

Therapeutic Effects of Aqueous Extract of Pheonix Dactilyfera on Lead Acetate Induced Sperm Toxicity in Adult Male Wistar Rats

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Abstract: *The therapeutic effects of aqueous extract of Pheonix dactilyfera was evaluated on lead II acetate induced testicular toxicity. Twenty four (24) male adult Wistar rats were used for this experiment and divided into six (6) groups of four (4) Wistar rats each. Group I was assigned as the control group and was administered distilled water; Group II was the toxic group and was administered 120mg/kg per body weight; Group III was the treated group 1 which was first administered the 120mg/kg of lead acetate for 14 days, followed by administration of 1000mg/kg per body weight of date palm for 14 days; Group IV was treated group 2 which was also administered the 120mg/kg of lead acetate and followed by administration of 1500mg/kg of date palm; Groups V and VI were both administered 1000mg/kg and 1500mg/kg of date palm respectively. Groups I, II, V and VI were sacrificed on the 15th day while Groups III and IV were sacrificed on 29th day. There was a significant increase in non motile sperms, non viability of sperm in lead group ($p<0.05$) with non significant increase in abnormal morphology. Date palm treated groups (Groups III and IV) had a non significant increase in normal motility, normal morphology, sperm viability, concentration. Date palm only groups had a significant increase in non motile and non viable cells ($p<0.05$) but with an increase in the weights, normal morphology of sperm cells. Based on the above observations and results, it can be concluded that date palm may have a possible curative effect on lead acetate induced testicular damage.*

Key words: *Pheonix dactilyfera, lead acetate, sperm cells*

I. Introduction

Approximately 15% of couples attempting their first pregnancy meet with failure. Most authorities define these patients as primarily infertile if they have been unable to achieve a pregnancy after one year of unprotected intercourse (Stephen, 2007). Conception normally is achieved within twelve months in 80-85% of couples who use no contraceptive measures, and persons presenting after this time should therefore be regarded as possibly infertile and should be evaluated. Data available over the past twenty years reveal that in approximately 30% of cases pathology is found in the man alone, and in another 20% both the man and woman are abnormal. Therefore, the male factor is at least partly responsible in about 50% of infertile couples (Brugh and Lipshultz, 2004).

Lead is a bright and silvery metal with a very slight shade of blue in a dry atmosphere. Lead is used in virtually all aspects of the environment and common sources include in building construction, as lead-acid batteries, bullets and shot, weights, as part of solders, pewters, fusible alloys, and as a radiation shield (Lide, 2004). Lead affects every one of the body's organ systems, especially the nervous system, but also the bones and teeth, the kidneys, and the cardiovascular, immune, and reproductive systems (White et al., 2007). Chronic high-level exposure has been shown to reduce fertility in males (Golub, 2005).

The role of traditional medicines in the solution of health problems is invaluable on a global level (Krentz and Bailey, 2005). **Date palms** (*Pheonix dactilyfera*) have been cultivated in the Middle East since at least 6000 BC (Copley et al., 2007). It is considered as native to countries around the Arabian Gulf (Chandra et al., 1992). The Palm family is a symbol of prosperity and love to Muslims and its legend dates back to Judeo-Christian mythology (Al Qarawi et al., 2004). Numerous studies involving date palms have shown that date palm possess unique properties such as antioxidant, hepatoprotective, nephroprotective, anti inflammatory, reproprotective effects (Al Qarawi et al., 2004).

II. Materials And Methodology

Plant Material

The date palm fruits were bought in Samaru market, Zaria from the farmers. The date palm fruits were then taken to the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria and were identified in its herbarium unit as date palm fruits. It was given the voucher number 8017.

Chemical

Lead (II) acetate was of analytical standard (Meyer and Baker England) and used for the experiment as the reproductive toxicant. The Lead (II) acetate compound was then taken to the Department of Chemistry, Faculty of Science, Ahmadu Bello University, Zaria for verification and identification.

Extraction Of Date Palm

Phoenix dactylifera fruit was macerated according to the method described by Al-Qarawi et al., 2004, in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

The flesh of the dried Phoenix dactylifera fruit were manually separated from the pits and pounded into powder using laboratory mortar and pestle. About 650g of the powder was soaked in 2 litres of cold distilled water in a conical flask. After 24 hours, the solution (mixture of date palm fruit powder and distilled water) was filtered using filter rag and funnel. The filtrate was allowed to settle for a whole day, followed by decantation of the supernatant.

The supernatant was heated (steamed) to dryness in an evaporating dish (Royal Worcester; made in England) using H-H Digital thermometer Water Bath (Mc Donald Scientific International -22050Hz1.0A international Number) at 60°C.

Experimental Animals

Twenty four (24) male adult rats of Wistar strain within the weights of 110-370g were purchased from the Animal House of the Faculty of Pharmaceutical science and Veterinary medicine, Ahmadu Bello University, Zaria.

The animals were kept and maintained under a standard laboratory condition of temperature, humidity and 12hours light and dark cycle. The animals were fed with standard animal feed pellets and water ad libitum. The animals were allowed to acclimatize for a period of two weeks in the Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria. The animals were grouped into 6 groups (4 animals per group) prior to the commencement of the experiment.

Experimental Protocol

The Group I (Control group) was given only distilled water for 2 weeks (14 days). Group II (Lead group) was given only lead (II) acetate at a concentration of 120mg/kg per body weight for 2 weeks (14 days). Group III was (first) given lead (II) acetate at a dose of 120mg/kg of body weight for 2 weeks (14 days) and was then given aqueous extract of date palm at 1000mg/kg per body weight. Group IV was (first) given lead (II) acetate at a dose of 120mg/kg of body weight for 2 weeks (14 days) and was then given aqueous extract of date palm at 1500mg/kg per body weight. Group V was given aqueous extract of date palm at a dose of 1000mg/kg per body weight while Group VI was given aqueous extract of date palm at a dose of 1500mg/kg per body weight. The rats were weighed before, during (3 days interval) and at the end of the experiment. The experiment (from the above data) lasted for 4 weeks (28 days).

Dilution Ratio

10g of the fruit extract was measured and dissolved in 10mls of distilled water from freshly prepared solution. This resulted in a ration 1:1 dilution of the extract. 1000 mg/kg (low dose) and 1500 mg/kg (high dose) of the extract were administered to the experimental animals per body weight orally using syringes and orogastric tubes for the experimental period.

5g of lead (II) acetate was measured and dissolved in 10mls of distilled water. 120mg/kg of lead acetate was then administered to the experimental animals per body weight for the experiment. This was administered orally through the use of syringes and orogastric tubes. LD₅₀ of lead II acetate is said to be 600mg/kg body weight (Sujatha et al., 2011).

Animal Sacrifice

Before the sacrifice, at the various stages of the experiment, the final weights of the animals were taken and recorded. After the first 14 days, 4 groups of Wistar rats were sacrificed; Groups 1(control), 2(lead group only), 5 and 6 (date palm groups only). On the 14th day, the animals were fasted overnight. Also, after the 28th day, groups 3 and 4 (date + lead) were sacrificed.

The animals were anaesthetized using chloroform and their blood was collected through the jugular vein. Incision was made through the abdomen along the midline down to the pelvic region to remove the testes. The testes collected were used for sperm analysis.

Determination Of Sperm Parameters

The caudate epididymis of the right testes was immediately taken for examination of the respective sperm parameters. Sperm parameters such as sperm motility, morphology, viability and concentration were all evaluated. Sperm motility was evaluated in 3 grades; A (progressive motility), B (non progressive motility) and C (non motility). Sperms were considered morphological abnormal if they had double tails, detached, double and round heads. Sperm viability was considered as the life and death ratio of the sperm cells. Sperm concentration was evaluated and calculated as $10^6/\text{ml}$ of semen.

Statistical Analysis

Data was reported as Mean \pm Standard deviation of mean. One way Analysis of Variance (ANOVA) was statistically used to compare the means with $p < 0.05$ which was deemed statistically significant. Sigmastat 2.0 (Systat Inc, Point Richmond, CA) was used for the statistical analysis.

III. Results

Morphological Studies

Analysis of the weight values of the animals after the experiment across the groups did not show any statistical significance as the $P > 0.05$ when comparing the mean values of the other groups to that of Group I. However, there is increase and decrease in the mean values of the weights across the groups as compared to Group I. There is an increase in the mean values of Groups II, IV and VI as compared to Group I while a decrease in the mean values of Groups III and V occurred as compared to Group I. But the analysis of the weight values of the animals before the experiment showed statistical significant difference as the $P < 0.05$. There was also a decrease from the initial weight of Lead group animals as compared to other groups which had an increase in mean values of weights.

Testicular Weight Of The Animals

Statistical analysis on the testicular weight of the animals was done for both the right and left testes. The results of the analysis for both testes showed no statistical significant difference as $P > 0.05$ ($P_{\text{left testes}} = 0.422$; $P_{\text{right testes}} = 0.400$). However, there was an increase and decrease in the mean values of the weight when compared to the mean values of Group I. The organ-body weight ratio clearly shows that lead has decreased the testicular weight as compared to other groups.

Sperm Parameters

Sperm Motility

The motility of the sperm cells are divided into three (3) grades: A (progressive directional movement), B (non progressive directional movement) and C (non motile movement) written in percentages (%). The analysis shows that the lead group (Group II) had a statistical significant change ($P < 0.05$) across the grades with the exception of the B grade as compared to Group I. Group III had no statistical significant difference as compared to Group I although increase and decrease in mean values were observed. Group IV on the other hand had statistical significant changes across the grades except for the B grade while Groups V and VI had statistical significant differences across the 3 grades ($P < 0.05$).

Sperm Morphology

The analysis of the results from the sperm of the Wistar rats shows a variety of morphology such as some with normal morphology, others with detached head, double head, double tail and round head. From Group II to VI, there was no statistical significant difference in the above parameters as compared to Control. Although, increase and decrease in the mean values of the other groups as compared to Control group was observed across the groups,

Sperm Viability And Concentration

Statistical analysis was also done for the viability, non viability and concentration of the sperm cells of the Wistar rats. Group II showed no statistical significant change in viability and non viability parameters but had a statistical significant change in the sperm concentration ($P < 0.05$). This is also similar with that of Groups III and IV but with Group III having no statistical significant difference in all the parameters. Groups V and VI were observed to have a statistical significant change in all the parameters as compared to the Control group (Group I).

Table 1 Statistical analysis of body weight (g) of the Wistar rats before and after the experiment.

GS	TREATMENT	INITIAL W.	FINAL W	
GS	TREATMENT	A (%)	B (%)	C (%)
I	Distilled water	70.75 ±10.24	19.75 ± 11.09	9.50 ± 3.42
II	LA(120mg/kg)	16.75 ± 5.38*	23.00 ± 11.63	61.25 ±14.20*
III	LA(120mg/kg)+ AFE(1000mg/kg)	49.00 ± 32.53	34.00 ± 22.63	17.00 ± 9.90
IV	LA(120mg/kg)+ AFE (1500mg/kg)	17.00 ± 14.53*	39.00 ± 11.00	44.00 ± 22.54*
V	AFE (1000mg/kg)	27.50 ±28.29*	10.25 ± 3.30*	62.25 ± 25.33*
VI	AFE (1500mg/kg)	10.75 ± 1.71*	8.75 ± 2.06*	82.00 ± 2.94*
I	Distilled water	175.00 ± 73.44	181.25 ± 70.75	
II	LA (120mg/kg)	306.25 ± 24.21	280.75 ± 30.65	
III	LA(120mg/kg) + AFE(1000mg/kg)	157.00 ± 15.13	162.00 ± 11.53	
IV	LA (120mg/kg) +AFE (1500mg/kg)	204.00 ± 48.17	207.00 ± 55.98	
V	AFE (1000mg/kg)	144.75 ± 42.48	164.75 ± 42.04	
VI	AFE (1500mg/kg)	209.75 ± 105.70	221.75 ± 86.21	

LA Lead acetate

AFE Aqueous fruit extract

Table 2 Statistical analysis of testicular weight of the Wistar rats

GS	TREATMENT	LEFT TESTES(g)	RIGHT TESTES(g)	ORGAN:BODY WEIGHT RATIO
I	Distilled water	0.97 ± 0.36	0.92 ± 0.30	0.0054
II	LA(120mg/kg)	1.09 ± 0.18	1.10 ± 0.19	0.0040
III	LA(120mg/kg)+ AFE(1000mg/kg)	0.99 ± 0.37	0.97 ± 0.36	0.0061
IV	LA(120mg/kg)+ AFE (1500mg/kg)	1.31 ± 0.08	1.30 ± 0.03	0.0063
V	AFE (1000mg/kg)	1.06 ± 0.12	1.03 ± 0.14	0.0064
VI	AFE (1500mg/kg)	1.22 ± 0.10	1.04 ± 0.18	0.0055

LA Lead acetate

AFE Aqueous fruit extract

Table 3 Statistical analysis of the motility grades of the sperm cells.

A Progressive motility	*P<0.05
B Non progressive motility	
C Non motility	
LA Lead acetate	
AFE Aqueous fruit extract	

Table 4: Statistical analysis showing the morphology of the sperm of Wistar rats

GS	TREATMENT	DTH(%)	DH(%)	RH(%)	DT(%)	NS(%)
I	Distilled water	9.25 ± 3.10	1.00 ± 0.82	0.50 ± 1.00	0.00 ± 0.58	89.75 ± 3.50
II	LA(120mg/kg)	16.75 ± 4.35	2.25 ± 0.50	3.50 ± 4.36	0.50 ± 0.00	79.75 ± 7.72
III	LA(120mg/kg) + AFE(1000mg/kg)	11.50 ± 7.78	2.00 ± 0.00	1.00 ± 4.24	0.00 ± 0.00	86.50 ± 3.54
IV	LA(120mg/kg) +AFE (1500mg/kg)	13.67 ± 1.53	3.33 ± 2.31	2.00 ± 0.00	0.00 ± 0.00	82.50 ± 4.50
V	AFE (1000mg/kg)	10.50 ± 4.04	0.00 ± 0.00	0.75 ± 0.96	0.00 ± 0.00	87.75 ± 3.59
VI	AFE (1500mg/kg)	15.25 ± 5.62	0.25 ± 0.50	1.25 ± 0.50	0.00 ± 0.00	84.50 ± 5.80

DTH Detached head; DH Double head; DT Double tail; RH Round head; LA Lead acetate
AFE Aqueous fruit extract

Table 5: Statistical analysis showing the viability and sperm concentration

GS	TREATMENT	VIABLE (%)	NON-VIABLE (%)	SPERMCONC (X10 ⁶ /MI)
I	Distilled water	54.50 ± 7.85	46.33 ± 8.50	53.4 ± 5.24
II	LA(120mg/kg)	45.50 ± 9.00	55.50 ± 9.00	48.43 ± 4.81*
III	LA(120mg/kg)+AFE(1000mg/kg)	49.00 ± 7.07	51.00 ± 7.07	50.65 ± 3.47
IV	LA(120mg/kg)+AFE (1500mg/kg)	47.00 ± 2.65	53.00 ± 2.65	36.50 ± 6.94*
V	AFE (1000mg/kg)	34.00 ± 5.10*	66.00 ± 5.10*	46.70 ± 9.68*
VI	AFE (1500mg/kg)	28.00 ± 5.10*	72.00 ± 10.44*	43.33 ± 5.31*

*p<0.05

LA Lead acetate
AFE Aqueous fruit extract

IV. Discussion

In the present study, we observed a significant decrease in the body and testicular weight of the animals treated solely with lead acetate (Group II). This is in tandem with the findings of Nabil et al. (2012). They found out that lead caused a decrease in the growth rate in rats when fed with lead. These effects on bodyweight could be associated with several factors, one of which is an imbalance in the metabolism produced by changing the zinc status in zinc-dependent enzymes that are necessary for many metabolic processes. Along with the decrease in body weight, a non significant reduction in testicular weight was also found in lead acetate-treated animals as compared to the date palm groups. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells. Hence a reduction in its weight might be due to the decreased number of germ cells and elongated spermatids (Chapin et al., 1997). It could also be that lead also has an effect on the hypothalamus of the brain affecting the thirst and hunger centers. This leads to a loss in appetite and therefore reduction in food intake causing weight loss. It was also found that it had a decrease in the mean values of the testicular weight as compared to those of the extract and extract treated groups and this supports the work of Marchlewicz et al (2004). However, the animals treated with date palm and date palm only groups experienced a significant increase in the body and testicular weights of the animals. This could be attributed to the high carbohydrate and fatty contents of the date palm.

Lead acetate group also had a significant statistical increase in non motile cells and decrease in motile cells (progressive and non progressive). This goes in line with the fact that lead acetate can induce infertility by the increase number of non motile sperms. This is in concord with the works of Jensen et al., 2006 and Berry et al., 2002.

However, date palm had a positive effect on the motility, morphology, viability and sperm concentrations in the animals administered to. The group treated with low dose date palm (Group III) had a significant increase in the number of motile and decrease in non motile cells. But, the group treated with high dose of date palm (Group IV) had a non significant increase in number of motile and decrease in non motile

cells. On the overall, date palm extract was able to counter the effect of lead acetate and increased sperm motility but better results were achieved with the low dose (Bahmanpour et al., 2006). Strikingly, the date palm only groups experienced a significant decrease in motile and increase in non motile cells. Several studies conducted on the effects of date palm only on sperm parameters have revealed the dose dependent nature of the extract. El Mougy et al., 1991 used doses such as 500 and 1000mg/kg and discovered that the low dose (500mg/kg) had a much better effect on the sperm parameters than the high dose (1000mg/kg). Bahmanpour et al., 2006 used doses such as 30, 60, 120 and 240mg/kg on sperm parameters but discovered that they had better results with doses 30 to 120mg/kg but had the best results with 120mg/kg. This clearly shows that the lower the dose, the more desirable it is but the higher the dose the less desirable it becomes (dose dependent).

Also, the lead acetate group showed no significant increase in the number of morphological abnormal sperm cells and a decrease in the percentage of normal sperm cells although there was no statistical significant decrease or increase. This is also in support of the work of Berry et al., 2002 and it could be accounted for the fact that lead has the ability to cross the cell membranes to cause structural abnormality (Thoreux et al., 1995). On the other hand, the date palm treated groups and the date palm only groups showed a non significant decrease in the amount of abnormal cells and increase in the number of normal cells as compared to the lead acetate and control groups. This shows that date palm was able to ameliorate the effects of lead on morphology of the cells and is in line with the work of Bahmanpour et al., 2006.

There was also a significant decrease in sperm concentration and sperm viability (non significant decrease) of lead acetate group as compared to the control group. This result is in accordance with Garcia Leston et al., 2010 who suggested that lead would induce a decrease in the levels of sperm concentration and sperm viability. The date palm treated groups (Group III) had a non significant increase in sperm concentration and viability when compared to that of the lead acetate group. This dose was still able to counteract the effects of Lead acetate. However, the group given high dose of date palm (Group IV) had a significant decrease in the sperm concentration. This is an indication that lower doses of date palm exhibit better results than higher doses.

V. Conclusion

From the research conducted, it is clearly evident that lead can serve as a reproductive toxicant in every ramification. Also, aqueous extract of date palm was able to ameliorate the effect of lead acetate induced testicular toxicity. This is evidenced by the improved sperm characteristics with the lower dose of the extract than the higher dose.

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