

Original
Article
Life ScienceAqueous Extract of Date Fruit (*Phoenix Dactylifera*) Protects Testis against Atrazine-induced Toxicity in RatGodson G AKUNNA^{1,*}, Chia L SAALU¹, Oluwaseyi S OGUNMODEDE, Babatunde OGUNLADE¹, Ayomide J BELLO²

ABSTRACT [ENGLISH/ANGLAIS]

The importance of traditional medicine in solving health problems is invaluable on a global level since a lot of side effect is associated with modern medicine. Atrazine (ATZ), a commonly used herbicide in agricultural practice has been reported to cause several health problems. Herein, the ability of aqueous extract of Date fruit (DFEs), to protect the testis against Atrazine induced spermatogenic, and oxidative derangements in rats were evaluated. Sprague-Dawley rats (n=10/group) were divided into control group (soya oil treated), Date fruit extract (DFEs) group (orally with 4 ml/kg bw daily for 16 days), Atrazine (ATZ) treated groups (200 mg /kg bw for 16 days) and Atrazine/DFEs treated groups. Testicular characteristics, sperm parameters, testicular tissue level of lipid peroxidation (MDA), Reduced glutathione (GSH), Catalase activity (CAT), Glutathione peroxidase (GPx) activity and Superoxide dismutase (SOD) activity were estimated in all groups. Atrazine produced a significant decrease in the testicular weight ($p<0.005$), Sperm count, Sperm motility and sperm with normal morphology ($p<0.005$) when compared to control group. It also decreased the activity level of GPx ($p<0.005$), SOD and CAT ($p<0.05$) when compared to control group. Further, a significant increase in testicular lipid peroxidation ($p<0.05$) was observed in Atrazine treated group when compared to control group. Concomitant treatment with Date fruit extract restored the level of SOD, CAT, GPx and reduced the testicular lipid peroxidation. Testicular weight, sperm count, sperm motility and percentage of abnormal sperm were also normal in groups treated with ATZ and DFEs. Moreover, Date fruit extract was capable of reducing the level of lipid peroxidation in the testis. Therefore, the present study suggests that Atrazine exposure can lead to oxidative damage in the testicular tissue in rats and concomitant treatment with Date fruit extract can protect the testis from oxidative damage induced by Atrazine.

Keywords: Atrazine, date fruit, testis, Sprague-Dawley

RÉSUMÉ [FRANÇAIS/FRENCH]

L'importance de la médecine traditionnelle dans la résolution de problèmes de santé est inestimable sur le plan mondial, depuis beaucoup d'effet secondaire est associée à la médecine moderne. L'atrazine (ATZ), un herbicide couramment utilisé dans la pratique agricole a été rapporté pour causer plusieurs problèmes de santé. Ici, la capacité de l'extrait aqueux de fruits Date (DFE), pour protéger contre le testicule atrazine induit la spermatogenèse, et dérangements oxydatif chez des rats ont été évalués. Rats Sprague-Dawley (n = 10/groupe) ont été divisés en groupe de contrôle (l'huile de soja traités), extrait de fruit Date (DFE) groupe (par voie orale avec 4 quotidiens ml / kg de poids corporel pendant 16 jours), l'atrazine (ATZ) les groupes traités (200 mg / kg de poids corporel pendant 16 jours) et l'atrazine / DFE groupes traités. Caractéristiques des testicules, les paramètres du sperme, le niveau de tissu testiculaire de la peroxydation lipidique (MDA), glutathion réduit (GSH), activité de la catalase (CAT), glutathion peroxydase (GPx) et la superoxyde dismutase (SOD) ont été estimés dans tous les groupes. L'atrazine a entraîné une diminution significative du poids des testicules ($p < 0,005$), nombre de spermatozoïdes, motilité des spermatozoïdes et du sperme avec une morphologie normale ($p < 0,005$) comparativement au groupe témoin. Il a aussi diminué le niveau d'activité de la GPx ($p < 0,005$), la SOD et CAT ($p < 0,05$) par rapport au groupe témoin. En outre, une augmentation significative de la peroxydation des lipides testiculaire ($p < 0,05$) a été observée dans le groupe traité lorsque l'atrazine comparativement au groupe témoin. Un traitement concomitant avec de l'extrait de fruits Date de restaurer le niveau de SOD, CAT, GPx et réduit la peroxydation lipidique des testicules. Poids des testicules, de spermatozoïdes, la motilité des spermatozoïdes et du pourcentage des spermatozoïdes anormaux ont également été normale dans les groupes traités avec ATZ et DFE. Par ailleurs, extrait de fruit date a été capable de réduire le niveau de peroxydation des lipides dans les testicules. Par conséquent, la présente étude suggèrent que l'exposition L'atrazine peut conduire à des dommages oxydatifs dans les tissus testiculaires chez le rat et le traitement concomitant avec de l'extrait de fruits de date peuvent protéger les testicules contre les dommages oxydatifs induits par l'atrazine.

Mots-clés: Fruits atrazine, la date, du testicule, Sprague-Dawley

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INTRODUCTION

Atrazine (ATZ) is an organophosphate herbicide commonly used in agricultural practice [1]. . . Animal studies have shown that Atrazine (ATZ) causes body weight loss by interfering with food absorption

mechanism [2, 3] , lead to testicular weight loss and androgen producing organs; likewise, it can diminish sperm counts and motility and cause important changes in testicular tissues as well as reducing the testicular protein concentrations [4]. High level of Atrazine (ATZ)

caused testicular weight loss, reduction in the pituitary weight and secretion of GnRH [5-14].

A study done to ascertain the effect of Atrazine (ATZ) exposure on reproduction indicated an increase in incidence of premature birth in families using Atrazine for farming, babies with low birth weight, and increase in birth defects with incidence of limb reduction was higher [1, 15-17]. Female rabbits treated with Atrazine (ATZ) had smaller litters and more miscarriages than unexposed rabbits, had offspring with low birth weight [18, 19]. . Atrazine (ATZ) decreases the production of interleukin in blood cells [19], enlarges and thickened the valves of the heart [11, 12], exerts a toxic action on the CNS [12].

In vitro studies indicated that Atrazine (ATZ) reduces embryo survival, decreases testosterone production and induces cardiovascular disorders [20-23].

Date palm (*Phoenix dactylifera*) is a member of the monocot family *Arecaceae* called 'Nakhla' [24]. It is well cultivated and considered an important source of food in Middle East and North African countries which is why it is seen as native to countries around the Arabian Gulf [24-28]. The various parts of this plant are widely used in traditional medicine for the treatment of various disorders [29, 30]. The Hausa speaking part of Nigeria use the date palm fruit as a deterrent and astringent in intestinal troubles, treatment for sore throat, colds, to relieve fever, cystitis, gonorrhoea, edema, liver and abdominal troubles and to counteract alcohol intoxication.

Numerous studies has shown that aqueous extract of date (DFEs) exhibits a potent superoxide and hydroxyl radical scavenging activity, inhibits lipid peroxidation and protein oxidation [31]. Date fruit extract (DFEs) contains carbohydrates, alkaloids, steroids, flavonoids, tannins, ascorbic acid, and thiamine, riboflavin, nicotinic acid and vitamin A [32]. Date fruit extract (DFE) has been shown to ameliorate liver damage in rat, suppress swelling and tumors, inhibit growth of *Streptococcus pyogenes* and improve sperm parameters [26, 33-35].

In view of the fact that Atrazine has been shown to induce testicular toxicity in rats, , we therefore aimed at investigating the protective efficacy of aqueous extract of Date fruit (DFEs) on Atrazine-induced testicular derangement in rat.

MATERIALS AND METHODS

Chemical

Atrazine was obtained from the Department of crop protection, Faculty of agriculture, University of Ilorin, Kwara state, Nigeria in the month of September, 2010.

Plant Materials and the Aqueous Extraction Procedure

Date fruit (*Phoenix dactylifera*) was purchased from a local market in Lagos, Nigeria.

The flesh was manually separated from the pits and soaked in cold distilled water and kept for 24 h at a temperature of 4°C [33].

Acute Oral Toxicity Study of Aqueous Extract of Date Fruit

The acute oral toxicity study for DFEs was conducted as earlier described by Saalu et al [36] using the Organization for Economic Cooperation and Development (OECD) (2000) Guidance Document on Humane End points that should lessen the overall suffering of animals used in this type of toxicity test.

Animal Grouping and Experimental Design

Forty male adult (13 to 15 weeks old) Sprague-Dawley rats weighing 195-215 g were used for this study. The animals were randomly divided into four groups of ten rats each such that the average weight difference between and within groups did not exceed $\pm 23\%$ of the average weight of the model population. The first group of animals which served as the control were given 2.5 ml/kg bw of soya oil orally for 16 days. The second group were treated orally with 4 ml/kg body weight of DFEs daily for 16 days [33]. The third group received 200 mg /kg body weight of ATZ dissolved in soya oil for 16 consecutive days [11, 52]. Finally, the fourth group of animals received concomitant treatment of DFEs and ATZ orally for 16 days with both 4 ml of DFEs /kg bw/day and ATZ 200 mg/kg bw dissolved in Soya oil. The appropriate quantity of aqueous extract of date fruit and ATZ were given orally through an orogastric cannula into the stomach via the esophagus [53]. The extract and drugs were administered once daily by 12 noon for 16 days consecutively. All the animals were sacrificed 24 hours after the last dosing. Experimental procedures involving the animals and their care were conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (CCAC, 1985). Further, the animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals [37].

Animal Sacrifice and Sample Collection

The rats were at the time of sacrifice first weighed and then anaesthetized by placing them in a closed jar containing cotton wool sucked with chloroform anesthesia. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. Then the testes were excised and trimmed of all fat. The testes weights of each animal were evaluated. The testes were weighed with an electronic analytical and precision balance (BA 210S, d=0.0001- Sartoriusen GA, Goettingen, Germany). The testes volumes were measured by water displacement method. The two testes of each rat were measured and the average value obtained for each of the two parameters was regarded as one observation. Serum and the testes of each animal were stored at -25°C for biochemical assays.

Determination of Sperm Characteristics

The sperm parameters were estimated as earlier described by Saalu et al [38]. In this study spermatozoon was considered morphologically abnormal (%) if it has a rudimentary tail, round or detached head.

Biochemical Assay

Catalase activity (CAT) was estimated using the method of Aebi [41] as described by Saalu et al (36). Superoxide dismutase activity (SOD) was measured according to the method of Winterbourn et al. [42] as described by Rukmini et al. [43]. Plasma testosterone (TT) was determined using the method described by Saalu et al. [38]. Glutathione (GSH) was determined by the method of Ellman [44] as described by Saalu et al. [36]. Lipid peroxidation (LPO) in the testicular tissue was estimated colorimetrically by thiobarbituric acid reactive substances TBARS method of Buege and Aust [45]. A principle component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. Glutathione peroxidase activity (GPx) was measured by the method described by Rotruck et al.[46].

Statistical Analysis

All data were expressed as mean ± SD of number of experiments (n = 10). The level of homogeneity among the groups was tested using Analysis of Variance (ANOVA) as done by Snedecor and Cochran [47]. Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A value of $p < 0.05$ was considered to indicate a significant difference between groups [48].

RESULTS

Acute Oral Toxicity Studies

There were no deaths of rats dosed 3000mg/kg body weight of the plants extract both within the short and long outcome of the limit dose test of Up and Down method (Table 1). The LD50 was calculated to be greater than 3000mg/kg body weight/orally.

Table 1:

This table shows the results of Acute Toxicity Test for DFEs (Up and Down Procedure) in Rats

Test serial number	Animal Identity	Dose of DFEs mg/kg	Short term result (48hrs)	Long term results (14days)
1	REP	2000	S	S
2	LEP	2000	S	S
3	TC	2000	S	S
4	RLT	2000	S	S
5	LLT	2000	S	S
6	I	2000	S	S

S = Survival; REP = Right ear pierced; LEP = Left ear pierced; TC = Tail cut; RLT = Right leg tagged; LLT=Left Leg tagged, I = Intact rat

Body Weight Changes

Table 2 shows that rats in control and DFEs groups had significant ($p < 0.05$) increase in weight. Both Atrazine-administered groups lost weights when compared with their initial weights. However the weight loss by the Atrazine-alone group of rats was significantly ($p < 0.005$) higher than the losses by the group that received both Atrazine and DFEs.

Weights and Volume of Testes Mean

The testicular weights, testis weight/body weight ratio and volumes of the Atrazine-alone rats were the least, being significantly ($p < 0.005$) lower compared to the mean testicular weights, testis weight/body weight ratio and volumes of the rats treated with Atrazine and DFEs (Table 2).

Sperm Count

As shown in Table 3, the Atrazine-alone group had marked oligospermia with their sperm concentration being significantly lower ($p < 0.005$) compared to the control and DFE-alone groups. The Atrazine with DFEs group, however, showed only moderate oligospermia; the sperm concentration being significantly lower ($p < 0.05$) than the control group.

Sperm Motility

Even though sperm motility of both the Atrazine-alone and Atrazine with DFEs groups were significantly lower ($p < 0.005$ and $p < 0.05$, respectively) compared to the control group, the Atrazine with DFEs group still had a significantly higher ($p < 0.05$) sperm motility than the Atrazine-alone group (Table 2).

Sperm Morphology

The control and DFEs-alone group of rat showed normal sperm morphology. The Atrazine-alone group showed significant ($p < 0.005$) decrease in normal sperm and a significant ($p < 0.005$) increase in abnormal sperm compared to the control and DFEs-alone group (Table 3).

Plasma testosterone (TT)

Figure 1 showed a significant ($p < 0.005$) decrease in the testosterone (TT) level of the ATZ-alone treated group compared to the control and DFEs-alone treated groups. Following co-administration of ATZ and DFEs, the level of TT increase significantly ($p < 0.05$) when compared to the ATZ-alone treated groups.

Activities of testicular enzymes SOD, CAT and GPx

As shown table 4, treatment with DFEs alone caused no significant change in testicular SOD, CAT and GPx activity compared to the control group, however, rats treated with Atrazine alone showed a significant decrease in SOD activity ($p < 0.05$), CAT ($p < 0.05$) activity and GPx activity ($p < 0.005$) when compared to the control group.

Table 2: This table shows the gross anatomical parameters

Anatomical Parameters	Control	DFEs-alone	ATZ-alone	ATZ/BLEs
Initial Body weight (g)	220±3.5	198.4±3.3	217.3±5.1	205.2±3.3
Final Body weight (g)	215.54±2.2	205±4.1	173.5±2.1	190.5±2.1
Bodyweight difference	4.6	6.6	43.8**	14.4**
Testicular weight(g)	1.69±1.6	1.7±5.1	0.3±2.2**	1.02±4.1*
Testicular Volume(mL)	1.65±2.2	1.72±6.1	0.47±2.1**	1.07±0.21*
Testis w.t/B.w ratio	0.008	0.008	0.002**	0.005

* $P < 0.05$, ** $p < 0.005$ compared to control group (n=10).

Table 3: this table shows the sperm parameters

Treatment Groups	Sperm count($\times 10^6$ /ml)	Sperm motility%	Sperm morphology %Normal	%Abnormal
Control	136.7±5.2	95.2±4.2	82.7±1.1	15.7±1.8
DFEs-alone	140±1.2	95.8±0.7	85.4±2.1	10.3±3.1
ATZ-alone	55.6±2.5**	20±1.2**	25.1±1.8**	70.2±2.2**
DFEs/ATZ	109.33±3.1*	70.3±0.1*	69.1±1.13	36.2±1.7**

** $p < 0.005$, * $p < 0.05$ significantly different from control; A sperm is alleged to be morphologically abnormal if it has a detached head or undeveloped tail.

Values are expressed as mean \pm SD for n=10 in each group.

Table 4: Effect of DFEs on biochemical parameters in ATZ exposed rat.

Biochemical Parameters	Control	DFEs-alone	ATZ-alone	ATZ/BLEs
SOD (u/mg protein)	5.82±1.5	5.73±2.3	3.88±2.1*	5.01±3.10
CAT (u/mg protein)	397.81±2.2	395.34±1.10	378.01±4.1*	390.21±6.1
MDA (nmol/mg protein)	21.17±1.7	20.01± 6.1	46.34±2.13*	30.42*
GSH (nmol/mg protein)	8.33±5.1	7.4±1.23	8.25±1.34	9±2.11
GPx (nmol/mg protein)	0.95±2.2	0.9±2.2	0.35±3.21**	0.56±1.3*

* $P < 0.05$, ** $p < 0.005$ compared to control group (n=10).

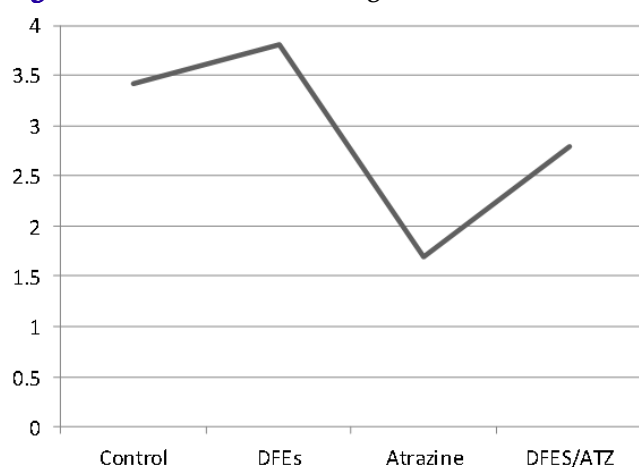
Group treated with Atrazine and DFEs showed significant increase in the testicular SOD activity ($p < 0.05$) and GPx

activity ($p < 0.05$) compared to animals that were treated alone with Atrazine.

Testicular content of Glutathione (GSH) and Malondialdehyde (MDA)

As shown in Table 4, no significant change was observed in testicular GSH activity of all treated groups compared to control animals. The control and DFEs-alone group showed no significant change in testicular content of lipid peroxides expressed as MDA. However, testicular MDA significantly ($p < 0.05$) increased in Atrazine-alone group compared to the control group of rats. After Concomitant administration of ATZ and DFEs, there was a significant reduction in testicular MDA compared to ATZ -alone treated rats.

Figure 6: Plasma testosterone (ng/mL)



DISCUSSION

It has been largely reported that Atrazine -induced organ toxicity could be as a result of its metabolites which may be involved in electron transfer, reactive oxygen species formation, and oxidative stress [49]. The present study was designed to evaluate the protective potential of date fruit extract as an antioxidant-rich nutraceutical on Atrazine-induced testiculotoxicity. Our results herein indicate that administration of Atrazine (200mg/kg /bw. for 16 days) caused body weight loss; decrease in the absolute testicular weights, testicular weight/body weight ratio and testicular volumes of rats. This is in agreement with several reports [2, 3, 6, 13, 50-53] , providing substantial evidences of testicular weight loss and derangement using animal models. Reports on testicular weights in ATZ-induced toxicity have been controversial, as some previous studies reported no differences in weight [2, 9], while others observed significant increase in weight [14, 54]. The observed body weight loss in our study might be due to reduction in the food consumption [2, 55], and the role of ATZ in pituitary weight loss by

degradation which could have lead to reduction in growth hormone and hence body weight loss [2, 13] . On the other hand, animals that were treated with ATZ (200mg/kg /bw. for 16 days) and DFEs (4ml/kg body weight/orally for 16 days) demonstrated largely preserved body weight, testis weights, and testis weight/body weight ratio and testis volumes.

The ATZ-alone group of animals suffered a drop in sperm motility, sperm count and viability. The observed decrease in sperm parameters indicates the effect of ATZ on androgen-secreting capacity [56-58] , hence the observed decrease in testosterone level of ATZ-alone group. The Reduction in plasma testosterone level was corrected following concomitant administration of DFEs (4ml/kg body weight/orally for 16 days).

Lipid peroxidation (MDA), SOD, CAT, GPx and GSH are some of the biomarkers used in investigating tissue damage and oxidative stress [58-60]. In this present study, significant ($p < 0.05$) reduction in the activity level of antioxidant enzymes and testicular content of SOD, CAT and GPx were observed in the ATZ-alone group. There was also a significant ($p < 0.05$) increase in the level of MDA indicating lipid peroxidation but with the activity level of GSH remaining unchanged. Strikingly, co-treatment with aqueous extract of date fruit (4ml/kg body weight/orally for 16 days) significantly ($p < 0.05$) increased the activity level of GPx and reduced lipid peroxidation which is evidenced by significant ($p < 0.05$) reduction in level of MDA.

Reduction in sperm count and motility with an associated increase in the percentage of abnormal sperms is directly related to infertility [53, 61-63]. The ATZ-alone group of animals suffered impaired spermatocytogenesis with a significant ($p < 0.005$) reduction in sperm motility, sperm count and an increase in percentage of abnormal spermatozoa. Our result is consistent with the report of Hayes et al [63]. There has been avalanche of reports that ATZ exerts its organ toxicity by generation of free radicals and reactive oxygen species [49, 64]. Sperm membranes are principally susceptible to oxidative stress due to elevated content of polyunsaturated fatty acids and need for Sertoli cell barrier protection [36, 65].

The initiation of oxidative stress by ATZ might have damaged the sperm membranes, proteins and DNA. Also, the decrease in activity of CAT might have allowed more H_2O_2 to be converted to toxic hydroxyl radicals [66], which could have contributed to severe oxidative damage of the cellular membrane of spermatozoa in atrazine-alone treated animals. These explains the reduced sperm

concentration and sperm motility with accompanying increase in abnormal sperm rates observed in ATZ -alone treated rats. Co-treatment with DFEs containing phytochemicals and other strong antioxidants resulted in a remarkable attenuation of the deranged sperm parameters.

Numerous reports have shown elevation of several antioxidant enzymes and testicular biomarkers as a result of treatment with DFEs [67, 68]. This could provide an explanation for the finding in our study why animals treated concomitantly with DFEs and ATZ showed improved sperm parameters compared to group treated with ATZ-alone. Faizi et al [69] indicated that enhancing the antioxidant system levels can favor reproductive potentials since the sperm cytoplasm contained very low concentrations of scavenging enzymes.

CONCLUSION

Despite avalanches of data relating Atrazine to reproductive toxicity, little is known about the molecular mechanisms of action. However, aqueous extract of Date fruit (*Phoenix dactylifera*) was able to ameliorate the effect of Atrazine testiculotoxicity and this is evidenced by the improved sperm characteristics, testicular oxidative enzymes and reduction in lipid peroxidation.

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CONFLICT OF INTEREST

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