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SODIUM ARSENITE INDUCED REPRODUCTIVE PERTURBATIONS IN WISTAR STRAIN ALBINO RATS: PROTECTIVE ASSESSMENT OF *CASSIA FISTULA*

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Key words. *Cassia fistula* . Livability . Phytochemical screening . Sodium arsenite

Abstract

Arsenic is a naturally occurring ubiquitous toxic compound. This study investigated the possible sperm protective potential of leaf extract of *Cassia fistula* Linn (Fabaceae) against sodium arsenite induced reproductive damage reflected as reduced sperm motility, livability and concentration. Sixty-four adult male wistar rats (180 to 220g) were used. The rats were separated into 8 groups (A-H) of eight rats each treated for 60days with 0.2ml corn oil (A), 2.5 mg/kg^{-1} body weight sodium arsenite (SA) (B), pre-administered 100mg/kg^{-1} *Cassia fistula* and 2.5mg/kg^{-1} SA (C), 100mg/kg-1 *Cassia fistula* (D), pre-administered 200mg/kg^{-1} *Cassia fistula* and 2.5mg/kg^{-1} SA (E), 200mg/kg-1 *Cassia fistula* (F), pre-administered 300mg/kg^{-1} *Cassia fistula* and 2.5mg/kg^{-1} SA (G), 300mg/kg^{-1} *Cassia fistula* (H). Phytochemical screening of *Cassia fistula* extract revealed the presence of carbohydrates, alkaloids, anthraquinones, cardenolides, tannins, saponins, phenols and steroids. Group G rats had a significant ($P<0.05$) decrease mean right testicular weight compared to the rest of the groups. There was a significant ($P<0.05$) decrease in sperm motility of groups B, C, E, G and H compared to groups A, D and F rats. The mean sperm count obtained across the groups also followed similar trend. Arsenic exposure led to a significant increase in sperm abnormalities and testicular damage evidenced by vacuolation of secondary spermatocytes, loss of spermiogenic epithelium. In conclusion, *Cassia fistula* at a dose of 100mg.kg^{-1} was found to attenuate Arsenic-induced testicular damage and sperm abnormalities. *Cassia. fistula* may therefore represent a potential therapeutic option to protect testicular tissues against arsenic intoxication.

Introduction

Arsenic exposure is a major environmental pollutant with multiple toxic effects. Arsenic in ground water is the major source of arsenic intoxication (Adnan, 2010). Many systems within the body are affected by inorganic arsenic exposure. Some of these toxic effects range from skin lesions, cardiovascular, haematological, hepatic, reproductive and renal defects (Adnan, 2010). Previous studies also revealed that several antioxidants agents protected against tissue damage due to arsenic intoxication (Harbone,1984; Gupta and Raina,1998).

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Cassia fistula Linn is a plant, which belongs to the family Fabaceae. Common names include Golden shower, Indian laburnum, and Purging cassia among others. The plant is native to India, the Amazon, and Sri Lanka but is now widely cultivated worldwide as an ornamental tree due to its beautiful showy yellow flowers and medicinal use (Patel *et al.* 1965).

Cassia fistula is used extensively in various parts of the world against a wide range of ailments, the synergistic action of its metabolite production being most probably responsible for the plant's beneficial effects. The plant has a high therapeutic value and it exerts an antipyretic and analgesic effect (Patel *et al,* 1965). The extract of the plant is used as an antiperiodic agent and in the treatment of rheumatism (Biswas and Ghosh, 1973).

Seed diet of the plant produced marked hypoglycaemic effect on normal albino rats but caused no hypoglycaemic effect on alloxan induced diabetic albino rats (Singh, 1975). Wound healing potential of the plant in the rats has been reported by Senthilkumar (2005). The plant is very effective in the treatment of intestinal disorders like Ulcer (Biswas and Ghosh, 1973).

Aqueous and methanol extracts of the bark were found to possess significant anti-inflammatory effect in both acute and chronic models (Ilavarasan, 2005). The plant has antibacterial action against *E. coli, B. mycoides, B. subtilis, Mycobacterium smegmatis, Klebsiella aerogenes, Pseudomonas aerogenes and Proteus vulgaris* (Perumal *et al.* 1998; Ali, 2003).

Methanol extract of the leaves was found to possess significant antifungal activities against three pathogenic fungi like *Microsporum gypseum, Trichophyton rubrum* and *Penicillium marneffei* (Souwalak Phongpaichit, 2004). Methanol extract of the seed has been reported to decrease the tumour volume and viable tumour cell count in the Ehrlich ascites carcinoma tumour hosts (Gupta and Raina, 1998).

A review on the plant indicated the hepato-protective, wound healing and antioxidant properties which were attributed to the presence of total phenolic, proanthocyanidin and flavonoid content of the plant. (Moshahid, 2009). The hepatoprotective effects were reported by Perumal *et al* (1998) by Adnan *et al.* (2010).

Cassia fistula leaf extract has been used in humans and animals as treatment for various conditions but there is paucity of research reports on its sperm protective potential in rats exposed to sodium arsenite toxicity. Therefore, this study was conducted to evaluate the sperm protective potential of the ethanol leaf extract of *Cassia fistula* in arsenic-treated wistar strain albino rats.

Materials and Methods

Chemicals

Sodium arsenite (Ioba, Chemie Co. India) was dissolved in glass-distilled water. A dose of 2.5mg/kg^{-1} body weight (equivalent to the 1/10th LD_{50} of $NaAsO_2$) was administered according to the guidelines for *in vivo* assays in rats (Preston, 1887).

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Freshly prepared stock solution was used for the experiment.

Plant materials

Fresh leaves of *Cassia fistula* were collected from the Department of Agronomy, University of Ibadan, Nigeria. The plant was identified and authenticated with voucher no: UIH-22396 at the Department of Botany, University of Ibadan, Nigeria. The voucher specimen of the plant is preserved at the herbarium in the University of Ibadan.

Plant extract preparation

The collected fresh leaves of *Cassia fistula* plant were air-dried under shade and powdered. The dry powder particles were soaked in hexane for about twenty-four hours to de-fat the leaves. The hexane-soaked ground particles were sieved to recover the particles then subsequently air-dried. When fully dried, the particles were soaked in 70% ethanol for 72 hours. The soaked particles were removed and the supernatant was removed. The filtered solution was then passed into rotary evaporator at 40°C for drying.

Phytochemical screening and plant extract

The leaf extract of *Cassia fistula* was chemically tested, using standard methods (Harbone,1984; Evans 2002) for the presence of carbohydrates, proteins and amino acids, alkaloids, anthraquinones, cardiac glycosides, tannins, flavonoids, saponins, phenols and steroids.

Experimental animals

Sixty-four adult male Wistar strain albino rats, weighing 180-220g, were obtained from the Experimental Ani House of the Department of Veteri Physiology, Biochemistry Pharmacology, Faculty of Veterin Medicine, University of Ibadan, Ibad Nigeria.

Animals were apparently healthy a kept in eight groups of eight animals ea according to their weights in animal ca (60 x 60 x 50cm). All animals were k under controlled conditions of temperat (25±02°C), relative humidity (50±15 and normal photoperiod (12 hour light a 12 hour dark). The animals were fed o standard rat diet (commercial pellet d from Vital Feeds Industry, Nigeria) a water provided *ad libitum*.

Compliance with ethical standards

The care and manipulations of a animals were done in accordance with Animal Practice requirements of the Anim Ethics Procedures and Guidelines a approved by the Animal Care and Us Ethics Committee of the University Ibadan, Nigeria.

Experimental protocol

The animals were randomly assign into eight (A-H) groups of eight rats eac and they were treated orally daily for days as below:

Group A : 0.2ml corn oil (vehicle)

Group B: Positive control, 2.5mg/ $NaAsO_2$ equivalent to the $1/10^{th}$ LD_{5} $NaAsO_2$

Group C: 100mg/kg^{-1} *Cassia fistu* extract and 2.5mg/kg^{-1} sodium arsenite

Group D:100mg/kg^{-1} *Cassia fistu* extract

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Group E: 200mg/kg^{-1} *Cassia fistula* and 2.5mg.kg^{-1} sodium arsenite

Group F: 200mg/kg^{-1} *Cassia fistula* extract

Group G: 300mg/kg^{-1} *Cassia fistula* extract and 2.5mg/kg^{-1} sodium arsenite

Group H: 300mg/kg^{-1} *Cassia fistula* extract

All treatments were carried out daily for 60 days in order to evaluate their effects. The rats were subjected to diethyl ether anesthesia using sliding top chamber (Kent Scientific corporation) during sample collection.

Semen collection and analysis

The rats were anaesthetized with diethyl ether before sacrifice and evaluated as previously described (Narayana, 2005; El-Desoky, 2013). Briefly, the mid caudoventral abdominal incision was made with sterilized scissors, permitting instant access to the testis once pushed upward from the scrotum. The testes were then separated from the epididymis. The right and left epididymides were trimmed off the body of the testes and semen sample was collected from the tail of the epididymis through an incision made with a scalpel blade. The semen was dropped on warm glass slide and stained using warm Wells and Awa stains for morphological studies for live/dead ratio was done using Eosin-Nigrosin stain. Also, percentage motility was carried out using 2 to 3 drops of 2.9% warm buffered sodium citrate kept at body temperature as described by Zemjanis (1977).

Percentage viability

This was performed by staining one drop of semen with one drop of warm Eosin-Nigrosin stain on a warm slide. A thin smear of semen and stain mixture was then made. The smear was air dried and observed under the microscope. The ratio of the *in vitro* dead sperm cells was observed. It is based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm repel the stain (Zemjanis, 1977).

Percentage motility

It was evaluated with a drop of semen in a warm 2.9% buffered sodium citrate on a warm glass slide covered with a glass slip and viewed at a magnification of ×40. Only sperm cells moving in a unidirectional motion were included in the motility rating, while sperm cells moving in circles, backward direction or pendulating movement were excluded (Patel *et al.* 1965; Zemjanis, 1977).

Histological study

The testes samples were collected in bouin solution for histopathological analysis. The tissues were processed, embedded and sections were made of about 4-6 µm. After staining with haematoxylin and eosin (H&E), slides were examined under the microscope (Olympus, Japan) for histopathological changes (Oloye *et al*, 2013).

Statistical analysis

All values were expressed as Mean ± Standard Error and analyzed using one way analysis of variance (ANOVA). SPSS Version 15 for Windows (SPSS Inc, 2006)

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and Microsoft Excel Professional Plus Microsoft Corporation, 2010) were used to carry out all procedures.

Results

Qualitative phytochemical analysis of ethanol leaf extract of *Cassia fistula* revealed the presence of carbohydrates, alkaloids, anthraquinones, cardenolides, tannins, flavonoids, saponins, phenols and steroids (Table I).

There were no significant changes in the testicular weight and diameter obtained across the groups except for the rats in group G which had a significant ($P<0.05$) decrease in the mean weight of the right testis compared to the rest of the groups. (Table II).

Arsenic intoxication decreased the sperm motility and count compared to the normal control (Table III). There was a significant ($P<0.05$) decrease in the mean percentage motility of groups B {treated 2.5mg/kg^{-1} Sodium arsenite: (SA)}, C (treated 100mg/kg^{-1} *Cassia fistula* extract and SA), E (treated 200mg/kg^{-1} *Cassia fistula* extract and SA), G (treated 300 kg-1 *Cassia fistula* extract), H (treated 300mg.kg^{-1} *Cassia fistula* extract and compared to group A rats (normal control) and groups D and F (treated 100 and 200mg/kg^{-1} *Cassia fistula* extract respectively). The mean sperm counts obtained across the groups also followed similar trend. It was observed that treatment with *C. fistula* only at dosage rate 100mg/kg^{-1} (D) and 200mg/kg^{-1} (F) had significant positive effect on the motility and sperm count but as it increased to 300 kg^{-1} the values dropped significantly (Table III). There were no significant ($P>0.05$) changes in the mean percentage livability of spermatozoa of rats across the groups (Table III). In addition, a significant increase of sperm abnormalities was found in rats treated with arsenic and 300mg/kg *Cassia fistula* extract. However, administration of *C. fistula* reduced the toxic effects of arsenic on sperms (Table IVa and IVb).

Table I. Qualitative phytochemical screening of ethanol leaf extract of *C. fistula*

Constitutents	Qualitative tests	Result
Carbohydrates	Molisch's	+ve
	Benedict's	+ve
Proteins and amino acid	Xanthoprotein	-ve
	Ninhydrin test	-ve
Alkaloids	Dregendoff's	+ve
Anthraquinones	Borntragers	+ve
Cardenolides	Killer-killanins	+ve
Tannins	Ferric chloride	+ve
Flavonoids	Ferric chloride	-ve
Saponins	Frothing	+ve
Phenoids	Sodium hydroxide	+ve
Steroids	Salkoski's	+ve

+ve=present, -ve=absent

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Table II. Mean (+SEM) testicular biometric values of Wistar rats exposed to different concentrations of *C. fistula* and Sodium arsenite

Groups	Weight (kg)		Diameter (cm)	
	Left testes	Right testes	Left testes	Right testes
A	1.01+0.13	1.06+0.06	3.06+0.23	3.28+0.19
B	1.12+0.04	1.10+0.05	3.30+0.19	3.22+0.13
C	1.11+0.03	1.11+0.02	3.18+0.13	3.16+0.21
D	1.11+0.08	1.09+0.02	3.32+0.15	3.36+0.21
F	1.08+0.49	1.08+0.02	3.30+0.12	3.28+0.19
G	1.07+0.05	1.04+0.03*	3.28+0.37	3.26+0.11
H	1.07+0.05	1.06+0.04	3.18+0.27	3.18+0.13

Mean values are significant at ($P<0.05$) level

Table III. Mean (+SEM) values of semen characteristics of Wistar rats exposed to different concentrations of *C. fistula* and Sodium arsenite

Groups	Motility (%)	Livability (%)	Sperm count ($x10^6$) sperm ml^{-1}
A	89.00+5.48*	97.40+1.34	138.6+9.63*
B	74.00+8.94	96.80+1.64	98.20+15.5
C	76.00+11.4	96.80+1.64	104.8+13.7
D	88.00+4.47*	96.20+1.64	141.8+11.7*
F	80.00+7.07	96.80+1.64	100.0+11.5
G	72.00+8.37	96.80+1.64	97.80+20.4
H	76.00+5.48	96.20+1.64	98.00+7.38

Mean values are significant at ($P<0.05$) level

Table IVa. Mean (+SEM) values of sperm morphological parameters (abnormalities of the tail) of Wistar rats exposed to different concentrations of *C. fistula* and sodium arsenite

Groups	Tailess head (%)	Headless tail (%)	Rudimentary tail (%)	Bent tail (%)	Curved tail (%)	Looped tail (%)
Group A	0.92+0.32	0.75+0.17	0.13+0.25	1.00+0.20	0.94+0.13	0.25+0.29
Group B	1.92+0.32	1.75+0.17	1.20+0.27	2.15+0.52	2.10+0.29	1.20+0.27
Group C	0.73+0.28	1.50+0.17	1.20+0.27	0.35+0.34	1.00+0.25	0.20+0.27
Group D	0.73+0.28	0.77+0.22	1.30+0.27	0.30+0.41	0.20+0.45	0.30+0.27
Group E	1.73+0.28	0.57+0.28	1.20+0.27	1.25+0.35	1.15+0.38	1.10+0.22
Group F	0.67+0.27	1.63+0.29	0.25+0.29	0.06+0.24	0.13+0.32	1.25+0.29
Group G	1.83+0.33	1.87+0.38	1.20+0.27	2.50+0.31	2.40+0.22*	1.20+0.27
Group H	0.87+0.18	0.47+0.19	1.30+0.27	1.85+0.60	1.05+0.27	1.20+0.27

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Table IVb. Mean (+SEM) values of sperm morphological parameters (abnormalities bodies) of Wistar rats exposed to different concentrations of *C. fistula* and sodium arsenite

Groups	Curved midpiece (%)	Bent mid piece (%)	Total abnormal cells (%)	Total normal cells (%)
Group A	0.25+0.20	0.13+0.14	4.37	95.63
Group B	2.25+0.35	2.25+0.18	14.82	85.18
Group C	0.25+0.40	0.25+0.25	5.48	94.52
Group D	0.30+0.27	0.30+0.21	4.20	95.80
Group E	1.00+0.25	0.30+0.27	8.30	91.70
Group F	0.25+0.29	0.19+0.35	4.43	95.57
Group G	2.30+0.27	2.35+0.14	15.65	84.35
Group H	2.50+0.18*	2.20+0.11	11.44	88.56

Histopathological evaluation of the testes of the control animals showed no lesion with normal spermatogenic cells and normal architecture (Figure 1). The testes of arsenic treated rats showed thickening of the basement membrane, significant decrease in spermatogenic cells, vascular degeneration and leydig cells deformation (Figure 2). Co- treatment with *C. fistula* however prevented the arsenic toxicity and maintain the testicular architectural integrity (Figure 3A and B).

Discussion

The mean testicular biometric values obtained in this study showed that there were no significant (P>0.05) changes in the testicular weight and diameter obtained across the groups except for group H. This implies that treatment of rats with *Cassia fistula* extract at 100mg/kg^{-1} and 200mg/kg^{-1} for 60 days had no negative effect on the testicular integrity for sperm production and sperm reserves. This finding validates the report of Raji and Njidda, (2014) in which testicular weight and diameter were shown to have a high correlation with sper reserves in the testes and epididymis a also the testicular integrity f spermatogenesis. The significant (P<0.0 reduction in the right testicular weig noticed in group G rats (treated 300n *Cassia fistula* extract and Sodium arseni (SA) might be due to a severe parenchyn atrophy in the seminiferous tubules followi arsenic challenge reported by Saalu *et a* (2009). This might imply that the extract 300mg/kg^{-1} had no protective effect again arsenic toxicity.

The percentage motility score obtain in this study ranges from 72.00% (grou G) to 89.00% (group A) and are all with the normal range required for potenti fertility in rats (Patel *et al,* 1965; Zemjani 1977).

However, the sperm motility and sper count values in groups A, D and F we significantly (P<0.05) higher than the valu in groups B, C, G and H. Since rats groups A, D and F were treated with bo arsenite and various doses of the extrac

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Fig 1. Histology of the testes of control animals showing no lesion with normal spermatogenic cells and normal architecture

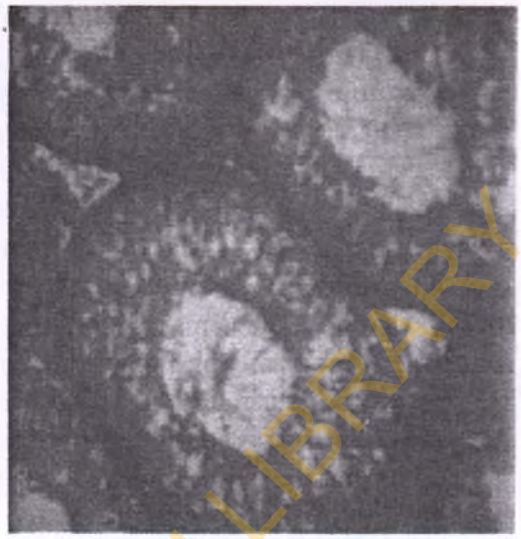

Fig 2. Histopathology of the testes of arsenic treated rats showing thickening of the basement membrane and vascular degeneration

A

B

Fig 3A &B. Histology of the testes of animals co-treated with C. fistula showing normal testicular architectural integrity indicating that the treatment prevented arsenic toxicity

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of *C. fistula*, it further shows that *Cassia fistula* extract may have some protective properties in itself but not at higher concentration above 200mg/kg^{-1}. The observed increase in sperm abnormalities and decreased sperm motility and count may be as a result of increased level of reactive oxygen species (ROS) generation from testicular lipid peroxidation (Vernet *et al.*, 2004). It has been documented that abnormal sperm morphology can be induced by ROS generation (Ramya *et al.*, 2010). The total morphologically abnormal sperm cells in groups B, G, H were higher than the 10% normal range proposed by Reece, (1997). This implies that the spermatozoa of all arsenic exposed rats were structurally affected. This finding is similar to a report by Ola-Davies *et al.* (2014) in which arsenite-exposed rats were treated with ethanol extract of *Spondias mombin*. It appears that *C. fistula* which is reported to contain antioxidants (Ilavarasan, 2005), significantly ameliorated the deleterious effects of arsenic on the sperms and testes architecture. Thus, this antioxidative prowess of *C. fistula* may play a positive role in the defense mechanism against arsenic induced testicular toxicity.

This present study revealed that the treatment of the wistar rats with *C. fistula* at 100mg.kg^{-1} best improves testicular characteristics as evidenced by increased sperm motility, sperm count, decrease in sperm abnormalities and maintenance of normal testicular architecture.

Thus, it is concluded that oral treatment of ethanol extract of *Cassia fistula* at 100mg/kg^{-1} for 60 days has better sperm protective effect against arsenic induced testicular injury which may be due to its antioxidant activities. Hence, *C. fistula* represents a potential agent to prevent arsenic induced testicular injury.

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Conflict of Interests

These authors (O.E. Ola- Davies, A.A. Oloye, and A. Adeoye) declare that they have no conflict of interest regarding the publication of this manuscript.

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