistical analysis

Values reported are means ± SEM (number of observations). Differences between the group means are evaluated by one-way analysis of variance, followed by Dunnett's test. $p < 0.05$ was considered significant.

Table 1
Composition of major dietary components in date pits

	% Dry matter
Dry matter	91.0
Crude protein	06.1
Crude fat	10.7
Crude fibre	14.4
Ash	2.4
NFE[a]	66.4

Each value is the mean of two analyses.

[a] Nitrogen-free extract = 100−(crude protein + crude fat + crude fibre + ash).

Table 2
The effect of inclusion of date pits in the feed for 28 days on the body and testicular weight of rats

Group	Date-pit concentration	Initial body weight (g)	Final body weight (g)	% Increase	Absolute testes weight	Relative testes weight (as%) body weight
1	Zero (control)	196.2 ± 6.5	272.8 ± 6.5	39.0 ± 2.1	1.49 ± 0.05	0.54 ± 0.03
2	7% normal date pits	180.5 ± 1.8	266.4 ± 7.1	47.6 ± 4.5*	1.49 ± 0.08	0.56 ± 0.05
3	14% normal date pits	180.0 ± 0.8	275.1 ± 8.2	52.0 ± 3.9*	1.61 ± 0.05*	0.59 ± 0.03
4	14% acid-treated date-pits	190.4 ± 0.9	265.9 ± 7.9	39.7 ± 5.0	1.46 ± 0.03	0.55 ± 0.03

Values are means ± SEM; $n = 6$ rats.
Rats were individually housed and given powdered feed mixed with the powdered date pits at different concentrations.
*$p < 0.05$ (compared to control).

3. Results

3.1. Chemical composition of the diet used

The chemical composition of the date pits used is shown in Table 1. The rat basal feed contained crude protein (24%), crude fibre (5%) and ash (8%).

3.2. Effect of date pits on male rats

As shown in Table 2, the treatment of male rats with normal date pits for 28 days at a level of 7 and 14% increased the final body weight significantly by 48.0 ($p > 0.01$) and 52.0 ($p < 0.01$), respectively.

The absolute testicular weight in the group fed with 14% normal date pits was significantly higher ($p < 0.05$) than in the control and other groups. However, there were no significant differences between the different groups in the relative testis/body weight ($p > 0.1$).

The concentrations of testosterone, oestradiol and LH in the plasma of rats treated with date pits are shown in Fig. 1. The testosterone levels in rats given the date pits at levels of 7 and 14% in the feed were about 3 and 5 times that of the control, respectively. The acid-treated date pits increased the testosterone concentration by 50% but this increase was not significant. The concentration of oestradiol was not affected significantly by any of the treatments ($p > 0.1$), nor was the concentration of LH in rats treated with 7 and 14% of the normal date pits. However, the acid-treated date pits (14%) caused a significant increase in the concentration of LH, amounting to 23% ($p < 0.05$).

Treatment with the date pits did not cause significant changes in any of the biochemical plasma parameters measured.

Macroscopic examination of the organs of treated rats revealed no obvious abnormality and there was no evidence of any pathological alterations in the sections examined.

3.3. Effect of date-pit fractions on female rats

Treatment of rats for 10 days with lyophilized date-pit extract or with a polar or a non-polar fraction did

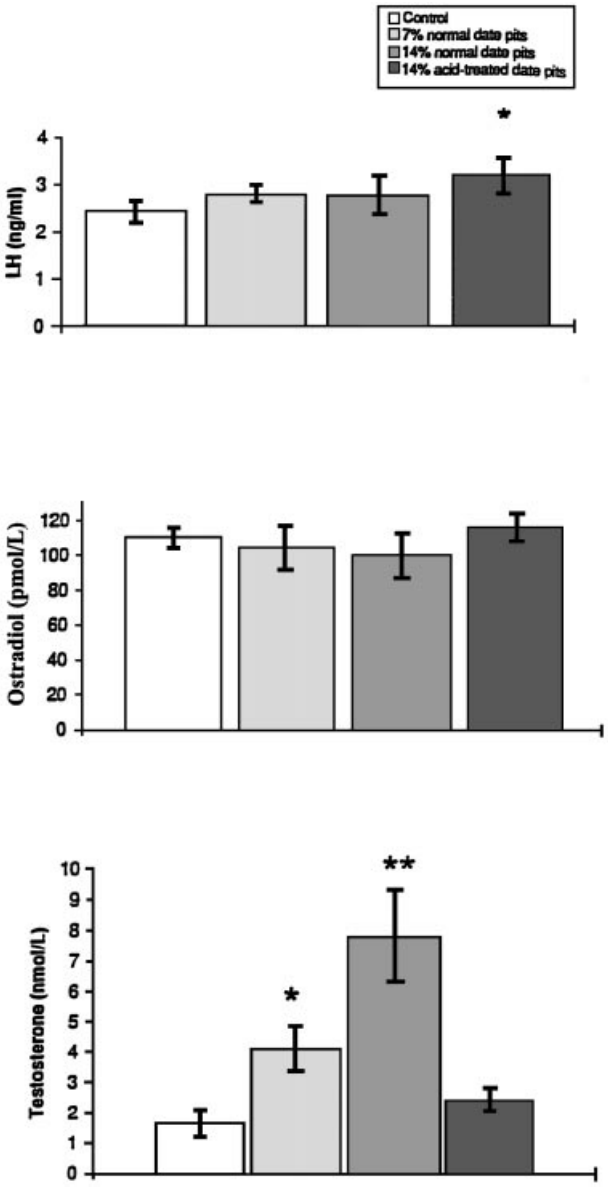

Fig. 1. The concentrations of lutenizing hormone (LH), oestradiol and testosterone in the plasma of male rats treated with normal pits (control), or with 7 and 14% powdered normal date pits or 14% acid-treated date pits added to the diet for 28 consecutive days. The hormones were measured on the 29th day. Each column and vertical bar represent mean ± SEM of six rats. *$p < 0.05$; **$p < 0.01$ (compared to control).

Table 3
The effect of treatment of immature female rats with various date pit extracts

Group	Treatment[a]	Initial body wt (g)	Final body wt (g)	Uterine wt (mg)	Degree of opening vaginal orifice
1	Control[b] (saline, 2 ml kg^{-1})	36.1 ± 0.3	68.3 ± 6.3	38.1 ± 0.9	0/12
2	Lyophilized extract (500 mg kg^{-1}, p.o.)	35.3 ± 0.3	61.6 ± 4.1	37.0 ± 0.9	0/12
3	Lyophilized extract (500 mg kg^{-1}, i.p.)	36.3 + 0.4	67.3 ± 5.4	41.2 ± 2.3	0/12
4	Polar flaction (500 mg kg^{-1})	35.2 ± 0.4	67.5 ± 2.1	46.4 ± 4.3	0/12
5	Non−polar fraction (500 mg kg^{-1})	35.8 ± 0.3	74.9 ± 1.1	37.0 ± 3.9	0/12
6	Oestradiol[c] (2 mg kg^{-1}, s.c.)	36.3 ± 0.4	70.1 ± 4.9	69.3 ± 5.7[d]	10/12
7	Corn oil (2 m kg^{-1}, s.c.)	36.3 ± 0.5	69.3 ± 6.1	38.7 ± 3.2	0/12

Values are means ± SEM $n = 12$ rats.

[a] Intraperitoneal (i.p.) or oral (p.o.) treatment was given to rats in each group daily for 10 consecutive days, and rats were killed 24 h after the last dose.

[b] Six rats were injected with the saline (i.p.) and six were given the saline orally (p.o.) and the results combined.

[c] Oestradiol was suspended in corn oil and injected subcantaneously (s.c.).

[d] $p < 0.05$ (compared to control).

not significantly affect the body or uterine weight or the rate of opening of the vaginal orifice. In contrast, treatment with oestradiol (2 mg kg^{-1}) for 10 days caused a significant increase in the uterine weight ($p < 0.05$) and the rate of opening of the vaginal orifice. The vehicle of the hormone (corn oil) did not have a significant effect on these variables (Table 3). Compared to the oestradiol plasma concentration in the control (77.0 ± 5.2 pmol per litre), the hormone levels in rats treated with the lyophilized extract and the polar fraction were 58.0 ± 5.6 and 49.5 ± 5.7 pmol per litre, respectively ($p < 0.05$). In the rats treated with the non-polar fraction, however, the hormone level was increased, but not significantly, to 86.3 ± 12.5 pmol per litre.

4. Discussion

These results show that the addition of powdered date pits at a concentration of 14% significantly increase the body weight of rats. This finding is in agreement with previous studies in sheep and chickens (Al-Kinani & Alawash, 1975; Elgasim et al., 1995). However, the reason for the weight-enhancing effect is not known. The chemical composition of the powdered datepits showed that they contain only modest amount of protein and fat, which argues against any additional nutritional effect of date pits. Also, the pits were not effective in enhancing the appetite of the treated rats, as the feed intake of these rats was not significantly different from the controls. It is reasonable to assume, therefore, that the weight-increasing effect was as a result of a component(s) in the date pits that improved the feed utilization in the rat. In sheep, the increase in weight after treatment with date flesh and pits was hypothesized to be the result of an oestrogenic effect. However, at least in rats, we did not find any pharmacological (uterus weight) or endocrinological (plasma hormonal measurement) evidence for an oestrogen-like action attributable to date pits.

Unexpectedly, however, we observed a significant increase in the concentration of testosterone in the plasma of rats fed with the normal, but not the acid-treated, date pits. Among other actions, testosterone is well known for its anabolic effect on metabolism, producing a positive nitrogen balance with an increase in the bulk of tissues such as muscles, bone, skin and liver. Although we are not suggesting that the increase in body weight was wholly the result of the increase in testosterone, our findings do indicate that oestrogen may not be involved in the action of date pits on growth, as has been hypothesized by Elgasim et al. (1995). Moreover, anabolic hormone testosterone and testes weight were found to increase significantly following treatment with date pits.

The treatment of date pits with acid to remove the fibrous coat resulted in the loss of the enhancing effects on body weight, testes weight and plasma testosterone concentration. However, a small but significant increase in plasma LH concentration was observed. The reason for this increase is not known and it is unlikely to be of biological significance, considering its small size and any absence of an effect on the other hormones measured. Phytoestrogens may act as antioestrogens. In this study this contention has been confirmed with the lyphilized extract and polar fraction, which caused significant reductions in the plasma hormone levels. It would be of interest to investigate the effect on the reproductive hormonal levels of feeding rats and humans date flesh for different periods.

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