

AMELIORATIVE EFFECTS OF ADANSONIA DIGITATA LEAF EXTRACT ON CARBON TETRACHLORIDE (CCl₄) INDUCED TESTICULAR TOXICITY IN ADULT MALE WISTAR RATS

Oyewopo A. Oyetunji, Ibrahim R. Babatunde, Saalu L. Chia, Osinubi A. Abraham, Adewale F. Benard, Eweoya Olugbenga, Williams F. Esemekiphoraro

Correspondence to: Ibrahim, Ridwan Babatunde Department of Anatomy, Kampala International University, Ishaka, Uganda Tel no: +256751437502. Email: geniusridwan@gmail.com.

ABSTRACT

Adansonia digitata is locally consumed as food in Nigeria. In the present study, the ameliorative effect of the aqueous leaf extract of *Adansonia digitata* (AeAD) was evaluated in carbon tetrachloride (CCl₄)– induced testicular toxicity in Wistar rats. To evaluate the effect of AeAD in CCl₄ induced testicular toxicity, 20 adult male Wistar rats were equally divided into 4 groups (n=5). Group A animals received 1 ml olive oil *per os* (p.o) for two weeks, Group B animals received 2.5 ml/kg CCl₄ (50% in olive oil, p.o) for two days, Group C animals received 500 ml/kg AeAD (p.o) for two weeks while Group D animals received 2.5 ml/kg CCl₄ (50% in olive oil, p.o) for two days followed by 500 ml/kg AeAD (p.o) for two weeks. The ameliorative effects of AeAD were observed on reproductive hormonal parameters, activity of an antioxidant enzyme and cyto-architecture of the testis. Carbon tetrachloride treatment significantly (P<0.05) reduced levels of testosterone, follicle stimulating hormone, luteinizing hormone and superoxide dismutase levels with distortions in the cyto-architecture of the testes in treated animals. These effects were ameliorated with AeAD treatment. The results demonstrated that the AeAD has the ability to ameliorate against carbon-tetrachloride-induced testicular toxicity suggesting it may have a therapeutic role in free radical mediated diseases.

Keywords: Antioxidant; SOD; Testes; Histology.

INTRODUCTION

Certain aspect of man's well-being and some forms of life depend directly or indirectly on plants. The medicinal properties of some plants used locally have been scientifically proven over the years to be potent in curing different prevailing diseases and ailments (Sofowora, 1993). *Adansonia digitata* (Baobab) is a tropical plant of African origin belonging to the *Malvaceae* family (Beckier, 1983). The baobab (*Adansonia digitata* Linn) is important to the livelihood of the people in arid zones of Africa (Beckier, 1983). In Nigeria, the Hausas and Yorubas commonly refer to the tree as Kuka and Ose respectively. Its stem-bark is regarded as a 'heart tonic' with diuretic properties (Ashorobi and Joda, 1998). Its immunostimulant (El-Rawy et al., 1997), hepatoprotective (Al-Qarawi et al., 2003), anti-inflammatory and analgesic (Ramadan et al., 1994) effects have been reported.

Carbon tetrachloride (CCl₄) is an industrial solvent that causes multiple organ damages (Abraham et al., 1999). It triggers oxidative damages through free radicals production (Bruckner et al., 2002). The role of oxidative stress in CCl₄-induced testicular toxicity have been reported (Khaki et al., 2011; Al-Olayan et al., 2014)

Free radicals from CCl₄ bind to polyunsaturated fatty acid (PUFA) of the sperm membrane producing alkoxy and peroxy radicals that generate highly reactive lipid peroxides that changes sperm concentration, reduces enzyme activity, alter hormonal levels and causes degeneration in the seminiferous tubules (Sikka et al., 1995; Ogeturk et al., 2005; Guo et al., 2005).

Sexual dysfunction in males characterised by low testosterone levels, reduced sperm

concentration and motility have been implicated in chronic alcoholism, drug abuse and exposure to toxic chemicals (Brock et al., 2002; Khaki et al., 2011). Currently, medicinal plants are being investigated as a source of alternative medicine against chemical induced

oxidative stress. The present study was carried out to access the ameliorative effect the aqueous leaf extract of *Adansonia digitata* (AeAD) will have on CCl₄-induced testicular toxicity in adult Wistar rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing between 130-145g were obtained from Ladoké Akintola University of Technology. The rats were housed at the animal house of the College of Health Sciences, University of Ilorin, Nigeria, kept in ordinary cages at room temperature of 24 ± 3°C under a 12 h dark/ light cycle. They were fed with standard rat pellets and allowed access to water. Study was performed in accordance with the ethical guidelines stipulated by the ethical committee of the College of Health Sciences, University of Ilorin, Nigeria. These guidelines were in accordance with the internationally accepted principles for laboratory animal use and care.

Plant collection and the extraction

The plant material (*Adansonia digitata* leaves) was collected at oke-ose area in Ilorin, Kwara state Nigeria. Identification and authentication was done by Mr. Bolu Ajayi of the Department of Plant biology, University of Ilorin, Nigeria. A voucher with specimen number UIH/951 was deposited at the herbarium in the department of plant biology, University of Ilorin, Nigeria. The leaves were air-dried and extracted in accordance with the methods of Nwafor *et al.*, (1996) with slight modifications. The dried leaves were pulverized by mortar and pestle into powdery form. The powder was sieved to obtain a fine powder. 2 kg powder was soaked in distilled water for 48 h and stirred intermittently. After 48 h the extract was filtered with a white clean cloth and the filtrate was evaporated till dryness under a regulated temperature (37°C).

Experimental design

Twenty adult male Wistar rats were equally divided into four groups of 5 rats each. Single dose treatment of animals was carried out follows:

- Group A (control) animals were orally administered with 1 ml olive oil (p.o) for two weeks.
- Group B animals were administered 2.5 ml/kg CCl₄ (50% in olive oil; p.o) for two days.
- Group C animals were administered 500 ml/kg AeAD (p.o) for two weeks.
- Group D animals were administered 2.5 ml/kg CCl₄ (50% in olive oil; p.o) for two days followed by subsequent administration of 500 ml/kg AeAD (p.o) for two weeks.

Sacrifice

24h post treatment, animals were weighed and exposed to chloroform inhalation (in a closed jar). Blood was collected into heparinized tubes and centrifuged at 3000 rpm for 15 min using a bench centrifuge. Plasma was stored at 4°C until analysed. The peritoneal cavity was opened through a lower transverse abdominal incision to expose the reproductive organs. The testis were dissected and cleared free of the surrounding tissue. The left testis were weighed independently and fixed in 10% formol saline for histological examination while the right testis were weighed and homogenised for tissue antioxidant assay.

Serum analysis of hormone

Serum level of testosterone (TT), luteinizing hormone (LH) and follicle stimulating

hormone (FSH) was estimated using the enzyme-linked immunosorbent assay (ELISA) kits from Fortress diagnostics.

Assessment of Superoxide dismutase (SOD) level

Testicular tissues were transferred into 5 ml ice-cold sucrose solution (0.25 M) and homogenized. The homogenates were further centrifuged at 3000 rpm for 15 min to obtain the supernatant, which was then aspirated with Pasteur pipette into sample bottle, stored overnight at 4°C before being used for assays. Tissue activities of superoxide dismutase (SOD) were determined by the method of Marklund and Marklund, (1974).

Histological studies

Body and testicular weights

The body weights of the animals increased proportionally in all four groups (Table 1) with the control group gaining most weight. The testicular weights of animals exposed to CCl₄ alone decreased (P<0.05) when compared to other groups (Table 1).

Serum hormonal levels

The effects of AeAD on follicle stimulating hormone, luteinizing hormone and testosterone are shown in Table 2. CCl₄ administration in rats significantly reduced (P<0.05) levels of testosterone, luteinizing hormone and follicle stimulating hormone compared to control group. Alterations in both testosterone and luteinizing hormone was significantly reversed (P<0.05) by administration of 500 mg/kg AeAD in CCl₄ treated rats. No significant reversal in altered level of FSH was seen following administration of 500 mg/kg of AeAD in CCl₄ treated rats.

Effects of AeAD on testicular superoxide dismutase level following CCl₄ administration.

The left testes from each group were fixed in 10% formol saline for 48 h. The organs were dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin and sectioned. Testicular tissue sections were stained in haematoxylin and examined at magnifications of X400 under a light microscope.

Statistical Analysis

Data were analyzed by Microsoft Excel program for windows software. Results are expressed as mean ± standard error (SEM) and subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test. The significant level considered was p < 0.05.

RESULTS

The effect of AeAD treatment on testicular superoxide dismutase (SOD) level following CCl₄ administration is shown in Table 3. Activities of SOD were significantly (P<0.05) reduced following CCl₄ treatment when compared to control group. This reduction was improved significantly (P<0.05) by post-administration of AeAD at 500 mg/kg near to control rats. However, no significant changes (P<0.05) in SOD levels were found in the administration of AeAD alone compared to the control group.

Histological Observation

Histologically, there are varying observations in the testes of the experimental animals compared to the control group. Administration of CCl₄ caused reduction in spermatogenic cells with sparsely populated Leydig cells (Fig. 2). Oral treatment with AeAD ameliorated testicular changes induced by CCl₄ treatment (Fig. 4). The administration of AeAD alone to animals (Fig. 3) has no adverse effect on the histology of the testis when compared to control group (Fig. 1).

Table 1: Effects of AeAD on body and testicular weights in male Wistar rats exposed to CCl₄.

Groups	Final weight (g)	Initial weight(g)	Weight difference (g)	Testicular weight (g)	Testicular-Body weight ratio
A: Control	181.08± 5.67	142.10 ± 3.07	38.98 ++	1.15 ±0.15	0.0064
B: 2.5 ml/kg CCl ₄	144.16 ± 3.25	123.46 ± 3.56	20.7	0.80± 0.80*	0.0055
C: 500 ml/kg AeAD	165.36 ± 8.1	143.82 ± 8.08	21.54	1.00 ±0.00	0.0060
D: 2.5 ml/kg CCl ₄ + 500 ml/kg AeAD	162.0 ± 3.49	128.48 ± 6.24	33.52	1.05 ± 0.05	0.0065

Mean ± SEM (n=5)
 ++ indicate significance from CCl₄ and extract treated groups
 * indicate significance from the control group at P<0.05

Table 2: Effect of AeAD on FSH, LH and testosterone levels in male Wistar rats exposed to CCl₄.

Treatment	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)
Control	0.0690±0.01 ⁺⁺	0.4950± 0.03 ⁺⁺	2.5380±0.20 ⁺⁺
2.5 ml/kg CCl ₄	0.0200±0.01*	0.0300 ± 0.0*	2.0170±0.00*
500 ml/kg AeAD	0.0935±0.01 ⁺⁺	0.3500 ± 0.08 ⁺⁺	2.5515±0.13 ⁺⁺
2.5 ml/kg CCl ₄ + 500 ml/kg AeAD	0.0440±0.01	0.0825 ± 0.03*	2.4185±0.40 ⁺⁺

Mean ± SEM (n=5). * Indicate significance from the control group at P<0.05; ++ Indicate significance from CCl₄ group at P<0.05.

Table 3: Effects of AeAD on testicular superoxide dismutase activity in male Wistar rats exposed to CCl₄.

Animal grouping	SOD (U/mg protein)
Control	65.0±10 ⁺⁺
2.5 ml/kg CCl ₄	26.5±1.5*
500 ml/kg AeAD	68.0±6.0 ⁺⁺
2.5 ml/kg CCl ₄ + 500 ml/kg AeAD	64.0±4.0 ⁺⁺

Mean ± SEM (n=5). * indicate significance compared to control (p<0.05). ++ Indicate significance from CCl₄ group at P<0.05.

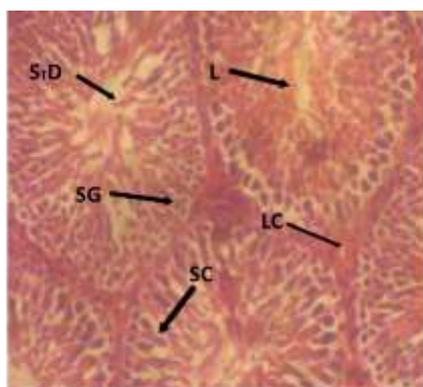


Figure 1: Photomicrograph of the transverse section of testis in the control group showing normal cross section of the seminiferous tubules (ST), with stratified epithelium consisting of spermatogenic cells (SG); spermatid (S₁D); moderate lumen (L); normal interstitial connective tissue (ICT) present consisting of Leydig cells (LC) and Sertoli cells (SC). H&E x 400.

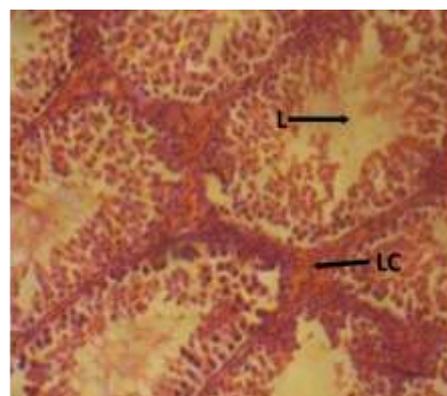


Figure 2: Photomicrograph of the transverse section of testis in the CCl₄ group showing closely packed seminiferous tubules with a wider lumen (L), reduction in spermatogenic cells, and the Leydig cells (LC) were sparsely populated. H&E x 400.

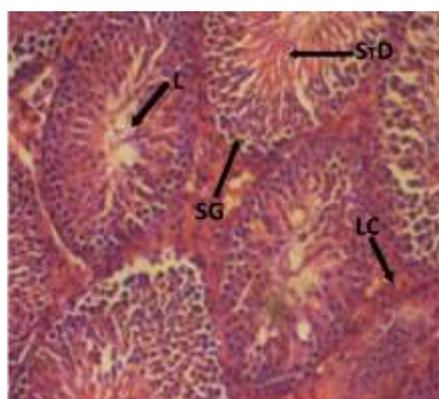


Figure 3: Photomicrograph of the transverse section of testis in the extract (500 mg/kg AeAD) group showing the stroma consisting of interstitial Leydig cells (LC), seminiferous tubule consisting of spermatogenic cells (SG), spermatid (S₁D) and a closer lumen (L). H&E x 400.

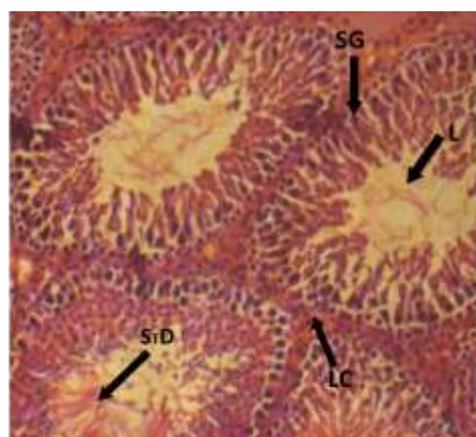


Figure 4: Photomicrograph of the transverse section of testis in the CCl₄ + 500 mg/kg AeAD group showing a seminiferous tubule with a closing lumen (L) and spermatogenic cells (SG), sertoli cells (SC), spermatids (S₁D) and less sparsely Leydig cells (LC). H&E x 400.

DISCUSSION

Previous reports have established the induction of oxidative stress in animal models as a result of increased free radicals production and/or decrease in antioxidant defence capacity (Sahreen et al 2013).

Carbon tetrachloride induced injuries reduces antioxidant enzyme and their substrates to induce oxidative stress, which is key in both

acute and chronic injuries seen in various tissues (Szymonik-Lesiuk et al., 2003).

Herbal remedy originating from plants and plant extracts is an alternative form of medicine that has been around for centuries. It is usually the first line of remedy in rural communities. *Adansonia digitata* possess bioactive components, which play important

role in protection in rats (Al-Qarawi et al., 2003). Infertility, which is a major cause of broken marriages, has been linked to testicular oxidative stress suggesting the need to develop potent antioxidant therapies for this condition (Turner and Lysiak, 2008).

It has been reported that CCl₄ cause reduction of reproductive hormonal levels of follicle stimulating hormone, luteinizing hormone and testosterone in male rats (Khan, 2012). The result of the present study revealed that AeAD significantly improved reproductive hormonal levels of testosterone and luteinizing hormone, which were altered following CCl₄ exposure.

Antioxidant enzymes play essential role in oxidative infertility. Overproduction of oxygen radicals and/or decreased efficiency of the antioxidant system results in oxidative stress (Khan, 2012). Accumulation of free radicals in testicular tissues leading to reduction of antioxidant enzymes and enhanced lipid peroxidation following CCl₄ administration has been reported (Khan and Ahmed, 2009). From our study, treatment of animals with 2.5 ml/kg CCl₄ caused significant reduction in the activity of antioxidant enzyme SOD. Improvement of testicular SOD levels in rats treated with *Adansonia digitata* extracts in comparison to CCl₄ exposed rats suggests the antioxidative properties of the plant. These antioxidative effects may be due to the

presence of α -myrin, β - sitosterol, β -amyryn palmitate (Al-Qarawi et al., 2003) that may possess different functional properties such as scavenging reactive oxygen species and inhibition of the generation of free radicals.

Histological examination of the testes revealed that CCl₄ treatment causes the seminiferous tubules to be closely packed with wider lumen, reduction in spermatogenic cells and sparsely populated Leydig cells; however treatment with *Adansonia digitata* showed noticeable improvement in the histological changes induced by CCl₄. These histological changes seen in the testes of animals exposed to CCl₄ are in agreement with Khan and Ahmed (2009) who reported alterations in the seminiferous tubules and reduction in spermatogenic cells in rats following CCl₄ administration.

In conclusion, our data indicated that AeAD is useful in managing reproductive hormonal dysfunction in male. Further work towards the isolation of its bioactive constituents responsible for these activities is advised.

Acknowledgements: The authors acknowledge Mr Bolu Ajayi for identification and authentication of *Adansonia digitata* leaves.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

1. Abraham P, Wilfred G, Cathrine SP. 1999. Oxidative damage to lipids and proteins of the lungs, testis and kidney of rats during CCl₄ intoxication. *Clinical Acta* 289:177–179.
2. Al-Olayan E, El-Khadragy MF, Othman MS, Aref A, Kassab R, Abdel Moneim AE. 2014. The potential protective effect of *Physalis peruviana* L. against carbon tetrachloride-induced hepatotoxicity in rats is mediated by suppression of oxidative stress and downregulation of MMP-9 expression. *Oxid Med Cell Longev*. Article ID 381413.
3. Al-Qarawi AA, Al-Damegh MA, El-Mougy SA. 2003. Hepatoprotective influence of *Adansonia digitata* Pulp. *Journal of Herbs, Spices & Medicinal plants* 10(3): 1-6
4. Ashorobi RB, Joda AO. 1998. Positive inotropic effect of the extract of *Adansonia digitata* (Linn) on isolated atrial muscle of the rat. *Discovery and Innovation* 10: 3-4.
5. Beckier B. 1983. The contribution of wild plants to human nutrition in the Ferlo (Northern Senegal). *Agroforestry-Systems* 1(3): 257-267.
6. Brock GB, McMahan CG, Chen KK. 2002. Efficacy and safety of tadalafil for the treatment of erectile dysfunction, results of integrated analysis. *J Urol* 168:1332–1336.

7. Bruckner JV, Ramanathan R, Lee KM, Muralidhara S. 2002. Mechanisms of circadian rhythmicity of carbon tetrachloride hepatotoxicity. *J Pharmacol Exp Therap* 300:273–281.
8. El-Rawy M, Eman SM, Gergis SB and ElMougy SA. 1997. The immunostimulant effect of *Adansonia digitata* on the immune response of chicken vaccinated with avian cholera vaccine. *Journ. Egypt. Veterinary Med Asso.* 57: 959-970.
9. Guo C, Lu Y, Hsu GSW. 2005. The influence of aluminium exposure on male reproduction and offspring in mice. *Environ Toxicol Pharmacol* 20:135–141.
10. Khaki A, Fathiazad F, Nouri M, Khaki AA, Ghanbari Z, Ghanbari M, et al. 2011. Anti-oxidative Effects of Citro_flavonoids on Spermatogenesis in Rat. *Afr J Pharm Pharmacol* 5(6):721–725.
11. Khan MR, Ahmed D. 2009. Protective effects of *Digera muricata* (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. *Food Chem Toxicol* 47:1393–1399.
12. Khan RA. 2012. Protective effects of *Launaea procumbens* on rat testis damage by CCl₄. *Lipids in Health and Disease* 11:103.
13. Marklund S and Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.* 47: 469-474.
14. Nwafor PA, Effraim K D and Jacks T W. 1996. Gastroprotective effects of aqueous extract of *Kaya sinegalensis* on indomethacin induced ulceration in rats. *West African journal pharmacol. Drug. Res.* 12: 45-50.
15. Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M. 2005. Caffeic induced nephrotoxicity and protective effect of betaine in SpragueDawley rats. *Urology* 62:353–356.
16. Ramadan A, Harraz FM, and ElMougy SA. 1994. Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of *Adansonia digitata*. *Fitoterapia* 65: 418-422.
17. Sahreen S, Muhammad RK, Rahmat AK, Naseer AS. 2013. Effect of *Carissa opaca* leaves extract on lipid peroxidation, antioxidant activity and reproductive hormones in male rats. *Lipids in Health and disease* 12:90
18. Sikka SC, Rajasekaran M, Hellstrom WJ. 1995. Role of oxidative stress and antioxidants in male infertility. *J Androl* 16(6):464–468.
19. Sofowora AE. 1993. Medicinal plants and traditional medicine in Africa. 2nd ed. Ibadan: Spectrum Book Ltd, p 2.
20. Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Słomka M, Madro A, Celin´ski K, et al. 2003. Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. *J Hepato-Biliary-Pancreatic Surg.* 10:309–315.
21. Turner TT, Lysiak JL. 2008. Oxidative stress: A common factor in testicular dysfunctions. *J Androl* 29:488–498.