Stem Bark Extract of Cinnamomum Zeylanicum (Cinnamon) could Serve a Protective Role on Opioids Induced Toxicity on Male Fertility using Sprague Dawley Model of Rat

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ABSTRACT

Cinnamon is a natural herb that has a long history of safety and has so many health benefits. Fertility is the natural capacity to conceive a child. The issue of infertility affects both sexes equally. This study was therefore aimed at evaluating the potential of cinnamon to protect against opioid-induced toxicity in the male Sprague-Dawley rat model. A total of 30 rats were used in this study. The rats were weighed and divided into three groups. Group A (Control) received only normal feed (growers' mash) and distilled water, Group B received tramadol, rohypnol, and codeine, Group C received tramadol, rohypnol, and codeine and Cocosnucifera extract for 42 days (6 weeks). The animals were weighed, euthanized, and sacrificed, after which the serum was collected for testosterone evaluation and testicular tissue for histological processing. A highly significant reduction was seen in groups B, and degeneration was seen in the testes; however, improvement was seen in group C. The cell alterations caused by these substances could be alleviated using Cinnamomum zeylanicum.

Keywords: Sprague Dawley Rats, Cinnamon, Testosterone, Testicular Tissues, Narcotics

INTRODUCTION

The scientific name of Cinnamon is Cinnamomum zeylanicum, a member of the Lauraceae family and a natural herb with a long history of safety. It has a wide range of biological functions.[1] According to studies, cinnamon increased spermatogenesis and enhanced reproductive system performance in rats. [2]Aljuaid and Amin (2016) discovered that the pathological, degenerating, and atrophied seminiferous tubules and the biochemical changes brought on by HFD in the testes of Wister rats were all improved by Cinnamon aqueous extract.[3] In mice given Cinnamon extract for two weeks, a significant rise in spermatogonial, spermatocyte, spermatid, Sertoli, and Leydig counts was observed.[4] Additionally, it was noted that the testosterone levels in the treated animals had significantly increased.[4]

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The ability to conceive a child naturally is known as fertility. Not everybody is naturally fertile. Infertility, or the inability to conceive naturally after a year of unprotected sexual activity, affects about 11% of couples.[5]

The issue of infertility affects both sexes equally. Infertility can affect people of any gender, and everyone can take measures to increase their fertility. The male factor was reported to be the cause of 42.4% of infertility cases in the southwest of Nigeria, while in Maiduguri, northeastern Nigeria, infertility accounts for about 40% of all gynecological consultations.[7,8]

Opioids (narcotics) are addictive drugs that reduce the perception of pain and give users an inflated and false sense of well-being (euphoria).[9] These include drugs like opium and its derivatives, morphine, heroin, and codeine that alleviate pain, anxiety, and tension. [9]

This is the first study of its kind to address the issue of tramadol, Rohypnol (flunitrazepam), and codeine abuse in combination, and it was motivated by the spread of abuse with these drugs as well as the lack of studies on it and the low incidence.

Tramadol's exact mode of action is unknown, but the two most prevalent hypotheses are sedation and respiratory depression.[10,11,12] Similarly, tramadol, a common opioid of abuse, has been shown to significantly reduce sperm count, sperm viability, and normal morphology.[13]Although tramadol can cause fatal overdoses, these cases are rare, especially when considering how the drug affects various biochemical markers like testosterone, oxidative stress indices, urea and creatinine, liver function tests, and electrolytes.[14]

Codeine can lessen withdrawal symptoms from harsher opiates like heroin, and problem drug users are more cognizant of its abuse potential than the general public, according to research.[15]

The uncontrolled use of these drugs by youths in Nigeria and the increasing rate of infertility amongst male folks of today, coupled with the need to find possible remedial means to this menace in the country and rare studies on the use of cinnamon in treating narcotic-related tissue damages, alteration, and injury, necessitated this study. Therefore, the study is aimed at assessing *Cinnamomum zeylanicum* (Cinnamon) as a possible treatment measure or protective measure against narcotic-induced toxicity in male fertility using an animal model.

MATERIALS AND METHOD

Research Design

Fifty (50) adult male albino rats were used for this study. A simple Randomized Design was adopted.

Study Area

This study was carried out at the University of Nigeria and the University of Benin Teaching Hospital, Benin City, both in the Edo state. A state in Nigeria with a land area of 17,450 square kilometers, Edo State is situated between the longitudes 06° 04IE and 06° 43IE and the latitudes 05° 44IN and 07° 34IN.

Animal Housing and Acclimatization Process

A total of fifty (50) adult Wistar rats, five (5) weeks old, were obtained from the animal houseof Anatomy Department, University of Benin, Benin city, where they were allowed to acclimate for two weeks. To avoid contamination, they were housed in cages made of wire mesh with a tripod separating the animal from its feces. The mice were fed grower's mash and given water throughout the acclimation period. The standard guide for the care and use of laboratory animals was followed in maintaining and using the animals.

Animal Grouping

The experimental animals were separated into three groups (A-C). Each group contains ten rats (n=10), using ten (10) big cages to house them. Group A served as the control, while groups B-C served as the test groups.

Substance of Study

A considerable amount of tramadol, codeine, and Rohypnol was purchased from pharmaceuticals and stored at a temperature below 30 o C in a cool place pending usage.

Substance Administration

The medications were given orally in the following ways:

Group A (Control) received only regular food (grower's mash) and distilled water for 6 weeks.

Group B received feed, distilled water, 0.2mg/kg bw of tramadol, 0.2mg/kg bw of Rohypnol (6 weeks), and 0.2mg/kg of codeine (6 weeks).

Group C received 0.2mg/kg body weight of tramadol, Rohypnol, codeine, and cinnamon extract (6 weeks).

Sample Collection and Analysis

Before and following acclimatization, similar weight assessments were made after

each week. The average weight was then recorded. Under chloroform anesthesia, the testes of each rat were removed after 2, 4, and 6 weeks and fixed in 10% formalin for histological processing. After the experiment, the rats' growth performance and feed consumption were assessed.

Hematoxylin and Eosin Staining

Deparaffinize, ethanol-treat, and water-wash. Stain the sample for two minutes at room temperature with Harris hematoxylin. Let the color develop in the lukewarm water for 10 minutes, carry out eosin staining at room temperature for 3 minutes, distinguish, drench, and clear and mount [16].

Immuno histochemistry Staining Procedure

Deparaffinize, ethanol-treat, and water-wash; put a distilled water-filled stainless steel tray in a specialized autoclave; autoclave at 121°C for 20 minutes. remove and wash with PBS after it has cooled; apply endogenous peroxidase for 5 minutes at room temperature using a 3% hydrogen peroxide solution; and complete the blocking procedure (10% normal goat serum in PBS, 5 minutes at room temperature). (This step can be left out), place the primary antibody (see below) on top, and let the mixture react at room temperature for 30 to 40 minutes. use PBS to wash, after 30 minutes of room-temperature Envision+ Solution reaction, wash the area with PBS, perform the DAB staining reaction, wash with tap water, then allow to react with Meyer's hematoxylin for 30 seconds at room temperature, allow color to develop in lukewarm water, then dehydrate, clear, and mount [16].

Data Analysis

Following data collection, statistical analysis was performed using SPSS (version 20). ANOVA (Scheffe) was used to compare the test groups' values to those of the control group with a 95% level of confidence.

RESULTS

Figure 1: Group A (Control): Photomicrograph a, of Sections of the testis of experimental animal showing normal circular seminiferous tubules (long arrow) with pyknotic Sertoli cells, Leydig and other cells of spermatogenic series (short arrow), Group B (0.2mg/kg tramadol, 0.2mg/kg of Rophynol & 0.2mg/kg codeine for 6 weeks): Photomicrograph of Sections of the testes of experimental animal showing fairly circular seminiferous tubules (long arrow) with depleted pyknotic Sertoli cells, Leydig and other cells of spermatogenic series (short arrow), Group C (0.2mg/kg codeine, 0.2mg/kg Rophynol, 0.2 codeine for 6 weeks &*Cinnamomum zeylanicum* extract): Photomicrograph of Sections of the testes of experimental animal showing almost empty circular seminiferous tubules (long arrow) with reactive pyknotic Sertoli cells, Leydig and other cells of spermatogenic series (short arrow). X400 magnification.

Figure 2: Group A (Control): Photomicrograph of Sections of the testis of experimental animal showing seminiferous tubules which are circular in outline (long black arrow) and nucleated spermatid pyknotic Sertoli cells; there is no excessive Giemsa stain pigmentation in the lumen (short arrow), Group B Photomicrograph of Sections of the testes of experimental animal showing seminiferous tubules which are fairly circular in outline (long black arrow) and nucleated spermatid pyknotic Sertoli cells; there is no excessive Giemsa stain pigmentation in the lumen (short arrow). Testis Geimsa Staining (X400 Magnification

Figure 3: Group A (Control): Photomicrograph of Sections of the testis of experimental animal showing seminiferous tubules which are circular in outline (long black arrow) and nucleated spermatid pyknotic Sertoli cells, Group B (0.2mg/kg tramadol & 0.2mg/kg codeine for 6 weeks): Photomicrograph of Sections of the testes of experimental animal showing seminiferous tubules which are fairly circular in outline (long black arrow) and nucleated spermatid pyknotic Sertoli cells; with diffused specific ABP immune expression (short arrow). Testis androgen Binding Protein (Abp) Immunohistochemistry Staining (X400 Magnification).

Table 1: Showing the values of Testosterone in different groups

	Group A	Group B	P-value	Group C	P-value
T(ng/ml)	2.51±0.01	0.68±0.31	0.0002**	1.00 ±0.00	0.003*

*Means levels statistically significant ($p<0.05$) with control and across the group
Key: T (Testosterone)

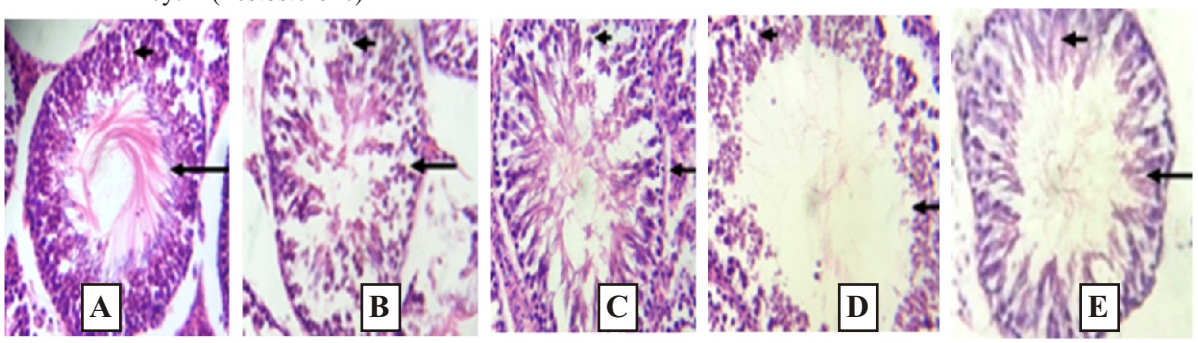

Figure 1: Group A (Control): Photomicrograph of Sections of the testis of experimental animal

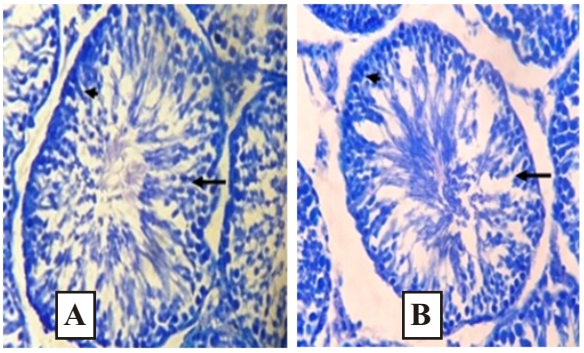

Figure 2: Group A (Control): Photomicrograph of Sections of the testis of experimental animal

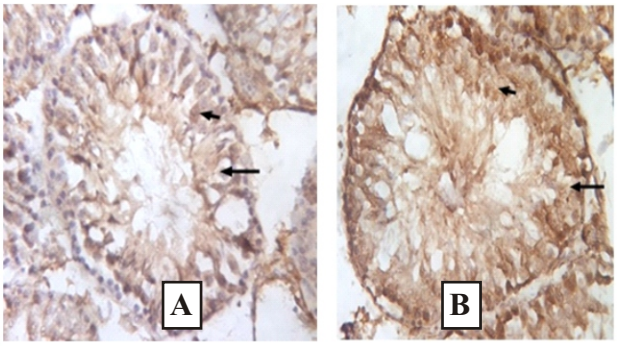

Figure 3: Group A (Control): Photomicrograph of Sections of the testis of experimental animal

DISCUSSION

In this study, the general tissue histology of group A shows normal histomorphology of the testes with circular seminiferous tubules, pyknotic sertoli cells, leydig cells, and other cells of the spermatogenic series. Group B shows testes histology with empty, fairly circular seminiferous tubules with depleted and reactive pyknotic sertoli cells, Leydig, and other cells of the spermatogenic series, and group C shows testes histology with normal circular seminiferous tubules, sertoli cells, Leydig, and other cells of the spermatogenic series.

This study and that of Ceccarell *et al.* (2006),[17] where it was reported that a single tramadol dose caused widely separated seminiferous tubules, are somewhat similar. The presence of intercellular vacuoles and the loss of intercellular bridges between the spermatogenic cells were both evident. Degenerated spermatogonia, spindle-shaped flat leydig interstitial cells, and broken tight junctions in Sertoli cells were all seen. This was in line with the findings of Ceccarell *et al.* (2006),[17] whose research shows that brain testosterone is significantly reduced four hours after taking a single tramadol dose. After receiving a single therapeutic dose of tramadol, Bastami *et al.* (2014)[18] reported that there was a significant variation in drug-related symptoms.

From Table 1 above, there was a significant reduction in the values of testosterone, but this significant reduction was reduced in group C, which is composed of codeine, Rohypnol, tramadol, and cinnamon extract. A previous study by Saleh and Ayuba (2021)[19], which demonstrated some seminiferous tubules undergoing hypertrophy by taking on a star-like shape

and having sparse interstitial connective tissue, is comparable to our findings. Rats given 60mg/kg of codeine displayed mild blood vessel oedema, focal mild degeneration of the seminiferous tubules, increased intertubular spaces, and focal seminiferous tubular hypertrophy (seminiferous tubules appeared star-shaped). Seminiferous tubular eruption, crumbling of the spermatogenic series cell, mild interstitial connective tissue oedema, increased intertubular spaces, mild focal oedema, scanty interstitial connective tissue, and disruption of the seminiferous tubular basement membrane were all observed in the testes of rats given 90mg/kg of codeine.

Similar to these findings was the work of Shen *et al*. (2002),[20] which attributed the protective effect of *C. zeylanicum* to the volatile oils in the bark, leaf, and root, which could explain the improvement in testosterone in Table 1. Cinnamaldehyde is very rich in antioxidants and helps in the elimination of reactive oxygen species. According to Chegini *et al*. (2019),[21] cinnamon acts as an antioxidant in food, increasing antioxidant enzymes and removing reactive oxygen species to improve testosterone levels, sperm counts, and motility. This helps to restore fertility through normal spermatogenesis. Reactive oxygen species (ROS) are a major contributor to sperm production and fertility rate optimization. Herbal antioxidants prevent and suppress ROS formation. Additionally, Cao (2008)[22] asserted that cinnamon's ability to reduce inflammation may be attributed to its high concentration of proanthocyanidins, which may be particularly effective at reducing inflammatory compounds and activating insulin signaling pathways through their antioxidant activity.

Our research data recorded a significant increase (P 0.05) in the level of serum testosterone hormone in the cinnamon-treated groups compared to the groups B, C, and D; this is associated with improved testicular structure. Similar to this finding was the work done by Shalaby and Mouneir (2004),[23] who claimed that after taking cinnamon, the amount of testosterone was raised by the zeylanicum extract. Additionally, it lessened the deteriorating lesions that diabetic rats' testes displayed. Furthermore, Fathiazad *et al*. (2013)[24] discovered that administering C. Due to the flavonoids in cinnamon, zeylanicum significantly raised the level of serum testosterone. Ismail claims that cinnamon extract's ability to reduce serum leptin levels is the cause of its antilipidemic effects. Leptin is a peptide hormone secreted by adipose tissue in proportion to its mass, according to Friedman's analysis in this regard.[25] Leptin regulates energy intake and expenditure by acting on the brain when it circulates in the blood. When the amount of body fat decreased, the plasma leptin level also decreased, which stimulated the appetite and reduced energy expenditure until the amount of body fat was restored[25].

The Greaves study,[26] which found that codeine causes testicular atrophy and degeneration of the seminiferous tubules in the testes of albino rats, was in line with our findings. The outcome revealed an irregular shape with widely spaced seminiferous tubules, disorganized interstitial tissue, and diffused vacuolations of the seminiferous tubules, which were also noted.[27] When tramadol was used in the study of Youssef and Zidan (2016)[28], histological analysis of the testicular tissues revealed atrophy of the seminiferous tubules with interstitial calcification. When tramadol dosage was gradually increased from 7.2mg/kg to 144mg/kg over 60 days, focal testicular degeneration with single and multiple layers of vacuolated spermatocytes was observed.[28]

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The histological changes in the testes caused by Rohypnol administration can be linked to a previous study that showed Rohypnol significantly increased mount latency, intromission latency, ejaculation latency, and postejaculatory interval, as well as lowered mount frequency, intromission frequency, and ejaculation frequency. Rohypnolinduced sexual dysfunction was found to be associated with significant suppression of circulatory folliclestimulating hormone, luteinizing hormone, testosterone, and oestrogen. According to the results of the current study, Rohypnol causes sexual dysfunction by suppressing the hypothalamic-pituitary-testicular axis. Rohypnol is also mentioned as a possible candidate for drug-induced infertility.[29]

CONCLUSION

Codeine, Rohypnol, and Tramadol were observed to have caused testicular damage and equally had a deleterious or reducing effect on testosterone, a hormone that controls male reproductive activities. However, cinnamon stem bark extract proves to be a potent improver of testosterone and also ameliorates the damaging effect meted out on the testicular tissue by the three opioid substances.

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