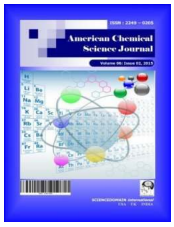

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Ameliorative Effect of Hydromethanolic Fraction of *Citrullus lanatus* Seeds on Biochemical and Histology Parameters of Female Wistar Rats Administered with Caffeine

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Authors' contributions

This work was carried out in collaboration between all authors. Author GIO designed the study, wrote the protocol. Author KWN wrote the first draft of the manuscript and managed the literature searches. Authors PCO and EU performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed at evaluating the ameliorative effect of hydromethanolic fraction of *C. lanatus* seeds on biochemical and histology parameters of female Wistar rats administered with caffeine. Oral administration of hydromethanolic fraction of *C. lanatus* seeds on experimental rats caused a significant reduction in serum aspartate, alanine and alkaline transaminases. The extract caused an increase dopamine and prolactin levels. The extract of *C. lanatus* at 200 mg kg^{-1} not only reversed the histopathological effects of caffeine but also counteracted the deleterious effects of caffeine - induced pituitary gland and hepatocyte injury by protecting pituitary gland tissues and hepatocyte cells. Thus hydromethanolic fractions of *C. lanatus* seeds have ameliorative effect.

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Keywords: Serum transaminases; dopamine; prolactin; C. lanatus; hepatocytes; pituitary gland.

1. INTRODUCTION

Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid that occurs naturally in coffee beans [1]. It is one of the most commonly ingested, pharmacologically active substances. It is present in coffee, tea, soda, cocoa, solid milk chocolate, and many medications [2]. Caffeine is rapidly absorbed from the digestive tract and distributes throughout all tissues [3]. Moreover, various mechanisms of action of caffeine like inhibition of phosphodiesterase [4], mobilization of calcium, binding to benzodiazepine receptors and blocking of adenosine receptors, mutagenesis [5], and inhibition of DNA poly-merase [6]. Psychotropic effects and tolerance to caffeine have also been reported [7,8]. Caffeine is readily absorbed following ingestion and is distributed in the body according to the water content of the tissues [9]. Caffeine has significant antioxidant ability in protecting membranes against oxidative damage and has ability to quench major reactive oxygen species [10]. Caffeine content in coffee varies widely depending on the type of coffee bean and the method of preparation used. Certain types of tea may contain somewhat more caffeine than other teas. Besides strength of the brew, growing conditions, processing techniques and other variables also affect caffeine content [11]. Chocolate derived from cocoa beans contains a small amount of caffeine. In a healthy liver, caffeine is mostly broken down by the hepatic microsomal enzymatic system. The resulting metabolites are mostly paraxanthines, theobromine and theophylline and a small amount of unchanged caffeine is excreted by urine [2].

The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism and its portal location within the circulatory system [12]. Caffeine is not an exemption and its constituents will be acted upon by the cells of the liver. Oral administration of caffeine at 50 mg kg^{-1} body weight on Wistar rat was said to increase plasma concentrations of AST, ALT and ALP and concentrations of about 100 mg kg^{-1} were found to be lethal [13]. However, there are reports of abnormally high concentrations of liver injury markers in a few patients who took caffeine. In these patients, cessation of caffeine consumption normalized liver function and resumption of caffeine drinking again elevated these biomarkers [14,15]. This study aimed at evaluating the ameliorative effect of hydromethanolic fraction of *C. lanatus* seeds on biochemical and histology parameters of female Wistar rats administered with caffeine.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total number of twenty-five (25) female Albino Wister rats weighing between 117-180 g were used for the study. The animals were purchased from the animal house of Department of Pharmacology, Niger Delta University Wilbeforce Island and kept in the animal house of the Deprtment of Medical Physiology Madonna University, Elele campus for three weeks to acclimatize. The animals were kept under room condition of temperature 25±2ºC, humidity of 50±5% and 12 hours light and day cycles. The animals were grouped into experimental and control groups (Table 1) and housed in sanitized wooden cages containing saw dust as bedding. They were also fed with standard rat chow pellet as diet and clean water ad-libitum was supplied.

2.2 Seeds Collection and Preparation

Mature watermelon pods were obtained from the local market in Elele, Rivers State, Nigeria in May, 2014. The seeds were extracted from the pods after it was allowed to rotting manually, by washing. Only healthy looking seeds (brown in colour, not floating on water, without mechanical

Table 1. Treatments groups used in this study

Group	Treatments	Durations	Number of rats
I	Normal feed + water	21 days	5
II	Normal feed + water + 200 mg kg^{-1} *C. lanatus*	21days	5
III	Normal feed + water + 50 mg kg^{-1} Caffeine	21days	5
IV	Normal feed + water + 100 mg kg^{-1} Caffeine	21days	5
V	Normal feed + water + 100 mg kg^{1} Caffeine + 200 mg kg^{-1} *C. lanatus*	21days	5

damage or sign of infection) were collected. The collected seeds were oven-dried at 35℃, until a constant weight was obtained. The dried seeds were reduced into fine powder using a laboratory grinding hand mill. The powder was weighed and kept away from light before extraction.

2.3 Seeds Extraction and Concentration

Extraction was by maceration over a period of 24 hours. After which 3398 g of the powdered seeds material was extracted with 4 litres of hydromethanol. The jar was tightly closed and thoroughly shaken intermittently. After 24 hours, the extract was filtered using Whatman No. 1 filter paper. The filtrate was collected in a glass jar as a brown colored liquid. The extract was concentrated using a Rotary Evaporator. The concentrated methanolic Extract of *C. lanatus* seeds were then quantitatively transferred into amber colored bottles covered with aluminiun foil and stored in a refrigerator at 4℃. The extract was thereafter placed in a water bath which made it concentrated to a gelatinous substance. After a week the concentrated hydromethanolic extract of *C. lanatus* seeds was dissolved in 2 litters of water and poured into a white bucket. A dose of 200 mg kg^{-1} was used for the administration. Caffeine anhydrous ($CH_{10}N_4O_2$; 1,3,7-trimethylxanthine), obtained from SIGMA Chemical Co. (St. Louis, MO, USA) was dissolved in physiological saline and made available in doses of 100 mg/kg and 50 mg/kg, these doses was used for the high dose and low dose administration respectively, as shown on Table 1.

2.4 Samples Collection and Analysis

After twenty-one (21) days of treatment, the animals were fasted for twenty-four (24) hours prior to sacrifice. The animals were anaesthetized using Chloroform and then sacrificed. Blood was collected via cardiac puncture with the aid of a syringe and transferred into tubes. Plasma samples were obtained by centrifugation at 860 g for 20 min and stored at -20℃ till measurement [16]. Liver and pituitary gland tissues were immediately removed, weighed and washed using chilled saline solution for histopathology study. Plasma alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) were assayed by the method of Reitman and Frankel [17]. Alkaline aminotransferase (ALP; EC 3.1.3.1) activity was measured at 405nm by formation of paranitrophenol from para-nitrophenylphosphate as a substrate [18]. Prolactin was assayed by enzyme-based immunoassay system method as described by Duhau et al. [19] and Grassi and Pradelles [20], while dopamine was assayed by enzyme-based immunoassay system method of Shome and Parlow [21]. The liver and pituitary gland tissues were fixed in formalin, after complete fixation the blocks was embedded in paraffin and sections cut at 5 µm (micron) using a microtome and then stained with haematoxylin and eosin and mounted in Canada balsam. Microscopic examination of the sections was then carried out under a light microscope and later the microscopic slides of the testes were photographed at magnification 40X.

2.5 Statistical Analysis

The results obtained from this study were analyzed using the statistical package for social science (SPSS) version 17.0 windows. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $P<0.05$. Post hoc multiple comparisons for differences between groups within groups were established using least significant difference (LSD), Turkey, scheffe and Duncan.

3. RESULTS

The results of hydromethanolic fraction of *C. lanatus* seeds and caffeine on liver enzymes plasma levels of AST, ALT and ALP of female Wistar rats are shown on Table 2. AST and ALT level of experimental animals in group II was significantly lower ($p<0.05$) when compared to groups I, III, IV and V. However, the groups III and IV statistically were significantly higher ($p<0.05$) when compared to the control group. Notwithstanding, the group V statistically showed no significant difference ($p>0.05$) with group I. ALP levels of experimental animals in group II was significantly lower ($p<0.05$) to group I and other experimental groups. On the other hand, the groups III and IV statistically were significantly higher ($p<0.05$) to group I.

The results of hydromethanolic fraction of *C. lanatus* seeds and caffeine on liver enzymes plasma levels of prolactin and dopamine of female Wistar rats are shown on Table 3. Plasma prolactin level of experimental animals in group II statistically shows no significant difference ($p>0.05$) when compare with group I. However, there was significant difference ($p<0.05$) in the groups III, IV and V with the group I experimental animals. Plasma dopamine level of experimental

animals in groups II, III, IV and V shows significant difference ($p<0.05$) in with group I, with group V having significant higher ($p<0.05$) levels of dopamine.

The results of hydromethanolic fraction of *C. lanatus* seeds and caffeine on the hepatocytes of female Wistar rats are shown on Figs. 1-5. Histological sections of liver tissues of group I exhibited normalization of cells and almost exhibited normal hepatocytes. The hepatocytes plates and the intervening sinusoids were not congested. No necrosis was observed as shown in Fig. 1. Histological sections of liver tissues (hepatocytes) of group II exhibited normalization of cells and reduced sinusoidal dilation along with mild inflammogens as shown in Fig. 2. While that of group III exhibited mild degree of necrosis, normalization of cells and reduced sinusoidal dilation. The hepatocytes showed moderate intracytoplasmic vacuoles, the hepatic lobules were not distinct. They were seen to radiate as single plate or cells from the central vein towards the portal tracts (Fig. 3). The hepatocytes of group IV had no presence of cytoplasm. Sinusoids were distorted, no necrosis was seen, the sections showed moderate architectural distortion. Some of the hepatocytes showed moderate intracytoplasmic vaculation along cell membrane. Other hepatocytes showed granular eosinophilic cytoplasm with little or no vacuoles. No necrosis was seen (Fig. 4). The hepatic lobules of group V were outlined at the edges by portal tracts (collection of portal arteries, portal vein and bile canaliculi) that were unremarkable. The hepatocytes plates and the intervening sinusoids were not congested. No necrosis was also observed as shown in Fig. 5.

Table 2. Effect of hydromethanolic fraction of *C. lanatus* seeds and caffeine on liver enzymes plasma levels of AST, ALT and ALP of female wistar rats

Groups	AST(U/L)	ALT(U/L)	ALP(U/L)
I	39.14±2.06	42.85±1.61	116.54±6.58
II	30.43±0.65**	32.93±2.15*	90.65±2.63**
III	59.44±3.12*	50.79±1.55*	136.62±4.37*
IV	67.31±3.73*	63.23±3.07*	155.48±4.37*
V	42.67±1.29	42.29±2.15	126.60±3.84

Data represented as Mean ± SEM; (*) p<0.05 significantly different in comparison with control group; (**) p<0.05 significantly different in comparison with control and other experimental groups; n=4

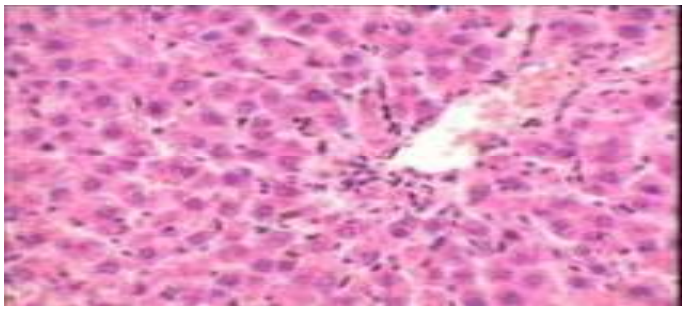

Fig. 1. Photomicrograph of hepatocytes of rats administered water (X40 H&E)

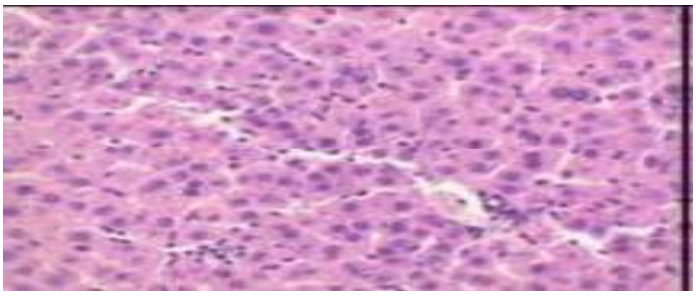

Fig. 2. Photomicrograph of hepatocytes of rats administered 200 mg/kg hydromethanolic fraction of *C. lanatus* seeds extract (X40 H&E)

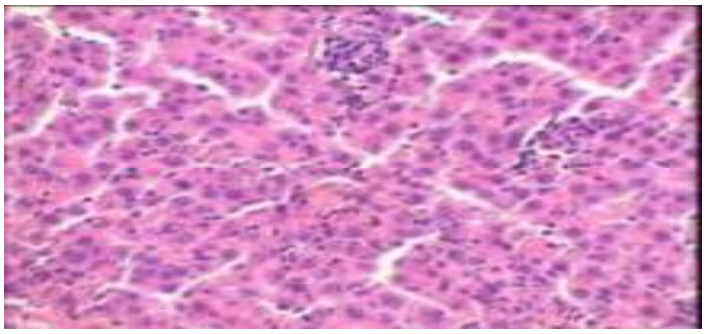

Fig. 3. Photomicrograph of hepatocytes of rats administered 50 mg/kg caffeine (X40 H&E)

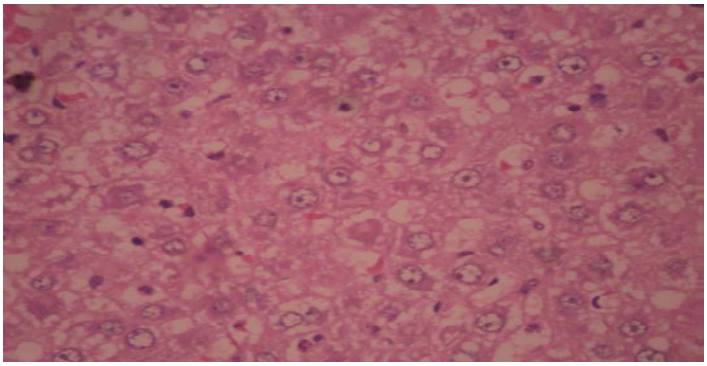

Fig. 4. Photomicrograph of hepatocytes of rats administered 100 mg/kg caffeine (X40 H&E)

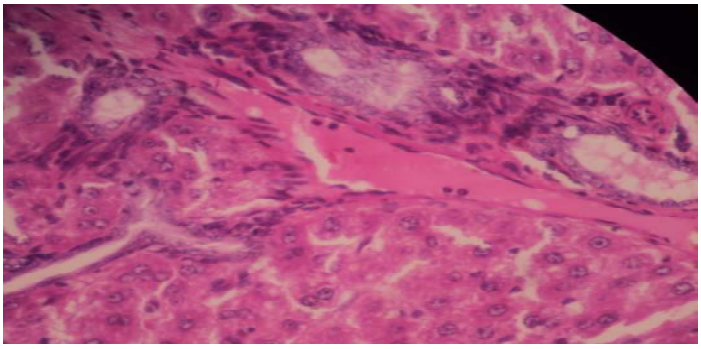

Fig. 5. Photomicrograph of hepatocytes of rats administered 200 mg/kg hydromethanolic fraction of *C. lanatus* seeds extract + 100 mg/kg caffeine (X40 H&E)

The results of hydromethanolic fraction of *C. lanatus* seeds and caffeine on the pituitary gland tissues of female Wistar rats are shown on Fig. 6-10. Histological sections in group I showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable as shown in Fig. 6. While the histopathological study of the anterior pituitary gland tissues of experimental animals in group II appeared normal with no irregularities or abnormalities (Fig. 7). However, group III showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable (Fig. 8). Also histopathological study of group IV appeared normal with mild irregularities or abnormalities. However, there was loss of

cytoplasmic contents and sparcity of the cells (Fig. 9). Group V showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer as shown in Fig. 10.

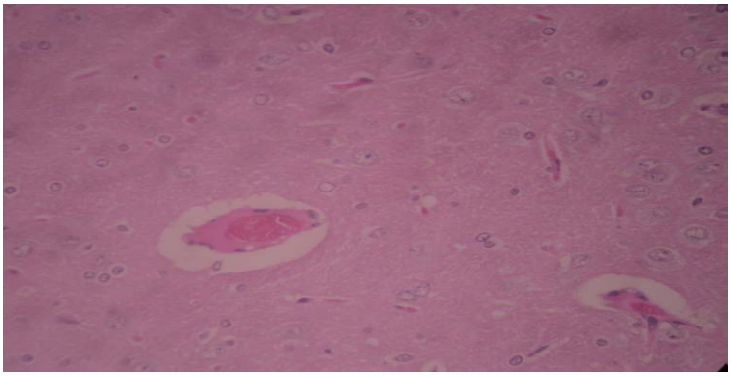

Fig. 6. Photomicrograph of anterior pituitary gland tissues of experimental control group (group I) (X40 H&E)

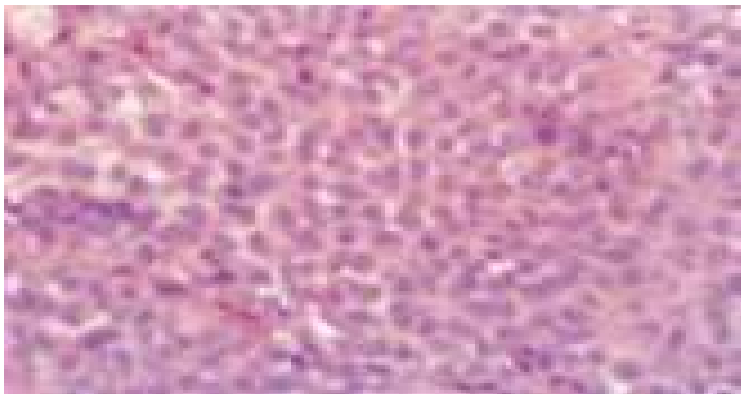

Fig. 7. Photomicrograph of anterior pituitary gland tissues of rats administered 200mg/kg hydromethanolic fraction of *C. lanatus* seeds extract (X40 H&E)

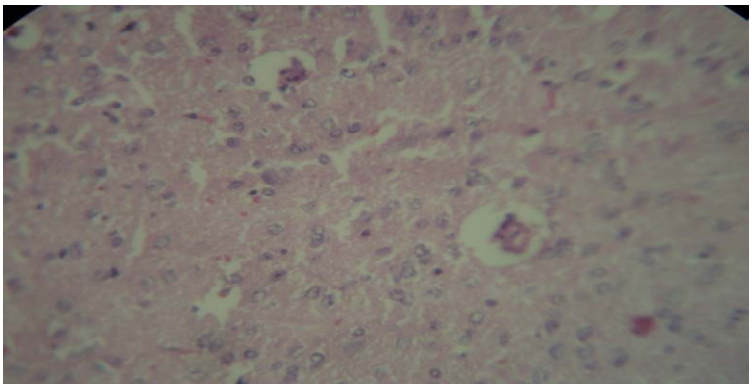

Fig. 8. Photomicrograph of anterior pituitary gland tissues of rats administered 50mg/kg caffeine (X40 H&E)

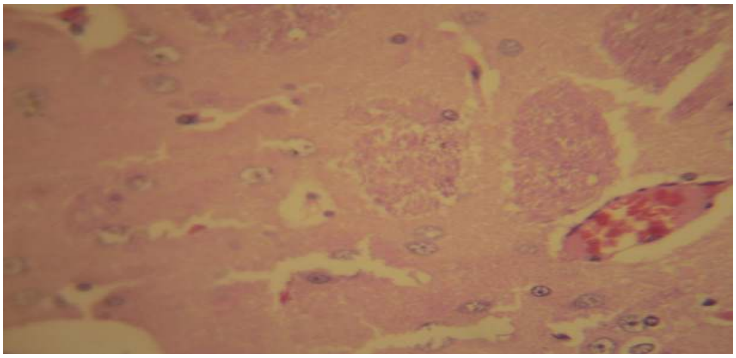

Fig. 9. Photomicrograph of anterior pituitary gland tissues of rats administered 100 mg/kg caffeine (X40 H&E)

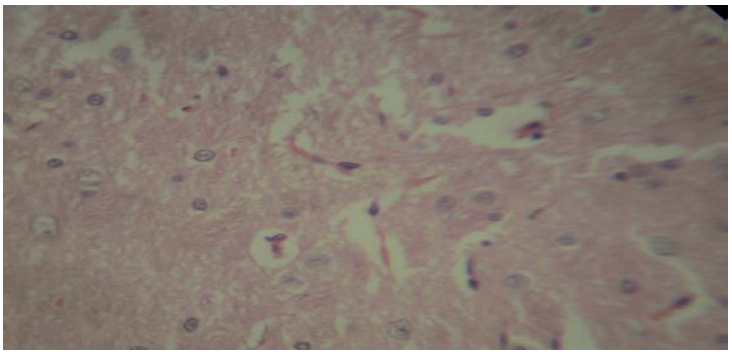

Fig. 10. Photomicrograph of anterior pituitary gland tissues of rats administered 200 mg/kg hydromethanolic fraction of *C. lanatus* seeds extract + 100 mg/kg caffeine (X40 H&E)

4. DISCUSSION

It has been hypothesized that liver enzymes are a target for caffeine or other components of coffee [22]. In this study, co-administration hydromethanolic fraction of *C. lanatus* seeds and caffeine resulted in a significant increase in mean plasma activity of ALT, ALP and AST over control values. It was observed that ALT was less affected by caffeine than AST levels. This study confirmed the findings of other investigators [23-27] who observed that subjects consuming coffee (caffeine) had a significant rise in mean concentrations of serum liver aminotransferases [28]. These results are also in agreement with Cheul Do et al. [29] who reported that there is an increase in the concentration of AST, ALT and ALP in serum of rats after treatment with caffeine. On the other hand, Ruhl and Everhart [30] and Cadden et al. [31] mentioned that, caffeine treatment leads to a mark decrease in serum ALT when compared with AST, this study also confirmed that. It is possible that the outer membrane of hepatocytes has become leaky but that cells are still largely intact. ALT is predominantly present in the cytoplasm of hepatocytes, whereas AST is predominantly present in the mitochondria. However, when hepatocytes sustain more severe injury, the serum levels of AST will exceed that of ALT [32] as reported in Table 2. These elevations of liver aminotransferase activity in serum may be indicative of disturbed integrity of liver cells caused by administration of caffeine at 50 and 100 mg kg^{-1}. However, it has been reported that serum activities of aminotransaminases returned to baseline after withdrawal of caffeine [27]. The results from this experiment are supported by other studies, which reported hepatic injuries related with high caffeine consumption [33-35]. The study showed that administration of *C. lanatus* at 200 mg kg^{-1} had significant ameliorative effect on the aminotransaminases (ALT, AST & ALP).

However, the effect of hydromethanolic fraction of *C. lanatus* seeds and caffeine on plasma levels of prolactin and dopamine of female Wistar rats are shown on Table 3. The results showed that treatment with high dose of hydromethanolic

extract of *C. lanatus* seeds on female wistar rats had a significant increase ($p<0.05$) in plasma dopamine level. Watermelon seeds contain phenylalanine, this could be responsible for the marked increase in dopamine level as phenylalanine is a precursor in the manufacture of dopamine. Phenylalanine is converted in the body into tyrosine which in turn is used to synthesize dopamine [36]. On the other hand, caffeine increases dopamine levels in the body system, acting in a way similar to amphetamines. It also produces increased concentrations of dopamine in the brain synapses [37]. More so, it excite dopaminergic neurons via glutamate neurons by removing an inhibiting effect due to metabotropic glutamate receptors thus by releasing this natural brake, would make the dopaminergic neurons more readily excitable [37]. *C. lanatus* did not have ameliorative effect on plasma prolactin and dopamine levels of experimental animals in experimental groups III, IV and V. Plasma dopamine and prolactin level of experimental animals in groups II, III, IV and V showed significant difference ($p<0.05$) with group I; group V having significant higher ($p<0.05$) levels.

Table 3. Effect of hydromethanolic fraction of *C. lanatus* seeds and caffeine on plasma levels of prolactin and dopamine of female wistar rats

Groups	Prolactin (ng/mL)	Dopamine (pg/mL)
I	19.99±2.03	13.73±2.13
II	23.33±2.26	25.65±1.44*
III	26.89±0.78*	24.61±1.87*
IV	31.17±1.16*	38.06±0.97*
V	33.45±1.76*	43.45±1.73*

Data represented as Mean ± SEM; (*) p<0.05 significantly different in comparison with group I; n=4

In the present study, the hepatic cells cytoplasm of rat's given 100 mg kg^{-1} body weight (Fig. 9) caffeine appeared vacuolized with presence of lipid droplets, which might be attributed to degenerative changes within the hepatocytes. Such observations were previously reported by Mubarak [38] in rat submandibular salivary glands induced by caffeine for 8 weeks. The distortion of the radial arrangement of the sinusoids from the central vein, the distortion of the hexagonal shape of the hepatocytes with evidence of hepatic necrosis and the desquamation of the wall of the central vein of the liver in the group IV may be due to the cleaving of hazardous substances in caffeine resulting to haemoglobin-free erythrocytes, plasma and the liver, thus exposing the liver to the damage noticed. But administration of 200 mg kg^{-1} of *C. lanatus* ameliorates such effect noticed in experimental groups III and IV when compared with group II and V. Fig. 10 exhibited hepatic lobules which were outlined at the edges by portal tracts (collection of portal arteries, portal vein and bile canaliculi) that were unremarkable. The hepatocytes plates and the intervening sinusoids were not congested and no necrosis was observed.

Histological sections in group III and IV showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable, while group IV appeared normal with mild irregularities or abnormalities. Also, there was loss of cytoplasmic contents and sparcity of the cells. These findings showed caffeine - induced pituitary gland injury. But group I, II and V showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable. *C. lanatus* at 200 mg kg^{-1} not only reversed the pathological effects of caffeine but also counteracted on deleterious effects of caffeine - induced pituitary gland injury by protecting pituitary gland tissues and triggering immune system [39,40].

5. CONCLUSION

Oral administration of hydromethanolic fraction of *C. lanatus* seeds on experimental rats caused a significant reduction in serum aspartate, alanine and alkaline transaminases. However, the extract caused an increase dopamine and prolactin levels. The seeds extract of *C. lanatus* at 200 mg kg^{-1} not only reversed the pathological effects of caffeine but also counteracted on deleterious effects of caffeine - induced pituitary gland and hepatocyte injury by protecting pituitary gland tissues and hepatocyte cells. Thus hydromethanolic fractions of *C. lanatus* seeds have ameliorative effect.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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