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Toxicological Profile of *Anacardium occidentale* Nut Shell Extract on Hematologic and Antioxidant Parameters in Brain and Testicular Tissues of Wistar Rats

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Abstract: ***The nut shell liquid of Cashew (Anacardium occidentale) has been reported to contain several compounds. The nut shell liquid possesses insecticidal activity in addition to several biological activities. Based on the report of insecticidal activity of Cashew nut shell liquid, the present study investigates the possible toxicological effects of Cashew nut shell extract (CNSE) in brain and testes of experimental rats. Cashew nut shell extract was administered to rats. The study used Forty-five male Wistar rats (average weight of 120g), being divided into nine groups (5 rats each), and given oral gavage as Groups A (corn oil), B (50 mg/kg), C (100 mg/kg), D (150 mg/kg), E (200 mg/kg), F (250 mg/kg), G (300 mg/kg), H (350 mg/kg) and I (400 mg/kg CNSE).Cashew nut shell was subjected to Soxhlet extraction using n-hexane, and then concentrated by a Rotary evaporator to obtain Cashew nut shell extract (CNSE). After 28 days, the rats were fasted overnight and subjected to anesthesia. Blood was collected by cardiac puncture for determinations of White blood cell (WBC), granulocytes and platelets. Brain and testes were harvested, rinsed and processed to obtain homogenates for determinations of Thiobarbituric acid-reacting substances (TBARS), superoxide dismutase (SOD), catalase, Glutathione-S-transferase (GST) and glutathione-S-transferase (GPx). Brain and testicular tissues were subjected to histological examination under light microscopy. The CNSE significantly reduced Total WBCs count, while platelets and granulocytes were increased compared with controls. The CNSE reduced catalase activities in brain and testes, while SOD was reduced in brain, but increased in testes. The GST activities in brain and testes were not significantly affected. However, GPx activity was increased in brain, but reduced in testes relative to controls. High doses of CNSE (250 - 400 mg/kg) showed neuronal changes in brain, while distortions were found in the interstitial space, seminiferous tubules and sertoli cells of testicular tissue. The findings from this study have suggested that Cashew nut shell extract may not cause hematological toxicity, but could potentially induce oxidative injury in both brain and testes of experimental rat models.***

Keywords: *Anacardium occidentale*, hematologic indices, redox profile, organ derangements

1. Introduction

Cashew (*Anacardiumoccidentale*) is a tropical evergreen tree plant that can grow up to 14m, while the dwarf cashew can be as high as 6m. The dwarf plant has been reported to have faster maturity and better yield compared with the tall type (Morton and Julia, 1987). The original place of cashew has been traced to the Caribbean Island, northern South America, Central America and northeastern Brazil (Duke, 1983; Morton *et al.*, 1987). In the mid-1950s, Nigeria witnessed the beginning of Cashew planting on for a commercial purpose Ogbe, Udi, Mbala and Oji, Eruwa, Iwo and Upper Ogun (Akinwale and Esan, 1989; Asogwa *et al.*, 2009). Studies have shown that the main producers of Cashew include Cote d'Ivore, Ghana, Guinea Bissau, India, Brazil, Mozambique, Nigeria, Benin Republic, Sri Lanka, Philippine, Vietnam and Tanzania (Asogwa*et al.*, 2009; Orwa *et al.*, 2009 and Hammed *et al.*, 2011). Cashew nut has served as food and industrial raw materials, as well as, a good source of income and foreign exchange in places like Latin America, Africa and Asia. The nut shell, however, has been used in the manufacture of paints and lubricants (Asogwa et al. (2009).

Cashew nut is rich in protein, carbohydrate, fats, minerals (such as manganese, potassium, copper, iron, magnesium, zinc, selenium) and thiamine, as documented by Blomhoff et al. (2006). Cashew plant has also been reported to be rich in polyphenols, dietary fibers and tannins (Maria Do Socorro et al., 2010). Cashew juice and its pulp have been documented to have a high level of vitamin C (Eca et al., 2015; Silva et al., 2017).Studies have shown that the stem back and leaves of Cashew have been used in traditional medicine, particularly against bacterial infections, fever, high blood pressure and high blood sugar (Konan and Bacchi, 2007; Brandao et al., 2008; Dahake, 2009; Olifeet al., 2013).

A study by Buxton et al. (2017) showed that Cashew nut shell oil is rich in cardol, anacardic acid and cardandol. The cashew nut shell liquid has been used as a good source of agrochemicals, varnishes, paints, surface coatings (Nyirenda et al., 2021). Furthermore, cashew nut liquid has shown several biological properties including anti-genotoxic and anti-mutagenic (Melo-Cavalcante et al., 2011), antioxidant, anti-inflammatory, anticancer and antimicrobial (Salehi et al., 2020) and antifungal (Jebapritha and Karpagam, 2017) properties. However, cashew nut shell liquid has been shown to possess insecticidal property in several insects (Oladejo et al., 2016;Acero, 2018, Adeleke et al., 2021).In addition, the

juice has been reported to reduce the levels of insulin and low-density (LDL) and elevate high-density lipoprotein (HDL) in individuals with type-2 diabetes mellitus (Darvish et al. 2019). *Anacardium occidentale* plant extract has been reported to exhibit antioxidant and anti-inflammatory (Fusco et al., 2020), antimicrobial (Murei and Kubo, 1996; Salehi et al., 2019).

The objective of the study was to investigate *in-vivo* toxicological effects of the extract of Cashew (*Anacardium occidentalis*) nut shell on blood and redox parameters of both brain and testes of experimental rat models.

2. Materials and Methods

2.1 Chemicals

Thiobarbituric acid, adrenaline (epinephrine), Sodium azide, Trichloroacetic acid, 5'-5'-dithiobis- (2-dinitrobenzoic acid) [DTNB], 1 chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione, dipotassium hydrogen phosphate and potassium dihydrogen phosphate, (KH_2PO_4).

2.2 Collection and extraction of Cashew nut shell

Cashew nuts were bought from Wazo market, Ogbomosho, Oyo state, Nigeria in June 2019. The nuts were de-shelled, and the shells were air dried for three weeks at the room temperature. The shells were then grinded using electric grinder. The pulverized cashew nut shell was subjected to Soxhlet extraction using n-hexane. The cashew nut shell extract (CNSE) obtained was then concentrated using rotary evaporator and then subjected to oven drying at 40^{o}C. The CNSE was kept 4^{o}C until use.

2.3 Experimental Design

Forty-five male Wistar rats (average weight of 120g) were purchased from the LAUTECH animal house, and then divided into nine groups (5 rats per group). After being acclimatized for one week, they were administered orally as Groups A (corn oil), B (50 mg/kg), C (100 mg/kg), D (150 mg/kg), E (200 mg/kg), F (250 mg/kg), G (300 mg/kg), H (350 mg/kg) and I (400 mg/kg CNSE), every other day for 28 days.

2.4 Blood and tissue collection

After 28 days, the experimental rats were sacrificed by anesthesia using chloroform. Blood was collected through cardiac puncture in to EDTA bottles for hematology. The brain and testis were excised and rinsed in 1.15% of KCl as washing buffer. Each of the two organs was cut into two portions; one portion was fixed using Bouin's solution, while the other portion was homogenized and centrifuged for biochemical assays.

2.5 Hematological analysis

Total white blood cell count (WBC), and differential white blood cell counts (lymphocytes, granulocytes and platelets) were carried out, and the mean values calculated according to Ugochukwu et al. (2015).

2.6 Biochemical Assays

The concentrations of total protein in brain and testis homogenates were spectrophotometrically measured as described by Lowry *et al. (*1951).The levels MDA in brain and testes were determined as described by Ohkawa *et al*. (1979). The absorbance of Thiobarbituric acid-reacting substances (TBARS) was measured at 532nm. The MDA concentrations were calculated using a molar extinction coefficient (Ɛ) of 1.56 x 10^5 Mcm^{-1}.Superoxide dismutase activities in brain and testes were determined by the epinephrine method described by Mistra and Fridovich (1975). The change in absorbance was monitored at 480nm.Catalase activities in brain and testes were spectrophotometrically determined by measuring the Change in absorbance at 240nm as described by Aebi (1984).Glutathione-S-transferase (GST) activity was determined according to the method described by Habig et al. (1974). The GPx activities were determined according to a modified method of Paglia and Valentine (1967). The decrease in absorbance was measured spectrophotometrically for 5 min at an interval of 5 min at a wavelength of 340 nm. The GPx activity was expressed as mmol/mg protein.

2.7 Histopathology

Ultra-thin sections of brain and testes were preserved with 10% formalin, and then examined under microscope after staining with hematoxyline and Eosin stains.

2.8 Statistical Analysis

Data were expressed in Mean ± Standard Deviation. Student t-test and ANOVA were used for statistical comparison and differences were taken as significant at $p < 0.05$.

3. Results and Discussion

3.1 Hematology

The result in table 1shows the hematological effects of CNSE in rats. Total white blood cells (WBCs) count was significantly reduced ($p<0.05$), while platelets were increased at all the doses applied in the study. The level of granulocyte was significantly elevated at most doses, while the CNSE treatment has no significant effect on lymphocyte level in the experimental rats compared with controls.Elevated level of white blood cells count has been associated with conditions such as inflammation, allergy, leukemia, infection, burn-induced tissue injury and systemic illness, as reported by Alomari et al. (2014).Platelets are small blood cell fragments that aid blood clotting, and a reduced level of these cells indicates infections (Osman, 2013).

3.2 Antioxidant indices

Living cells have inherent enzymatic and non-enzymatic antioxidant defense mechanisms for detoxification of free radicals (Phaniendra and Periyasamy, 2015). When there is an excess level of ROS above the capacity of the antioxidant system, oxidative stress occurs, leading to oxidation of

DNA, proteins and membrane lipids (Bansal and Simon, 2016). Malondialdehyde, a reactive molecule with mutagenic potential, is a marker of lipid peroxidation, which may be increased due to toxins and diseases (Hartman 1983; Ismail et al., 2015). The activities of SOD, GPx GST and catalase in prevention of ROS-induced cell damage have been well documented (Sukprasansap et al., 2017). As shown in table 2, the MDA levelsin both brain and testicular tissues were not significantly affected, compared with the control rats, across the doses of CNSE used in this study.In the brain of the experimental rats, the activities of SOD and catalase enzymes were significantly reduced relative to controls (Table 3). The reduction in SOD activity in brain was observed to be dose-dependent. However, in the testes, the activity of SOD was increased, while that of catalase was significantly lowered compared with the control rats (Table 3). Superoxide dismutase catalyzes the conversion of superoxide anion to hydrogen peroxide, while catalase, which is predominant in the peroxisomes, catalyzes the conversion of hydrogen peroxide to molecular oxygen and water (Yasui, 2006).A reduction in dismutation of superoxide anion to hydrogen peroxide by SOD has been linked to accumulation of superoxide anion (Veliogluet al., 1998), which interacts with tissue biomolecules to initiate oxidative stress and toxicity (Ismail et al., 2015).Many pathological conditions have been reported to be associated with superoxide anion, therefore, the use of SOD in treatment of degenerative diseases and cancer has been document by many study groups (Hayyan et al., 2016). A reduction in catalase activity has been linked to accumulation of hydrogen peroxide, malondialdehyde formation and oxidative modification of proteins (Dordevic et al., 2021).Hydrogen peroxide production has been reported to occur in target cells when NADPH oxidases are stimulated by several cytokines and growth factors (Geiszt and Leto, 2004; Park et al., 2004).This finding has shown that CNSE could potentially induce oxidative stress (OS) in the rat brain via a build-up of superoxide anion due to reduced SOD activity, whereas in the testes, the OS may occur due to a build-up of hydrogen peroxide due to reduced catalase and GP x activities. However, Duangian et al. (2021) reported that methanol extract of cashew plant significantly increased the endogenous expression of SOD, GPx and GST in experimental animals. Figures 1 shows the effects of CNSE treatment on the glutathione-S-transferase activities in brain and testicular tissues. The activity of the enzyme in the two organs was not significantly ($p > 0.05$) affected by the CNSE treatment compared with the control rats. The GSTs belong to a family of phase II enzymes in both eukaryotic and prokaryotic organisms. This enzyme family is associated with detoxification of xenobiotics via conjugation with reduced glutathione (Sheehan et al., 2001).In figure 2, CNSE increased the glutathione peroxidase activity in the rat brain, while there was decrease of activity in testes at most of the applied doses. The disparity in the effects of CNSE on GPx in the two organs of study may indicate organ specificity in the response of the enzyme to the extract. The reduction in catalase and GPx activities in testes may indicate an accumulation of hydrogen peroxide in this particular organ causing oxidative damage. Further study is however needed for the confirmation of this finding. The GPx is a family of isozymes (GPx1-8), responsible for the reduction of organic hydroperoxides by using GSH as an electron donor (Margis et al., 2008). Among the eight GPx isozymes, GPx 2 has been associated with cancer prevention (Haug et al., 2012; Naiki et al., 2018), and has also been targeted during the therapy for glioblastomemultiforme (Bangming et al., 2021).Reduced levels of SOD, catalase and GPx, with an increased level of MDA are associated with oxidative stress in living cells (Adeleke and Adaramoye, 2016; Ortega 2019).

3.3 Histopathology

The result of histopathology of the rat brain has been shown as the representative photomicrograph (Figure 3). Groups A1 (Control) and B4 (50 mg/kg) showed normal features with large pyramidal and granule neurons. Groups F2 (250 mg/kg), F3 (250 mg/kg), I2 (400 mg/kg) and I3 (400 mg/kg), which were the higher doses of the extract, showed pyramidal neurons with loss of cytoplasmic contents, succinctly expressed neurons with mildly appreciable axons and presence of some pyknotic cells (lesions are indicated with arrows). The cytological derangements observed in the brain tissue, at higher doses of the extract, may be due to oxidative stress, resulting from reduced activities of SOD and catalase in this organ, as earlier noted in the present study. A series of studies by Chakrabarti et al. (2013), Cobley et al. (2018) and Murray et al. (2021) revealed that the brain is rich in polyunsaturated fats, with a relatively weak antioxidant mechanism and a high rate of oxygen consumption, leading to a high rate of oxidative metabolism and oxidative stress. *A.occidentale* has been reported to show neuroprotective effects (Duangial et al., 2021), and this may be due to the presence of components, such as anacardic acid (Salehi et al., 2019), alpha- linolenic acid (Lee et al., 2018) and gallic acid (Oiram-Filho et al., 2018) in the plant. The photomicrographs of the representative testicular tissues are shown in figure 4.Groups A1 (Control) and B4 (50 mg/kg) showed intact seminiferous tubules and sertoli cells. Groups F2 (250mg/kg), F3 (250mg/kg), I2 (400mg/kg) and I3 (400 mg/kg), which were higher doses of the extract, showed lesions, including interstitial space distortion, pyknotic interstitial cells, empty seminiferous tubules, scanty sertoli cells and diminished membrane basement (lesions are indicated in arrows).The cytological derangements observed in testes, at higher doses, may be due to oxidative stress, resulting from reduced activities of catalase and GPx in this organ, as earlier noted in the present study. In male individuals, testicular oxidative stress has been reported to impair spermatogenesis, sperm capacitation and epididymal maturation, leading to infertility (O'Flaherty, 2020). Similar to the brain cells, spermatozoa membrane is rich in polyunsaturated fatty acids (PUFAs), which are highly prone to lipid peroxidation and oxidative stress (Martin-Hidalgo et al., 2019), resulting in morphological alteration, mitochondrial damage, and inability for fertilization (Ghaleno et al., 2021). The histopathological examinations thus show that CNSE could cause cytological derangements in both brain and testes at high doses.

3.4 Conclusions

The results from this study have indicated that Cashew nut shell extract may not exert hematological toxicity in

experimental rats. However, the extract could induce oxidative stress, by reducing the activities of antioxidant enzymes, in both brain and testes of experimental rat models

Consent

It is not applicable

Ethical Approval

An approval was obtained from the Ethical Committee of the University before the conduct of the Study.

Conflict of interests

There was no conflict of interests among the authors in the course of the study.

Table 1: Effects of Cashew nut shell extract on total and differential white blood cells count in Wistar rats

Treatment groups (mg/kg)	White blood cells (x 10^9)	Lymphocytes (%)	Granulocytes (x 10^{10})	Platelets (x 10^{11})
Control	16.43±2.56	68.92±7.87	15.80±6.17	437.23 ±18.31
50	13.23±1.81#	70.90±16.91	5.92±9.79	604.51±50.70*
100	13.58±2.51#	53.48±4.83	26.95±4.91*	616.25 ±80.11*
150	14.21±3.72#	61.11±5.88	20.45±3.33*	527.32 ± 55.99*
200	13.82±4.51#	59.96±8.28	20.98±7.11*	629.62 ± 61.30*
250	14.56±3.61#	67.15±5.26	16.13±3.56	677.55 ± 59.10*
300	13.34±1.63#	63.76±6.98	19.06±9.76*	630.20 ± 38.59*
350	12.32±2.97#	71.76±7.77	12.82±2.14#	607.52 ± 81.21*
400	14.51±6.60#	66.9±11.53	23.86±2.15*	519.33 ±32.21*

Values were expressed as mean ± standard deviation.
* Significantly higher compared with control (p>0.05)
# Significantly lower compared with control (p<0.05)

Table 2: Effects of Cashew nut shell extract on Malondialdehyde levels in Brain and Testis of Wistar rats

Treatment groups (mg/kg)	Brainx10-^{5}(µM MDA/mg protein)	Testis x 10-^{5}(µM MDA/mg protein)
Control	8.44±0.88	
50	7.11±1.00	8.34±0.80
100	6.97±0.25	8.67±0.50
150	7.88±0.71	6.80±0.55
200	7.64±0.67	7.44±0.25
250	8.04±0.96	7.32±0.43
300	7.75±0.99	7.50±0.50
350	7.67±0.84	8.40±0.52
400	7.73±0.75	7.82±0.72

Data expressed in mean ± SD, n=6,
*= significantly higher as compared to control (P>0.05)
= significantly lower compared to control (P<0.05)

Table 3: Effects of Cashew nut shell extract on activities of superoxide dismutase and catalase in brain and testis Wistar rats

Treatments (mg/kg)	Brain		Testis	
	Superoxide dismutase activity x 10^{-5} (U/mg/protein)	Catalase activity (U/mg/protein)	Superoxide dismutase activityx 10^{-5} (U/mg/protein)	Catalase activity (U/mg/protein)
Control	2.17±0.81	8.51±6.62	0.32±0.14	27.40±8.87
50	2.11±0.99	3.42±1.40#	0.65±0.48*	19.89±6.54#
100	2.03±0.85	3.50±1.15#	0.89±0.55*	12.45±7.69#
150	1.47±0.15#	3.68±2.31#	2.59±3.98*	7.04±5.34#
200	1.50±0.17#	1.79±1.29#	0.69±0.36*	9.65±5.24#
250	1.50±0.17#	2.42±1.14#	0.47±0.13*	6.93±2.21#
300	1.40±0.22#	3.19±3.16#	0.64±0.34*	8.54±2.87#
350	1.40±0.10#	6.02±4.80#	0.52±0.16*	6.94±5.26#
400	1.40±0.05#	4.13±1.12#	0.53±0.16*	6.79±2.45#

Values expressed as mean ± standard deviation
*- significantly higher as compared to control (P>0.05)
- significantly lower compared to control (P<0.05)

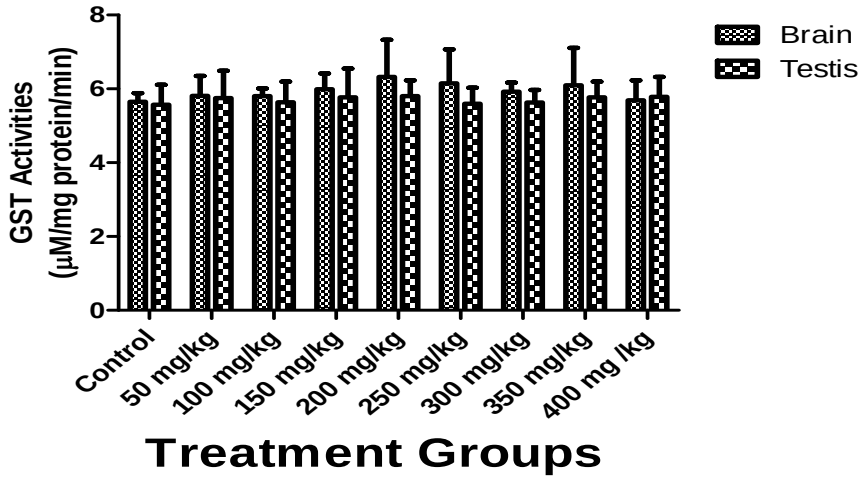

Figure 1: Effect of Cashew nut shell extract on GST activities in brain and testis of Wistar rats
Values expressed as mean ± standard deviation
GST- Glutathione-S-transferase

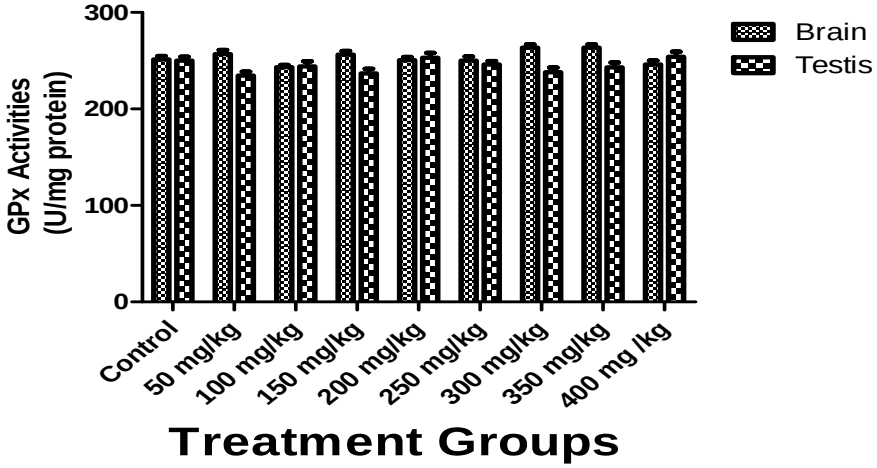

Figure 2: Effect of Cashew nut shell extract on GPx activities in brain and testis of Wistar rats
Values expressed as mean ± standard deviation
GPx- Glutathione peroxidase

Histopathological examinations

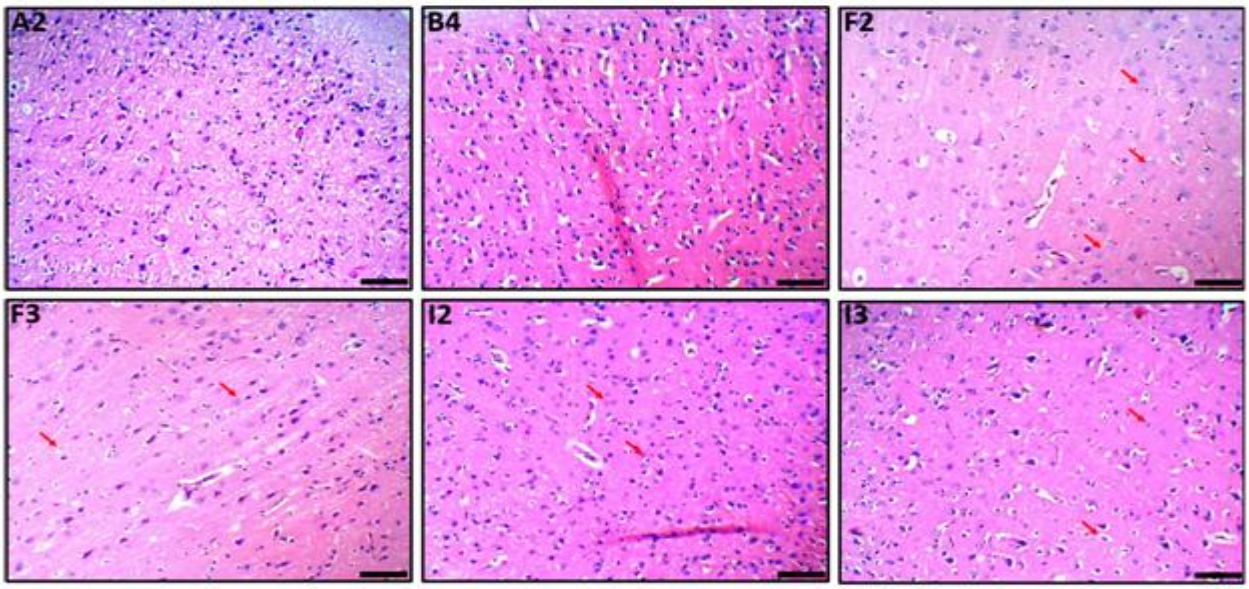

Figure 3: Representative micrographs of histology of brain of rats administered with Cashew nut shell extract (Scale bar: 10µm)

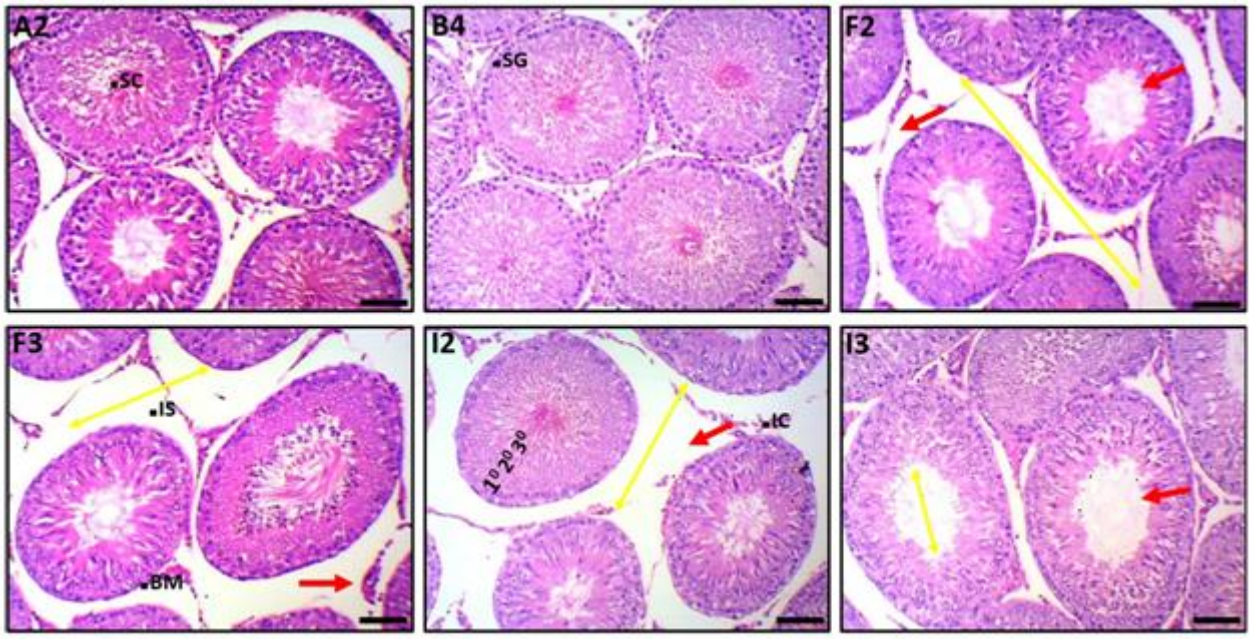

Figure 4: Representative micrographs of histology of brain of rats administered with Cashew nut shell extract (Scale bar: 10µm)

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