

## The influence of human chorionic gonadotropin on hormonal and haematological profile of postpubertal male albino rats exposed to chronic oral administration of alcohol

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### Abstract

This study evaluated the influence of human chorionic gonadotropin on hormonal and haematological profile of postpubertal male albino rats exposed to chronic oral administration of alcohol. Twenty-four mature male albino rats were assigned to four groups (n=6). Group A was the control, given distilled water, Group B was given 30% ethanol (8 ml/kg) orally 3 times a week, Group C was given human chorionic gonadotropin (HcG) (50 IU/kg) subcutaneously 3 times a week and Group D was given HcG (50 IU/kg) subcutaneously + 30% ethanol (8 ml/kg) orally 3 times a week. The study was for 10 weeks, and hormonal profile and haematology were determined. The follicle stimulating hormone of Group B decreased significantly ( $P<0.05$ ) when compared to Groups A, C and D. The luteinizing hormone was significantly lower ( $P<0.05$ ) in Group B when compared to Groups A, C and D. The testosterone level was significantly higher ( $P<0.05$ ) in Group D when compared to Groups A, B and C. The results obtained from haematology indicated that haemoglobin concentration of Group B was significantly lower ( $P<0.05$ ) when compared to Groups A, C and D. Ethanol significantly reduced ( $P<0.05$ ) platelets when compared to Groups A, C and D. There was a significant reduction ( $P<0.05$ ) in the neutrophils and lymphocytes in Group B when compared to Groups A, C and D. The study showed that HcG improves the level of male hormones and haematology in postpubertal albino rats exposed to chronic alcohol administration.

**Keywords:** ethanol; haematology; HcG; hormones; male; rats

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### Introduction

Male hormones play an organizational role, priming the body to behave in a certain way once puberty begins, and an activational role, referring to changes in hormones during puberty that trigger behavioral and physical changes (Mooradian *et al.*, 1987). At the onset of puberty, the hypothalamus secretes high pulses of gonadotropin-releasing hormone (GnRH). In response, the pituitary gland releases follicle stimulating hormone (FSH) and luteinizing hormone (LH) into the male system (Mooradian *et al.*, 1987). Follicle stimulating hormone, stimulates the Sertoli cells, which help to nourish the sperm cells that the testes produce, to facilitate spermatogenesis (Ronko and Llanos, 2000). Luteinizing hormone stimulates the interstitial cells, called Leydig cells, to produce and release testosterone into the blood (Petering and Brooks, 2017).

Testosterone, the hormone responsible for the development of secondary sexual characteristics, stimulates spermatogenesis. (Skinner *et al.*, 1989; Michael and Wojciech, 2006). Male hormones (androgens) promote erythropoiesis which is mediated by the ability of these steroids to increase erythropoietin (EPO) production (Gregory II and Finlay, 1999).

Alcohol is any organic compound in which the hydroxyl functional group (-OH) is bound to a carbon. Alcohol (ethanol or ethyl-alcohol) is the ingredient found in beer, urine and spirits. It is formed when yeasts ferment (breaks down without oxygen) the sugars in different foods. Alcohol is classified as a “sedative hypnotic” drug which means it acts to depress the central nervous system (CNS) at high doses and at lower doses acts as a stimulant inducing feeling of euphoria and talkativeness. Consuming too much alcohol at one session can lead to drowsiness, respiratory depression, coma or even death. Alcohol has great effects on every organ in the body and these effects depend on the blood alcohol concentration (BAC) over a period of time (Brust, 2005).

Chronic consumption of alcohol lowers the level of testosterone through its conversion of one of its precursors into oestrogens through a process called aromatization. Testosterone is metabolized into oestradiol and similarly the immediate precursor of testosterone – androstenedione can be converted into a less potent oestrone. This conversion is greatly increased in chronic alcoholics and it has been discovered that individuals with alcoholic liver disease have increased levels of blood oestrogen (Van Thiel *et al.*, 1974,1978; Gordon *et al.*, 1976, 1978). Alcohol consumption results to certain abnormalities of red blood cells (RBCs) such as grossly enlarged RBCs as well as abnormally shaped RBCs that are subject to haemolysis, leading to anaemia (Duarte *et al.*, 1995). Alcohol also interferes with normal production and function of white blood cells which form the body defense against microorganism and other foreign substances (Homaidan *et al.*, 1984).

Human chorionic gonadotropin is a glycoprotein composed of 237 amino acids with a molecular mass of 36.7 KDa, approximately 14.5 alpha HcG and 22.2 KDa beta HcG. It is a heterodimeric with an alpha subunit identical to that of luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and beta subunit that is unique to the HcG (Hoerman *et al.*, 1990). It has LH like activity, hence it is used to stimulate ovulation in ovaries as well as testosterone production in the testis. Human chorionic gonadotropin is naturally produced in human placenta after implantation by the syncytiotrophoblast. It can be extracted from the urine of pregnant women or produced from cultures of genetically modified cells using recombinant DNA technology. Blood or urine tests for pregnancy measures HcG in concentrations commonly reported as thousandth international units per millilitre (mIU/mL). Human chorionic gonadotropin is extensively used for final maturation induction *in lieu* of LH, in the presence of one or more mature ovarian follicles, thus it triggers ovulation following its administration (Gregory II and Finlay, 1999; Cole, 2009). Alcohol has been reported to have deleterious effects on blood and male reproductive hormones, while HcG is known to be used in the treatment of sex hormone linked conditions such as undescended testes and poor libido in male (Allen *et al.*, 1998). Male sex hormones have been shown to stimulate erythroid colony forming units in the bone marrow and promote their differentiation into erythropoietin responsive cells and testosterone enhances intestinal iron absorption, iron incorporation in red blood cells, and haemoglobin synthesis (Gregory II and Finlay, 1999). Hence, the aim of the present study was to evaluate the protective potentials of HcG on hormonal and haematological changes caused by alcohol in male rats.

## Materials and Methods

### *Chemicals and reagents*

Human chorionic gonadotropin (HcG) (Wellona Pharmaceuticals Ltd, India), Ethanol (Sigma, USA), Normal saline, WBC diluting fluid and Bouin fluid were obtained from the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka.

### *Animals*

Twenty-four male albino Wistar rats (11-12 weeks of age) weighing 150-180g were used for this study. They were housed in the laboratory animal house of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. They were kept in metallic cages and given water and feed (Chikun finisher) *ad libitum*. The rats were acclimatized for two weeks before commencement of the experiment. The investigation was carried out according to the approved procedure for the use and handling of laboratory animals as prescribed by the research and ethics committee of the University of Nigeria, Nsukka.

### *Experimental design*

Twenty-four mature male albino rats were assigned to four groups (n=6). Group A was the control, given distilled water, Group B was given 30% ethanol (8 ml/kg) orally 3 times a week, Group C was given human chorionic gonadotropin (HcG) (50 IU/kg) subcutaneously 3 times a week and Group D was given HcG (50 IU/kg) subcutaneously + 30% ethanol (8 ml/kg) orally 3 times a week. The study was for 10 weeks.

### *Determination of parameters*

For hormonal assay, blood samples were collected from 16 rats from the orbital venous plexus, 4 samples per group into plain sample bottles (without EDTA) and the serum was used for assay of hormones: testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The hormones were assayed using commercial kits of Enzyme linked immunosorbent assay (ELISA) (Rudolf, 2005).

The Packed Cell Volume (PCV) and haemoglobin concentration were determined by the microhaematocrit method of Coles, (1986) and cyanmethaemoglobin method of Kachmar, (1970) respectively. For red blood cell (RBC) and white blood cell (WBC) counts, the improved Neubauer haemocytometer was used, with formol citrate diluting fluid for RBC and Rus-Teller diluting fluid for WBC. The differential leucocyte counts were obtained by examining the thin blood smear stained with Leishman stain and observed under x100 magnification of the light microscope. The erythrocytic indices were deduced using routine standard procedures (Uchendu *et al.*, 2015).

### *Statistical analysis of data*

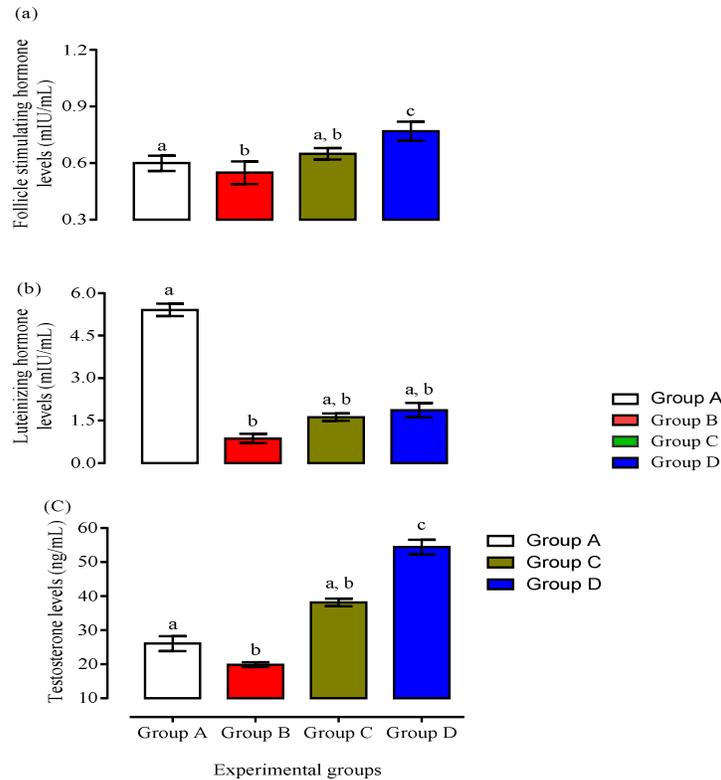
Data generated from the study were analysed using Graph pad® prism 6 statistical software. Results are presented as the means  $\pm$  standard error of the mean (SEM). Differences between the mean of the groups were compared using one-way analysis of variance (ANOVA) and Duncan's multiple range test. The statistical data were considered significant at  $P < 0.05$ .

## **Results**

The results obtained from hormonal assay (Figure 1) indicated a significant decrease ( $P < 0.05$ ) in FSH level in Group B when compared with Groups A, C and D. However, the value in Group D was significantly higher ( $P < 0.05$ ) when compared with Group C.

The mean value for LH was significantly lower ( $P < 0.05$ ) in Group B when compared with Groups A, C and D. However, the value of Groups C and D were similar.

The testosterone concentration significantly decreased ( $P < 0.05$ ) in Group B when compared with Groups A, C and D. However, values for Groups C and D were significantly higher ( $P < 0.05$ ) when compared to Group A, and there was a significant decrease ( $P < 0.05$ ) in Group C when compared with Group D.



**Figure 1.** The effects of HcG on hormonal profile of male rats exposed to chronic oral administration of alcohol

Note: Group A: control; Group B: alcohol administration; Group C: human chorionic gonadotropin and Group D: alcohol + human chorionic gonadotropin

Bars with different letters indicate significant difference ( $P < 0.05$ )

The results obtained from the haematological parameters are presented in Table 1. There was a significant decrease ( $P < 0.05$ ) in PCV of Group B ( $42.0 \pm 1.47$ ) when compared with Groups A ( $46.0 \pm 1.41$ ), C ( $46.5 \pm 1.32$ ) and D ( $45.8 \pm 0.85$ ). However, the mean values for Groups A, C and D were similar. The WBC count was significantly lower ( $P < 0.05$ ) in Group B when compared with Groups A, C and D. The mean value for Groups C and D were significantly higher ( $P < 0.05$ ) when compared with Group A. However, the values for Groups C and D were similar. The concentration of haemoglobin was significantly lower ( $P < 0.01$ ) in Group B when compared with Group A. The mean value in Group B was significantly lower ( $P < 0.05$ ) when compared with Groups C and D. The values in Groups C and D were similar. The RBC count of Group B decreased significantly ( $P < 0.05$ ) when compared with Groups A, C and D. The mean values for Groups A, C and D were similar. The mean value of platelet count was significantly lower ( $P < 0.01$ ) in Group B ( $107.5 \pm 2.50$ ) when compared with Groups A ( $115.0 \pm 2.89$ ), C ( $120.0 \pm 7.07$ ) and D ( $130.0 \pm 4.08$ ) (Table 1). The value in Groups C and D were significantly higher ( $P < 0.05$ ) when compared to Group A. However, the mean value of Group D was significantly higher ( $P < 0.05$ ) when compared to Group C. Neutrophils count was significantly lower ( $P < 0.01$ ) in Group B when compared with Groups A, C and D. The mean values for Groups C and D were significantly higher ( $P < 0.05$ ) than the normal (control). The values in Group D was significantly higher ( $P < 0.05$ ) when compared to Group C. The lymphocyte count decreased significantly ( $P < 0.001$ ) in Group B when compared with Groups A, C and D. The mean value in Group C was significantly lower ( $P < 0.05$ ) when compared to Group A, Group A was similar to Group D. The value in Group C was significantly lower ( $P < 0.01$ ) when compared with Group D.

**Table 1.** The effects of HcG on haematological profile of male rats exposed to chronic oral administration of alcohol

Parameters	Group A	Group B	Group C	Group D
Packed cell volume (%)	46.0±1.41 <sup>a</sup>	42.0±1.47 <sup>b</sup>	46.5±1.32 <sup>a</sup>	45.8±0.85 <sup>a</sup>
White blood cell count (x10 <sup>9</sup> /l)	8.55±0.29 <sup>a</sup>	7.15±0.21 <sup>b</sup>	10.50±0.17 <sup>c</sup>	10.95±0.21 <sup>c</sup>
Haemoglobin conc. (g/dl)	23.08±0.87 <sup>a</sup>	16.40±0.47 <sup>b</sup>	17.48±0.67 <sup>c</sup>	17.73±0.90 <sup>c</sup>
Red blood cell count (x 10 <sup>12</sup> /l)	7.30±0.06 <sup>a</sup>	4.63±0.05 <sup>b</sup>	7.50±0.04 <sup>a</sup>	7.53±0.03 <sup>a</sup>
Platelet count (x 10 <sup>9</sup> /l)	115.0±2.89 <sup>a</sup>	107.5±2.50 <sup>b</sup>	120.0±7.07 <sup>c</sup>	130.0±4.08 <sup>d</sup>
Neutrophils count (%)	63.0±2.08 <sup>a</sup>	55.5±2.22 <sup>b</sup>	66.50±1.50 <sup>c</sup>	69.50±1.89 <sup>d</sup>
Lymphocyte count (%)	39.50±2.36 <sup>a</sup>	27.50±1.50 <sup>b</sup>	32.00±1.82 <sup>c</sup>	38.00±2.94 <sup>a</sup>

Data on the same row with different superscript letters indicate significant difference (P<0.05)

## Discussion

The significant reduction in the levels of FSH, LH and testosterone concentrations in Group B when compared with Groups A, C and D may be attributed to the fact that alcohol inhibits the production and release of LH by inhibiting the movement of an enzyme protein kinase C from within the LH producing cells to the cell surface. This movement is essential for GnRH to stimulate the production and release of LH (Steiner *et al.*, 1997). Alcohol interferes with the complex mechanism in the chain of events involved in the binding of GnRH to the pituitary cell for the release of Luteinizing hormone (Van Thiel *et al.*, 1974, 1978; Gordon *et al.*, 1976, 1978; Salonen and Huhtaniemi, 1990). Alcohol also adversely affects Leydig cells resulting in reduced testosterone levels in the blood. It impairs the function of the testicular Sertoli cells that play an important role in sperm cell maturation (Emanuele and Emanuele, 1998). It was noted in this present study that testosterone level was significantly higher in Groups C and D (Figure 1) when compared to Groups A and B. This could infer that human chorionic gonadotropin enhances the stimulatory effect of LH surge in the release of testosterone by the Leydig cells in the testicular interstitium. This fits well with the findings of Skinner *et al.*, (1989); Michael and Wojciech, (2006) that a surge of luteinizing hormone promotes testosterone release which, in combination with the presence of follicle stimulating hormone, are needed to support spermatogenic process. In Group D, human chorionic gonadotropin also stimulated an increase in testosterone production by the Leydig cells. This increase in testosterone biosynthesis may be responsible for the drastic reduction in LH in this group through inhibition by negative feedback mechanism (Figure 1). Moreover, the reduction in serum testosterone level in Group B should expectedly, have led to an elevation in serum LH concentration even higher than in the control, due to the inadequate levels of testosterone to shut off further LH production from the pituitary gland. The increase in LH would have continued to stimulate the Leydig cells to make up for the short fall in testosterone production in a homeostatic manner. The over stimulation could even cause hypertrophy of the Leydig cells (Birken *et al.*, 1990; Ronco and Llanos, 2000). This, however, was not the case most likely due to the modulatory effect of alcohol on gonadotropin secretion. There is evidence that alcohol suppresses pituitary LH secretion via its inhibitory effect on LHRH release by the hypothalamus (Retorri and McCann, 1997). This may have accounted for the low levels of LH and testosterone in this group (that is, Group B). Earlier reports on chronic alcohol consumption indicated that ethanol induces changes in testicular activities in mature male rats. This corroborates the findings of Van Thiel *et al.* (1978) who stipulated that ethanol has a direct effect on the Leydig cells of the testis by decreasing LH level and a decrease in serum testosterone level through inhibition of testosterone biosynthesis. The Leydig cells are responsible for the production of testosterone in the presence of LH and supported by Sertoli cells. These Sertoli cells secretes androgen binding proteins (ADP) in the lumen of the seminiferous tubules which causes an increase in intratesticular testosterone concentration, it is this increase coupled with FSH that is needed for spermatogenesis.

The significant decrease in haemoglobin concentration in Group B in this study may be that excessive alcohol consumption results in suppression of blood cell production which reduces the number of blood cell precursors in the bone marrow and causes characteristic structural abnormalities in these cells resulting in nonfunctional mature red blood cells which adversely affects the concentration of haemoglobin (Ballard, 1989; 1993). The significant reduction in concentration of haemoglobin in Group B when compared with Groups A, C may have reduced the oxygen carrying capacity of the blood in that group, as haemoglobin is the pigment in blood that binds with oxygen. Also, the formation of red blood cell precursors with large vacuoles often leads to premature and accelerated cell death. It was also observed in this study that packed cell volume (PCV) and red blood cell (RBC) count were significantly ( $P < 0.05$ ) reduced in Group B when compared with other groups. This significant reduction in PCV and RBC counts could be that alcohol causes the production of defective red blood cells that are rapidly destroyed prematurely leading to anaemia (Ballard, 1993). Results obtained from this study correlates with the findings of Vonghia *et al.* (2008) who stipulated that chronic alcohol consumption results in certain blood abnormalities leading to anaemia (low haemoglobin).

Present findings indicate a significant reduction in leukocytes, neutrophils and lymphocyte counts in Group B which may be a consequence of alcohol causing suppression of the immune system. It interferes with the normal production and function of the white blood cells which forms the body defence against microorganisms and other foreign substances. Alcohol also impairs the function of neutrophils which is the primary defence against microorganisms and lymphocytes that mediates immune response (McFarland and Liebre, 1963). Neutrophils and lymphocyte counts were significantly reduced in Group B when compared to other groups which could be attributed to the deleterious effects of alcohol on these cells. The white blood cells also decreased significantly ( $P < 0.05$ ) in Group B when compared with Groups A, C and D which indicates that alcohol decreases body immunity. In Groups C and D, the concentration of neutrophils, lymphocytes and leucocytes was relatively high, which may indicate that HcG enhances immunity and body defence against microorganisms. Reduction in platelet counts in Group B could be the resultant effect of alcohol on platelets, this reduces the ability of the blood to clot. It is the function of platelets to form plugs that blocks ruptured blood vessels and capillaries (Lindenbaum and Hargrove, 1968; Duarte *et al.*, 1995). The mean value of platelet counts in Group C was significantly higher ( $P < 0.01$ ) when compared with Groups A and B, this may be that human chorionic gonadotropin improves platelet counts. However, Group D has the highest mean value of platelet counts which may be as a result of the influence of human chorionic gonadotropin in response to damage induced by alcohol to improve platelet counts. The mean values for the haematological parameters were relatively higher in Groups C and D except for haemoglobin concentration, where Group C and D were similar. This shows that HcG had a positive influence on haematological profile.

## Conclusions

The sexual behaviour and phenotypic appearances of an animal exposed to long term alcohol consumption may appear to be normal but the haematological, immunological and endocrine profile could be seriously compromised. This study has demonstrated that human chorionic gonadotropin has a positive influence on the haematological and hormonal profile of male albino rats exposed to chronic oral alcohol consumption.

## Authors' Contributions

Research conceptualization: CNU. Supervision, project administration, validation, visualization, writing - review and editing: CNU and RIO. Data curation, formal analysis, investigation and methodology: RIO and AMS. Resources: CNU, RIO and AMS. Software and writing - original draft: AMS.

All authors read and approved the final manuscript.

### **Ethical approval**

This research was approved by research and ethics committee of University of Nigeria, Nsukka. Ethical review was in conformity with the norms of researches involving animals.

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### **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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