Research article

Ameliorative Capacity of Rice Husk Extract on Codeine-induced Testicular Dysfunction in Male Wistar Albino Rats

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Abstract

Rice husks (RHs) is an agro waste generated from rice production with limited application. Codeine belongs to the numerous drugs of abuse worldwide and its impact on reproductive injury has been reported. This study was designed to establish the ameliorative potential of brown rice husk extract (RHE) against reproductive damage induced by codeine misuse. Experimental rats were grouped into six groups with six rats per group: Group I received only rat chow and clean water for 30 days. Group II received only codeine 10 mg/kg body weight (b.w) of rats. Group III received only RHE 500 mg/kg b.w of rats. The 4th, 5th and 6th groups received codeine 10 mg/kg/b.w alongside 250 mg, 500 mg and 1000 mg RHE, respectively. Treatments (RHE and codeine) were administered once during the early hours between 9-10 am daily for 30 days, the animals were sacrificed, and blood and tissue samples were harvested. Male reproductive hormones such as testosterone, Follicle stimulating hormone (FSH) and luteinizing hormone (LH), and sperm quality parameters were assayed. The testis tissues were excised and examined for histopathological changes. The result revealed that high dose codeine administration significantly decreased epidermal morphology, motility, and sperm count as well as testosterone, FSH, and LH concentrations, respectively. The histopathological examination revealed distinct abnormal changes in the codeine control group when compared with the other experimental groups. RHE administration significantly improved the altered assayed parameters.

Keywords

codeine;
reproductive hormones;
rice husk;
sperm quality;
testis

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The recorded data from the assays suggest that RHE can ameliorate poor sperm quality, with a therapeutic potential for the treatment and/or management of reproductive impairment in male associated with infertility.

1. Introduction

At the present time, inflexible economy has contributed greatly to the high rise in drug addiction in Africa, and this poses a serious concern to health workers. There are increasing addicted populace with limited health care personnel and limited drug and facility for treatment. Opioids remains the most misused drugs world-wide, possibly due to their strong analgesic effects and the ecstatic feelings they can produce in the user. Center for Behavioral Health Statistics and Quality [1] reported on the global rapid increase in opioids prescriptions for non-medical reasons, making opioid the second most used illicit drug. International Narcotics Control Board [2] classified codeine as the most over abused opioid globally. In Nigeria, Bakare and Isah [3] reported high codeine intake among the youths. According to Aldhamin and Hamid [4], the exposure route, a person's genetic make-up, nutrition, and age, are the determinants of the effects of poisonous substances on the human system. Opioids are recognized as disruptors of sexual activity in male humans and rodents [5], and Mckim [6] reported that opioids suppressed both male and female sex hormones and fertility. According to Akhigbe and Ajayi [7], codeine enhanced copulatory locomotor activity, reduced fertility indices and induced testicular toxicity. Several studies demonstrated that prolonged codeine intake may cause atrophy of the seminiferous tubules with interstitial calcification of the testis, and circulatory and intra-testicular testosterone suppression in male albino rat [7, 8]. Androgen deficiency has been associated with prolonged opioid intake in several studies. According to Rubinstein and Carpenter [9], the use of long-acting opioids leads to androgen deficiency and eventually suppresses testosterone synthesis. Currently, natural compounds from fruits, vegetables and herbs are recognized and adopted as allopathic medicines for the treatment and prevention of diseases including reproductive disorders. Agricultural by-products can be categorized as natural compounds as they possess substantial biological properties that may aid in the treatment of different diseases [10]. The epicarps of some farm products are often discarded or regarded as non-edible. Some of these epicarps are tagged phyto-waste, and they constitute about three-quarters of the total plant composition. Epicarps such as peels, covers, shells, and husks shield the seeds they contain from oxidative damage. This is a function of their high antioxidant capacity as they contain substantial amounts of isovitexin, flavonoids, phytic acid, hydrocinnamic acid derivatives, vanillin, anisole and syringaldehyde [11-13]. Thus, they are good sources of natural antioxidants. For the rice epicarp, apart from its protective capacity, it has also been applied as a fuel for biomass gasification [14], and as a feed for livestock due to its slow digestibility, distinct size, low bulk density, high ash/silica contents and abrasive feature [15]. Furthermore, studies have suggested that dietary fiber (especially a mixture of soluble and insoluble fibers) consumption has the proclivity to reduce the risk of cancer such as colon cancer [16], and to decrease post-meal blood cholesterol and sugar levels in people with diabetes [17]. The influence of rice by-products on the decline of cancer and diabetes may be partially explained by their high levels of dietary fiber. Various studies have reported its chemo-preventive potential against chemically induced early stage of carcinogensis, hepatocarcinogenesis, antimutagenicity and anticlastogenicity *in vitro* and *in vivo* [18-21]. Rice husk extract (RHE) has also shown promising antioxidant activity against DNA oxidative damage [12], and anti-inflammatory [22] and anti-diabetic [23] effects in mice. According to Mckim [24], a methanol extract of japonica rice husk (MEJRH) inhibited human colon cancer cell proliferation in dimethylhydrazine-induced rats. Okuno and Miyazawa [25] reported that momilactone-A from an ethyl acetate extract of rice husk (AERH) suppressed the SOS-inducing activity of Trp-P-1 in

the Salmonella antimicrobial usage (*Amu*) test. A dichloromethane extract of purple rice husk strongly inhibited aflatoxin B1 (AFB1) and MeIQ- (amino- imidazo- quinolines denoted IQ compounds), induced mutagenesis in the Ames test and decreased the number of micronuclei in the liver of diethylnitrosamine-induced rats [20]. Considering the challenges in the disposal of phyto-waste, and the limited data on the protective effect of RHE on the male reproductive function, this research was focused on the impact of high dose codeine intake on the male reproductive system, and the ameliorative influences of RHE on sperm quality, sex hormones and histopathology of the testis, using a rat model.

2. Materials and Methods

2.1 Ethical approval

Ethical approval was obtained from University of Port Harcourt Research Ethics Committee, with Reference Number (UPH/CEREMAD/REC/MM79/026).

2.2 Drugs and chemicals

Codeine sulfate was acquired from the Pharmacology Unit of the University of Port Harcourt Teaching Hospital (UPTH), River State, Nigeria with recommendation from the National Agency for Food and Drug Administration and Control (NAFDAC). All reagents used were of scientific grade. Codeine solution was prepared fresh by disolving a weighed amount of powdered codeine sulfate in a measured volume of distilled water at the ratio of 10:1.

2.3 Experimental rats

The research specimens comprised thirty-six (36) male Wistar rats, each weighing between 100-110 g. The rats were housed in a ventilated cage in the Animal house of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria. They received grower's mash (rat chow) and distilled water during the experimental period and were strictly monitored. The rats were left for 1 week to conform with the laboratory conditions and were properly handled according to the approved experimental protocol stated in the Guide for the care and use of Laboratory Animals by the National Institute of Health.

2.4 Experimental procedure

2.4.1 Sample collection

Rice husks were collected from a local rice mill at Awgu town, Enugu State, South-East zone, Nigeria.

2.4.2 Preparation of rice husk extract

The RHs were pulverized and filtered through a 4.8 mm mesh sieve. The powdered rice husk sample was weighed and subjected to extraction using a Soxhlet extractor apparatus, with methanol as the extraction solvent. The resultant extract was concentrated at 40-45^{o}C using a rotary vacuum evaporator. The brown paste semi-solid obtained was stored in an airtight container in the refrigerator at 4^{o}C till usage. Different concentrations of the extract were obtained by weighing

appropriate measures of the extract, which were diluted with distilled water before oral administration.

2.4.3 Groupings of experimental animals

The rats were selected randomly and grouped into six groups of six rats per group as follows:
Group I: Negative control rats that received only chow and distilled water.
Group II: Codeine control rats that received codeine 10 mg/kg body weight (b.w) of rats.
Group III: Rice husk extract (RHE) control rats that received only RHE 500 mg/kg b.w rats.
Groups IV: Rats received codeine 10 mg/kg b.w of rats + RHE 250 mg.
Groups V: Rats received codeine 10 mg/kg b.w of rats + RHE 500 mg.
Groups VI: Rats received codeine 10 mg/kg b.w of rats + RHE 1,000 mg.

After 30 days of treatment, the rats were fasted overnight, and blood samples collected via cardiac puncture under light diethyl ether anaesthesia. Blood samples were collected with plain sample bottles for the hormonal assay. Meanwhile, 50 µl of the sample was used for LH, 50 µL for FSH and 10 µL for testosterone assay. The rats testis were harvested for histology examination.

2.4.4 Assessment of testicular levels of reproductive hormones

The serum testicular testosterone was determined with a rat specific testosterone ELISA Kit (BioCheck, Inc. South san Francisco; Catalog Number: BC-1115) (Intra C.V. = 10.0%, Inter C.V. = 8.4%, Sensitivity = 0.05 ng/mL, Recovery = 95.3%); serum luteinizing hormone (LH) was determined with a rat specific LH ELISA Kit (Fortress diagnostics, United Kingdom; Product No: BXE0651A) (Intra C.V. = 4.2%, Inter C.V. = 5.3%, Sensitivity = 0.8 mIU/mL, Accuracy = 98.9%), and serum follicle stimulating hormone (FSH) was determined with a rat specific FSH ELISA Kit (Fortress diagnostic; United Kingdom; Product No: BXE0631A) (Intra C.V. = 4.3%, Inter C.V. = 3.0%, Sensitivity = 0.8 mIU/mL, Accuracy = 97.8%). These analyses were accurately carried out following the procedures provided by the reagent kits' manufacturers.

2.4.5 Assessment of sperm count

This was done according to an established method of the WHO [26]. Semen samples from the caudal epididymis of the testis were positioned in a petri dish. The epididymal spermatozoa was extracted from the epididymis. About 5 µL (an aliquot) from the suspended sperm was then combined with 95 µL diluent (consisting of 0.35% formalin made up of 0.25% trypan blue and 5% $NaHCO_3$). Each diluted sperm sample was introduced in a saline solution and warmed at 32°C for 10 min. Five mL of the diluted sperm was introduced onto the central square of a Neubauer hemocytometer, which was used to estimate the epididymal sperm count under a microscope at 400x magnification in an enhanced Neubauer chamber (Deep 1/10 m; Lambert, Munich, Germany). The resultant values were expressed in millions per 1 mL of sample.

2.4.6 Assessment of sperm morphological abnormalities and viability

Sperm morphology and viability were estimated following the established protocol of Wells and Awa [27]. The sperm samples were smeared onto glass slides and air dried overnight to spot morphological defects. The slides were stained with nigrosine (5%) in sodium citrate dehydrate mix (3%) and 1% eosin. The specimens were examined for morphological abnormalities under a microscope at 100x magnification. About 400 sperm cells were examined in each sample and the percentage of morphological defects was estimated.

2.4.7 Assessment of sperm progressive motility

Sperm motility was estimated following the protocol prescribed by Zemjanis [28]. The caudal epididymis was surgically opened with surgical blade and a droplet of the spermatozoa sample was suspended on a disinfected spotless glass slide. Then, 2.9% pre-warmed sodium citrate was used to dilute the sperm before it was thoroughly mixed and enclosed with a 24 x 24 mm coverslip. Thereafter, sperm motility was examined under a minimum of ten microscopic fields of 200x magnification and phase contrast within 2-4 min of their isolation from the epididymis. The motile and non-motile sperm were totaled, and values expressed as percentage of total sperm.

2.4.8 Histology examination

A testis was sliced and attached to a specimen holder having 10% formal saline. The 10% formal saline exterminates any bacteria present and ensures that the tissue does not rot or deteriorate. Water was displaced from the specimen using graded percentage of alcohol in ascending order from a lower concentration to the absolute. Thus, dehydration started with 50% alcohol for 2 h, 70% alcohol for 2 h, 95% alcohol for 12 h (overnight) and then absolute (100%) alcohol for 2 h. Agitation, which is one of the factors for tissue processing, was done using a Junior Orbit shaker, and the alcohol from the blocks or sections of tissue was emptied by dipping it in an ante-medium (xylene). Thin uniform sections for histological examination were generated using a rotary microtome. A grease-free slide was dropped on a warm plate and was flooded with distilled water. A section was set on the surface of the slide and grease was detached by stretching the surrounding wax carefully with mounted needles. As the H_2O warms, the section flattened out. The slide was then detached from the hot plate, labelled, and dried. The slips were stained using a combination of haematoxylin and eosin stains, which is the most adopted combination of stains for routine histology. After staining, the sections were arranged as a lasting preparation for microscopic examination. This was done by placing the section in a suitable medium under a glass cover slip using a mounting medium. The slips were then viewed under a microscope and photomicrographs captured with a camera.

2.4.9 Statistical analysis

All data were subjected to statistical analysis. Values were reported as mean±standard deviation (SD), while Duncan Test of One-way ANOVA was used to test for significant differences between groups. The results were considered significant at p-values of less than 0.05, that is, at the 95% confidence level ($p \leq 0.05$).

3. Results and Discussion

Rice husk, an agro-waste from the milling of rice, has been proven to have a wide range of biological activity [29]. However, there are limited report on its therapeutic potential on reproductive system injury/damage. A study reported that rice husk extract (RHE) contained numerous phytochemicals, antioxidants, and cellulose, which can be utilized for producing nanocrystals as part of drug design [30]. Testicular oxidative stress has been identified as a key mediator of reproductive toxicity in a number of studies on the effects of opioid usage on the male reproductive system. Opioids are common analgesics, but their adverse effects have become the subjects of extensive research. Thus, this study sought to investigate the ameliorative capacity of RHE on the sperm quantity and certain reproductive hormones in Wistar rats exposed to high dose codeine intake. As presented in Table 1, there was no significant difference in follicle stimulating hormone (FSH) and luteinizing hormone

(LH) level among the groups. The RHE control group recorded the highest level of FSH, and the normal control recorded the highest level of LH, while the codeine control group recorded the lowest FSH and LH levels, respectively. Furthermore, the testosterone concentration of the normal control group was significantly higher than that of the other groups. The codeine control group also recorded the lowest testosterone concentration. There were reports that opioid induced infertility in male reproductive system were mainly initiated by alteration of the reproductive hormone, which led to decreased sperm quality. A review article by Vuong *et al*. [31] reported that increased opioid abuse primarily leads to hypogonadism but may as well affect the secretion of other pituitary hormones. However, gonadotropin-releasing hormone (GnRH) stimulates the pituitary gland to secrete FSH and LH in male, which causes the testicles to produce testosterone [32, 33]. The wide misuse of opioids among youth and especially youths of male gender led to the assessment of the effects on testicular function and consideration of the histological implications of sub-chronic intake of codeine in young male rats. In this study, there were significant reductions in the circulatory levels of pituitary hormones (FSH and LH) and testosterone in the codeine treated groups when compared to that of the other experimental groups. This affirms the work of Sayed and Zidan [34], who reported significant decreased levels of LH, FSH and testosterone with increased prolactin hormone after tramadol administration in male rats. El-Gaafarawi *et al.* [35] reported significant reduced LH, FSH and testosterone in tramadol administered male rats. In our study, codeine administration for 30 days reduced the sex hormonal activity of the male albino rats, an effect that may have emanated from loss of testicular tissues, and/or testicular injuries. Reduction of the circulatory testosterone recorded in this study shows that codeine disrupts steroidogenesis. According to Riaz *et al.* [36], steroidogenesis results from impaired Leydig cell function. The Leydig cell impairment may be associated with ROS-induced oxidative damage. The result from this study is also in tandem with those of Katz and Mazer [37], who stated that prolonged opioids accumulation in the endocrine system had the tendency to suppress testosterone levels via decreased LH synthesis. LH has been reported to be regulated by numerous neurotransmitters, including endogenous opioid peptides [38]. Its alteration and inhibitory effect on spermatogenic functions recorded in this study may also be attributed to ROS and free radical generation, which has been reported to be a disruptor of spermatogenesis and steroidogenesis [32, 33].

Table 1. Impact of rice husk extract on male reproductive hormones of rats exposed to codeine

Groups	Group Treatment	FSH (mIU/mL)	LH (mIU/mL)	TET (ng/mL)
I	NC	3.41±0.15^{a}	17.76±22.08^{a}	0.36±0.25^{b}
II	COD Only	3.39±0.14^{a}	7.14±3.33^{a}	0.01±0.01^{a}
III	RHE Only	3.52±0.10^{a}	14.70±7.62^{a}	0.08±0.04^{a}
IV	COD + 250 mg RHE	3.42±0.05^{a}	8.36±6.90^{a}	0.02±0.02^{a}
V	COD + 500 mg RHE	3.41±0.12^{a}	8.61±1.38^{a}	0.02±0.00^{a}
VI	COD +1,000 mg RHE	3.46±0.15^{a}	12.04±1.60^{a}	0.10±0.02^{a}

FSH =follicle stimulating hormone; LH = luteinizing hormone; TET = testosterone
Data expressed as mean ± SD; (n = 3 replicate). Groups with different superscript(s) are significantly different at $p \leq 0.05$, while groups with same superscript(s) are not. Key: NC= negative control; COD Only = administered 10 mg codeine/kg b.w of rats; RHE Only = administered 500 mg RHE/kg b.w rats; COD + 250 mg RHE = administered 10 mg codeine/kg b.w + 250 mg RHE; COD + 500 mg RHE = administered 10 mg codeine/kg b.w of rats + 500 mg RHE; COD + 1,000 mg RHE = administered 10 mg codeine/kg b.w of rats + 1,000 mg RHE.

The results presented in Table 2 showed that the sperm volume of the RHE control group was significantly higher than that of the other groups, while the codeine control group and the group that received codeine with 250 mg/kg RHE recorded the lowest sperm volume. There were no recorded significant differences in sperm pH among the groups. The results also revealed that the RHE control, negative control, and the group that received codeine with 1,000 mg/kg RHE recorded more normal, viable and active sperm than the other experimental groups. Meanwhile, the codeine only treated group recorded more abnormal and dead sperm when compared to the remaining groups, while all the groups that received codeine with or without the RHE recorded more sluggishly active sperm than the negative control and the RHE control groups. The sperm cell counts of the RHE control, negative control, and the group that received codeine with 1,000 mg/kg RHE were significantly higher than those of the other groups. Meanwhile, the codeine only treated group recorded the lowest sperm cell count. The recorded data of the codeine control group is in line with that of a previous study which posited that synthetic opioid (tramadol) intake reduces sperm quality in male albino rats [39-41]. The recorded alterations in the assayed testicular markers and hormones of the codeine administered animals may be linked to elevated ROS damages of the mitochondria resulting in the functional disruption of the pituitary glandal axis responsible for the cellular processes and regulations involved in spermatogenesis and steroidogenesis, which may ultimately lead to infertility in the male rats. Moreover, another study by Shi *et al.* [42] revealed that a rise in aberrant sperm morphology and a decrease in sperm count and motility are two ways that ROS might cause oxidative damage to sperm. Ajayi and Akhigbe [43] demonstrated that codeine intake produces poor sperm quality as it causes decreased sperm count, motility, and viability. Furthermore, codeine intake causes changes to normal morphology and distortion of sperm membrane integrity, resulting in increase in sperm DNA fragmentation, oxidative damage, and apoptosis. The poor sperm quality recorded by the codeine only treated group in this study is also in consensus with a study by Nna and Osim [44], who demonstrated an association between poor sperm quality and tramadol intake. The reduced sperm count recorded in this study may have been induced by diminished testosterone levels recorded after codeine administration through spermatogenesis suppression [45]. It is obvious that for spermatogenesis to proceed, the levels of serum and testicular testosterone must be at the optimum; thus, the reduced testosterone level observed possibly led to impaired spermatogenesis, which in turn reduced sperm count.

The results of the histological examination of the testis tissues of the experimental animals at 400x magnification are presented in Figures 1-6. The photomicrograph of the codeine only treated rats revealed a histologically distorted testis with interstitial spaces containing Leydig cells, normal shaped seminiferous tubules containing spermatogonia, spermatozoa, spermatocytes, and vacuolated tubules in their lumen, while the photomicrograph of the codeine + RHE treated groups revealed normal histology of the testis. The negative control and the RHE control groups recorded a histologically normal testis with normal interstitial spaces Leydig cells (LC), and normal shaped seminiferous tubules lined by basement membranes (BMs) containing spermatogonia (SPG), primary spermatocytes (PSPC), secondary spermatocytes (SSPC), spermatozoa (SPZ) and Sertoli cells (SC). The histology results revealed that codeine administration caused vacuolated tubules in the lumen of the testis. This is in consensus with the report of Caju *et al.* [46], who noted that acute and chronic opioid (morphine) administration caused reduction in Sertoli and Leydig cells, and increased vacuolated tubules in the lumen of the testis of matured male rats. According to Sawy and Malak [47], sperm loss and lower fertility were found a studied rat as a result of tramadol infusion impaired the function of seminiferous tubules. Furthermore, it is known that changes in the thickness of the basement membrane can impair testicular metabolism, and thus promotes enhanced germinal cell hypoplasia and tubular atrophy [48]. Additionally, it is necessary to keep in mind that the extent of testicular injury is significantly influenced by how long a person uses drugs [49]. RHE

Table 2. Impact of rice husk extract on sperm indices of rats exposed to codeine

Parameter	NC	COD Only	RHE Only	COD+250 mg RHE	COD+500 mg RHE	COD+1,000 mg RHE
Sperm Volume (mL)	0.23±0.06^{a}	0.17±0.06^{a}	0.37±0.06^{b}	0.17±0.06^{a}	0.22±0.06^{a}	0.23±0.06^{a}
Sperm pH	8.00±0.00^{a}	8.00±0.00^{a}	8.00±0.00^{a}	8.00±0.00^{a}	8.00±0.00^{a}	8.00±0.00^{a}
Sperm Viability (%)	85.00±5.00^{b}	66.67±11.55^{a}	90.00±5.00^{b}	70.00±10.00^{a}	76.67±5.77^{ab}	85.60±5.00^{b}
Normal Sperm (%)	81.67±2.89^{bc}	65.00±13.23^{a}	88.33±2.89^{c}	70.00±10.00^{ab}	71.67±5.77^{ab}	81.67±2.89^{bc}
Abnormal Sperm (%)	18.33±2.89^{ab}	35.00±13.23^{c}	11.67±2.89^{a}	30.00±10.00^{bc}	28.33±5.77^{bc}	18.33±2.89^{ab}
Dead Sperm Cell (%)	10.00±0.00^{a}	30.00±13.23^{c}	8.33±2.89^{a}	26.67±5.77^{c}	23.33±5.77^{bc}	11.67±2.89^{ab}
Active Sperm (%)	81.67±2.89^{c}	60.33±13.23^{a}	86.67±2.89^{c}	63.33±5.77^{a}	66.67±5.77^{ab}	78.33±2.89^{bc}
Sluggishly Active Sperm (%)	8.33±2.89^{b}	10.00±0.00^{b}	5.00±0.00^{a}	10.00±0.00^{b}	10.00±0.00^{b}	10.00±0.00^{b}
Sperm Cell Count (x106)	650.00±50.00^{bc}	300.00±173.21^{a}	750.00±50.00^{c}	433.33±208.17^{ab}	450.00±50.00^{ab}	600.00±100.00^{bc}

Data expressed as mean ± SD; (n = 3 replicate). Groups with different superscript(s) are significantly different at $p \leq 0.05$, while groups with the same superscript(s) are not. Key: NC= Negative control; COD Only = administered 10mg codeine/kg b.w of rats; RHE Only = administered 500 mg RHE/kg b.w rats; COD + 250 mg RHE = administered 10mg codeine/kg b.w + 250 mg RHE; COD + 500 mg RHE = administered 10 mg codeine/kg b.w of rats + 500 mg RHE; COD. + 1,000 mg RHE = administered 10 mg codeine/kg b.w of rats + 1,000 mg RHE.

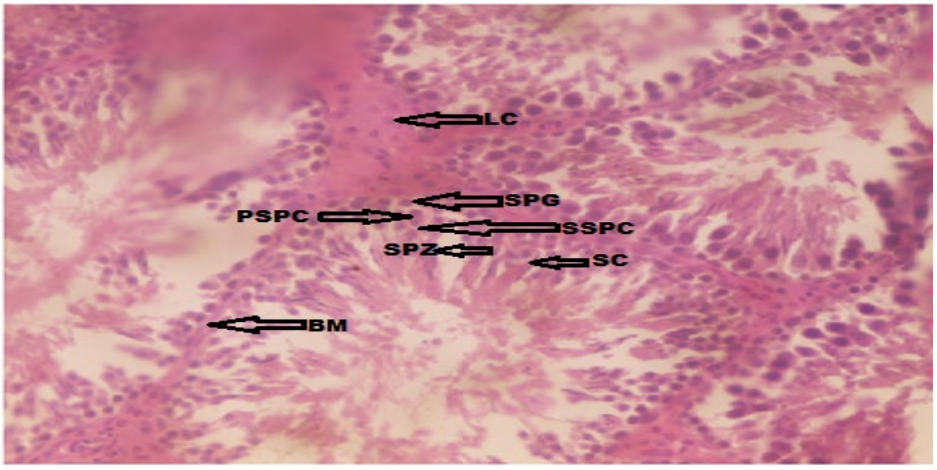

Figure 1. The photomicrograph of normal testis of Group I rats showing; Intact Leydig cells (LC) in interstitial space, normal shaped seminiferous tubules lined by basement membranes (BM) containing spermatogonia (SPG), primary spermatocytes (PSPC), secondary spermatocyte (SSPC), spermatozoa (SPZ) and sertoli cells (SC)

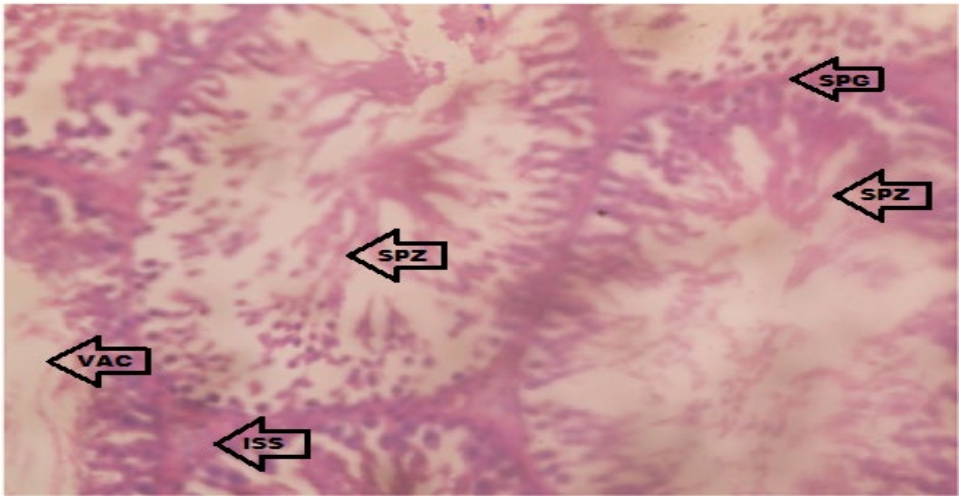

Figure 2. Photomicrograph of distorted testis of Group II rats showing interstitial spaces (ISS) containing Leydig cells (LC), normal shaped seminiferous tubules containing SPG, SPZ. Some tubules have vacuoles (VAC) in their lumens.

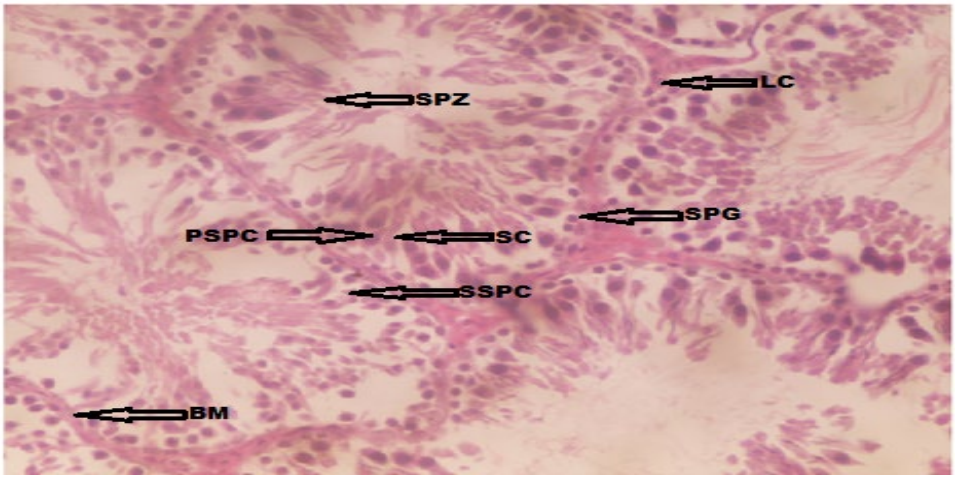

Figure 3. Photomicrograph of normal testis of Group III rats showing intact Leydig cells (LC) in the interstitial space, normal shaped seminiferous tubule containing SPG, PSPC, SPZ and SC, and seminiferous tubules lined by basement membrane (BM)

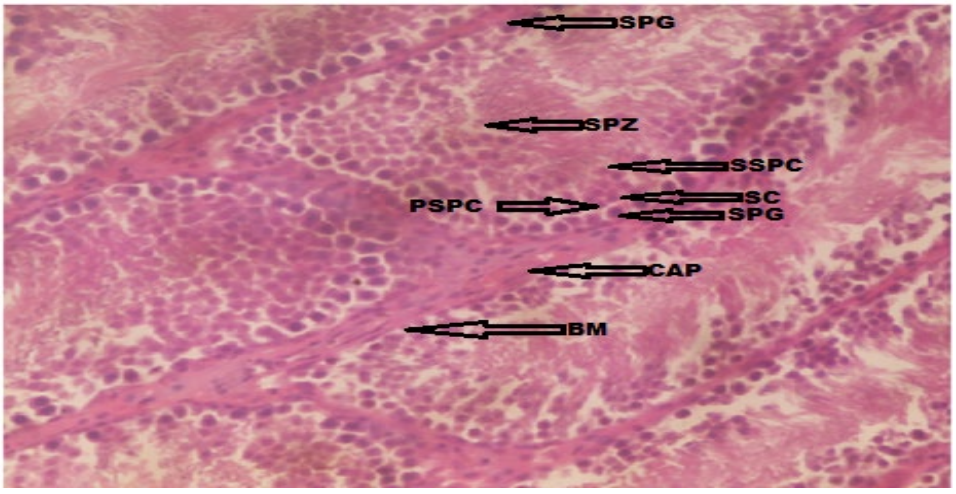

Figure 4. Photomicrograph of normal testis of Group IV rats showing normal shaped seminiferous tubules lined with capillary (CAP) and basement membranes (BM) containing SPG, PSPC, SSPC, SPZ and SC

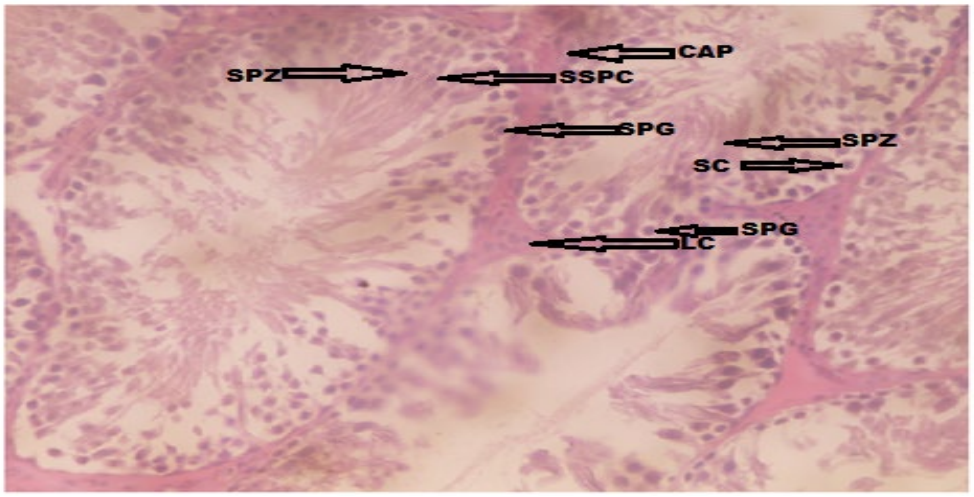

Figure 5. Photomicrograph of normal testis of Group V rats showing interstitial spaces containing Leydig cells (LC), normal shaped seminiferous tubule lined with capillary (CAP), SPG, SSPC, SPZ and SC

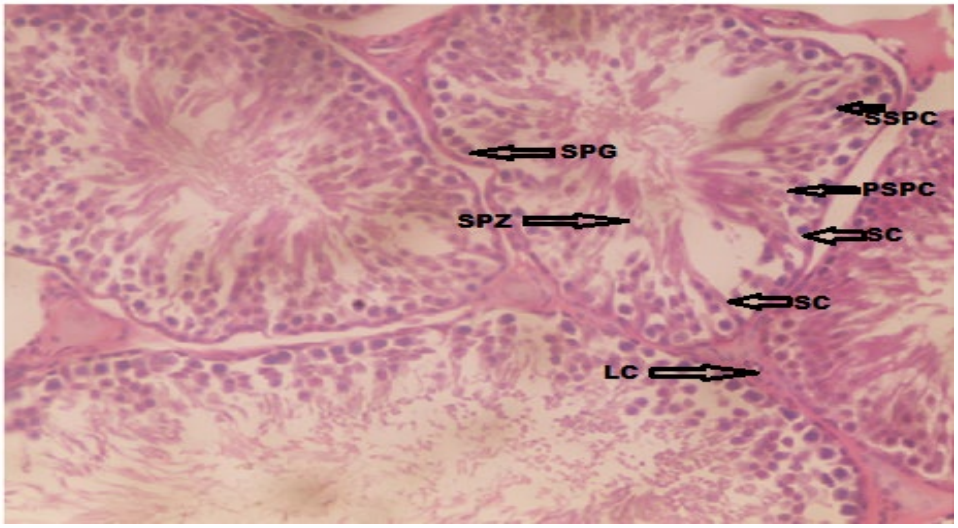

Figure 6. Photomicrograph of normal testis of Group VI rats

administration significantly improved the assayed hormones and also ameliorated the altered sperm quality induced by high codeine administration in a dose-dependent pattern. The recorded results may be linked to the phytochemical component of the extract and especially to its high antioxidant makeup that can deplete free radicals and further prevent oxidative damage to the reproductive organs. This report affirms the work of Lee *et al.* [50], who found that the high ROS depleting activity of rice hulls was a function of its phenolic content. The result of this study may also be attributed to the high flavonoid content of rice husk. This report is consonant with the report of Obianime and Uche [51], who observed that flavonoids may have a contributory effect to the

fertility properties and other pharmacological effects of plants. Flavonoids possess great antioxidant capacity that can deplete ROS and further prevent oxidative damage to the cells of the reproductive organs. According to Verma and Srivastav [52], RHE is rich in bioactive substances like phenols, polyphenols, flavonoids, glycosides, saponins, carotenoids, flavone, and flavonols, as well as saturated and unsaturated fatty acids, vitamins, and minerals. A review by Banihani [53] reported the role of vitamin B_{12} and B_6 in improving semen quality and sperm physiology in animal models. Another review article by Fallah *et al.* [54] reported on the significant contribution that zinc makes to antioxidant activity in male fertility and its prospective use as a nutritional marker in the management, detection, and intervention of male infertility. Additionally, vitamin C administration has been shown to lessen the damage caused by opioids in mice. According to Talkhooncheh *et al.* [55], vitamin C decreased opioid withdrawal symptoms and development of tolerance in morphine administered rats.

4. Conclusions

This study was carried out to establish the effects of subacute high dose codeine administration on the sex hormones and sperm quality of male Wistar rats, as well as the ameliorating impact of RHE against testicular dysfunction. This study showed that sub-chronic high dose codeine intake had the potential to alter male sex hormones and induce testicular injury. This study also demonstrated for the first time that RHE can improve male sex hormones and sperm quality, and as well can enhance the recuperation of codeine-induced testis injury. This study suggests that RHE may be employed as an effective therapy in boosting male sex hormones and treatment of male reproductive impairment and infertility.

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References

[1] Center for Behavioral Health Statistics and Quality, 2015. *Behavioral Health Trends in the United States: Results from the 2014 National Survey on Drug Use and Health*. [online] Available at: https://www.samhsa.gov/data/sites/default/files/NSDUH-FRR1-2014/NSDUH-FRR1-2014.pdf.

[2] International Narcotics Control Board, 2012. *Narcotic Drugs Estimated World Requirements for 2012.* Vienna: INCB.

[3] Bakare, A.T. and Isah B.A., 2016. Psychoactive substances use among in-patients in a Nigerian neuropsychiatric hospital: prevalence, pattern and presentation. *MOJ Addict Medical Therapy*, 2(1), 18-22.

[4] Aldhamin, A.S. and Hamid, N.M., 2022. Alterations in biochemical parameters and antioxidant enzymes in male mice as biomarkers of exposure to pollution with Cadmium. *Tropical Journal on Natural Product Research*, 6(11), 1794-1797.

[5] Cicero, T.J., Davis, L.A, Larigena, M.C., Meyer, E.R. and Schlegel, M.S., 2002. Chronic Pharmacol, opiate exposure in the male rat adversely affects fertility. *Biochemical*

Behaviour, 72, 157-163.

[6] Mckim, W.A., 2003. *Drugs and Behavior. An Introduction to Behavioral Pharmacology.* Vol. 1. 5th ed. New Jersey: Prentice Hall.

[7] Akhigbe, R. and Ajayi, A., 2020. Testicular toxicity following chronic codeine administration is via oxidative DNA damage and up- regulation of NO and caspase 3 activities. *PLoS One*, 15(3), https://doi.org/10.1371/journal.pone.0224052.

[8] Heba, Y.S. and Zidan, A.H., 2016. Histopathological and biochemical effects of acute and chronic tramadol drug toxicity on liver, kidney and testicular function in adult male albino rats. *Forensic Research and Criminology International Journal*, 2(4), https://doi.org/10.15406/frcij.2016.02.00060.

[9] Rubinstein, A.L. and Carpenter, D.M., 2017. Association between commonly prescribed opioids and androgen deficiency in men: a retrospective cohort analysis. *Pain Medicine*, 18, 637-644.

[10] Wang, X., Yuan, S., Wang, J., Lin, P., Liu, G., Lu, Y., Zhang, J., Wang, W. and Wei. Y., 2006. Anticancer activity of *litchi* fruit peri- carp extract against human breast cancer in vitro and in vivo. *Toxicology and Applied Pharmacology*, 215, 168-178.

[11] Asamarai, A.M., Addis, P.B., Epley, R.J. and Krick, T.P., 1996. Wild rice hull antioxidants. *Journal Agriculture Food Chemistry*, 44, 126-130.

[12] Jeon, K.-I., Park, E., Park, H.-R, Jeon, Y.J., Cha, S.-H. and Lee, S.-C., 2006. Antioxidant activity of far-infrared radiated rice hull extracts on reactive oxygen species scavenging and oxidative DNA damage in human lymphocytes. *Journal of Medicinal Food*, 9(1), 42-48.

[13] Butsat, S. and Siriamornpun, S., 2010. Phenolic acids and antioxidant activities in husk of different Thai rice varieties. *Food Science and Technology International*, 16(4), 329-336.

[14] Esa, N.M., Ling, T.B. and Peng, L.S., 2013. By-products of rice processing: an overview of health benefits and applications. *Rice Research*, 1, https://doi.org/10.4172/jrr.1000107.

[15] Saha, B.C. and Cotta, M.A., 2008. Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol. *Biomass and Bioenergy*, 32(10), 971-977.

[16] Oliveira, J.P.D., Bruni, G.P., Lima, K.O., Halal, S.L.M.E., Rosa, G.S.D., Dias, A.R.G. and Zavareze, E.D.R., 2017. Cellulose fibers extracted from rice and oat husks and their application in hydrogel. *Food Chemistry*, 221, 153-160.

[17] Salanti, A., Zoia, L., Orlandi, M., Zanini, F. and Elegir, G., 2010. Structural characterization and antioxidant activity evaluation of lignins from rice husk. *Journal of Agricultural and Food Chemistry,* 58(18), 10049-10055.

[18] Punvittayagul, C., Chariyakornkul, A., Sankam, P. and Wongpoomchai, R., 2021. Inhibitory effect of Thai purple rice husk extract on chemically induced carcinogenesis in rats. *Molecules*, 26(2), https://doi.org/10.3390/molecules26020360.

[19] Nilnumkhum, A., Punvittayagul, C., Chariyakornkul, A. and Wongpoomchai, R., 2017. Effects of hydrophilic compounds in purple rice husk on AFB1- induced mutagenesis. *Molecular and Cellular Toxicology*, 13, 171-178.

[20] Sankam, P., Punvittayagul, C., Sringam, K. Chaiyasut, C. and Wongpoomchai, R., 2013. Antimutagenicity and anticlastogenicity of glutinous purple rice hull using in vitro and in vivo testing systems. *Molecular and Cellular Toxicology*, 9(2),169-176.

[21] Tan, B.L. and Norhaizan, M.E., 2017. Scientific evidence of rice by- products for cancer prevention: chemopreventive properties of waste products from rice milling on carcinogenesis *in vitro* and *in vivo*. *BioMed Research International*, 2017, https://doi.org/10.1155/2017/9017902.

[22] Kim, S.P., Yang, J.Y., Kang, M.Y., Park, J.C., Nam, S.H. and Friedman, M., 2011. Composition of liquid rice hull smoke and anti- inflammatory effects in mice. *Journal of Agricultural and Food Chemistry*, 59(9), 4570-4581.

[23] Yang, J.Y., Kang, M.Y., Nam, S.H. and Friedman, M., 2012. Antidiabetic effects of rice hull smoke extract in alloxan- induced diabetic mice. *Journal of Agricultural and Food Chemistry*, 60(1), 87-94.

[24] Kim, S.-J., Park, H.-R., Park, E. and Lee, S.-C., 2007. Cytotoxic and antitumor activity of momilactone B from rice hulls. *Journal of Agricultural and Food Chemistry*, 55(5), 1702-1706.

[25] Okuno, Y. and Miyazawa, M., 2007. Suppressive components in rice husk against mutagens-induced SOS response using *Salmonella typhimurium* TA1535/pSK1002 Amu test. *Natural Product Research,* 21(9), 805-809.

[26] World Health Organization, 1999. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*. 4th ed. Cambridge: Cambridge University Press.

[27] Wells, M.E. and Awa, O.A., 1970. New technique for assessing acrosomal characteristics of spermatozoa. *Journal of Diary Science*, 53(2), 227-232.

[28] Zemjanis, R., 1970. *Diagnostic and Therapeutic Technigue in Animal Reproduction*. 2nd ed. Baltimore: William and Wilkins.

[29] Saad, N., Ismail, N., Mastuki, S.N., Leong, S.W., Chia, S.L. and Abdullah, C.A.C., 2022. Rice bran oil main bioactive compounds and biological activities. In: A.A. Mariod, ed. *Multiple Biological Activities of Unconventional Seed Oils*. Cambridge: Academic Press, pp. 195-213.

[30] Gao, Y., Guo, X., Liu, Y., Fang, Z., Zhang, M., Zhang, R., You, L., Li, T. and Liu, R.H., 2018. A full utilization of rice husk to evaluate phytochemical bioactivities and prepare cellulose nanocrystals. *Scientific Reports*, 8(1), https://doi.org/10.1038/s41598-018-27635-3.

[31] Vuong, C., Van Uum, S.H.M., O'Dell, L.E., Lutfy, K. and Friedman, T.C., 2010. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocrine Reviews*, 31(1), 98-132.

[32] Jin, J.-M. and Yang, W.-Y., 2014. Molecular regulation of hypothalamus-pituitary-gonads axis in males. *Gene*, 551, 15-25.

[33] Adedara, I.A., Ego, V.C., Subair, I.T., Oyediran, O. and Farombi, E.O., 2017. Quercetin imporoves neurobehavioral performance through restoration of brain antioxidant status and acetylcholinesterase activityin manganese-treated rats. *Neurochemical Research*, 42, 1219-1229.

[34] Heba, Y.S. and Zidan, A.H.M., 2016. Histopathological and biochemical effects of acute and chronic tramadol drug toxicity on liver, kidney, and testicular function in adult male albino rats. *Forensic Research and Criminology International Journal*, 2(4), https://doi.org/10.15406/frcij.2016.02.00060.

[35] El-Gaafarawi, I., Hassan, M., Fouad, G. and El-Komey, F., 2005. Toxic effects of paroxetine on sexual and reproductive functions of rats. *The Egyptian Journal of Hospital Medicine*, 21(1), 16-32.

[36] Riaz, M., Mahmood, Z., Shahid, M., Saeed, M.U.Q., Tahir, I.M., Shah, S.A., Munir, N. and El-Ghorab, A., 2015. Impact of reactive oxygen species on antioxidant capacity of male reproductive system. *International Immunopathology and Pharmacology*, 29(3), 421-425.

[37] Katz, N. and Mazer, N.A., 2009. The impact of opioids on the endocrine system. *Clinical Journal of Pain*, 25(2), 170-175.

[38] Gore, A.C., 2001. Gonadotropin releasing hormone neurons, NMDA receptors Cycle, and their regulation by steroid hormones across the reproductive life. *Brain Research Reviews*, 37, 235-248.

[39] Esua, I.S., Uno, U.U. and Ekaluo, U.B., 2019. Effect of tramadol on sperm profile of male albino rats. *Asian Journal of Research in Biochemistry*, 3(4), 1-6.

[40] Farag, A. G. A., Basha, M. A., Amin, S. A., Elnaidany, N.F., Elhelbawy, N. G., Mostafa, M. M. T., Khodier, S.A. and Mahfouz, R.Z., 2018. Tramadol (opioid) abuse is associated with a dose- and time-dependent poor sperm quality and hyperprolactinaemia in young men. *Andrologia,* 50(6), https://doi.org/10.1111/and.13026.

[41] Koohsari, M., Ahangar, N., Mohammadi, E. and Shaki, F., 2020. Ameliorative effect of melatonin against reproductive toxicity of tramadol in rats via the regulation of oxidative stress, mitochondrial dysfunction, and apoptosis-related gene expression signaling pathway. *Addict Health*, 12(2), 118-129.

[42] Shi, T.-Y., Chen, G., Huang, X., Yuan, Y., Wu, X., Wu, B., Li, Z., Shun, F., Chen, H. and Shi, H., 2012. Effects of reactive oxygen species from activated leucocytes on human sperm motility, viability and morphology. *Andrologia*, 44(Suppl. 1), 696-703.

[43] Ajayi, A.F. and Akhigbe, R.E., 2020. Codeine-induced sperm DNA damage is mediated predominantly by oxidative stress rather than apoptosis. *Redox Report*, 25(1), 33-40.

[44] Nna, V.U. and Osim, E.E., 2017. Testicular toxicity following separate and combined administration of PDE 5 inhibitors and opioid: Assessment of recovery following their withdrawal. *Andrologia*, 49(6), https://doi.org/10.1111/and.12669.

[45] Takzare, N., Samizadeh, E., Shoar, S., Zolbin, M.M., Naderan, M., Lashkari, A. and Bakhtiarian, A., 2016. Impacts of morphine addiction on spermatogenesis in rats. *International Journal of Reproductive BioMedicine*, 14(5), 303-308.

[46] Cajú, M.F., D'Angelo Queiroz, G.C., Torres, S.M., Tenório, B.M. and Júnior, V.A.S., 2011. Opioid system manipulation during testicular development: Result on sperm production and Sertoli cells population. *Acta Scienctiarum: Biological Sciences*, 33, 219-225.

[47] Sawy, M.M.E. and Malak, H.W.A., 2015. Effect of tramadol abuse on testicular tissue of adult albino rats: a light and electron microscopic study. *The Egyptian Journal of Histology*, 38(2), 356-366.

[48] Reuhl, J., Bachl, M., Schneider, M., Lutz, F. and Bratzhe, H., 2001. Morphometric, assessment of testicular changes in drug related fatalities. *Journal of Forensic Science International*, 115, 171-181.

[49] Sorge, R.E. and Stewart, J., 2006. Effects of long term chronic buprenorphine treatment on the locomotor and nucleus acumens dopamine response to acute heroin and cocaine in rats. *Journal of Pharmacology Biochemical Behaviour*, 84, 300-305.

[50] Lee, S.-C., Kim, J.-H., Jeong, S.-M., Ha, J.-U., Nam, K.C. and Ahn, D.U., 2004. Antioxidant activity of organic solvent extracts from far infrared-treated rice hulls. *Food Science and Biotechnology*, 13, 172-175.

[51] Obianime, A.W. and Uche, F.I., 2009. The phytochemical constituents and the effects of methanol extracts of *Phyllanthus amarus* leaves (kidney stone plant) on the hormonal parameters of male guinea pigs. *Journal of Applied Sciences and Environmental Management*, 13(1), 5-9.

[52] Verma, D.K. and Srivastav, P.P., 2017. Proximate composition, mineral content and fatty acids analyses of aromatic and non-aromatic Indian rice. *Rice Science*, 24, 21-31.

[53] Banihani, S.A., 2017. Vitamin B_{12} and semen quality. *Biomolecules,* 7(2), https://doi.org/10.3390/biom7020042.

[54] Fallah, A., Mohammad-Hasani, A. and Colagar, A. H., 2018. Zinc is an essential element for male fertility: A review of Zn role in men's health, germination, sperm quality, and fertilization. *Journal of Reproduction and Infertility*, 19(2), 69-81.

[55] Talkhooncheh, M., Alaei, H.A., Ramshini, E. and Shahidani, S., 2014. The effect of vitamin C on morphine self- administration in rats. *Advanced Biomedical Research*, 3, https://doi.org/10.4103/2277-9175.139524.