Modulatory Functions of *Craterispermumschweinfurthi*on the Hypothalamic-Pituitary-Gonadal Axis of Male Wistar Rats in Phenyl Hydrazine Induced Testicular Toxicity

ABTRACT

Introduction: Modulation of the hypothalamic-pituitary-gonadal axis **is** mediated by different factors which remain a major research interest.

Aim: To evaluate the modulatory functions of *Craterispermumschweinfurthi*leaf extracton the hypothalamic-pituitary-gonadal axis of male Wistar rats in phenyl hydrazine induced testicular toxicity.

Methodology: 40 male Wistar rats weighing between 100-250g were used for the study, and were randomly divided into 8 groups of 5 rats each. Testicular toxicity was induced intraperitoneally using 40mg/kg of phenyl hydrazine administration at 9am on day 0 and two additional injections at 9am and 6pm on day 1 in all rat groups except groups 1 and 8 and were treated as follows for 14 days; Group 1: Control group; rats in this group received distilled water only: Group 2: Untreated Phenyl hydrazine induced toxicity rats: Groups 3-5 received 250mg/kg, 500mg/kg and 750mg/kg body weight of the extract: Group 6: Rats in this group were administered 0.23ml/kg of Bioferon: Group7: Phenyl hydrazine + Phytosterol (2000mg/kg): Group 8: Phytosterol only (2000mg/kg). 24 hours after the last administration, the rats were anaesthetized using 3.5% chloroform soaked in cotton wool and blood samples collected through direct cardiac puncture for the estimation of serum concentration of reproductive hormones. Also, rats caudal epididymides containing sperm were excised for the determination of sperm indices.

Results: Administration of the hydromethanol leaf extract of C*raterispermumschweinfurthi*to rats in Groups 3-5, significantly increased serum concentration of luteinizing, Follicle stimulating hormones and Testosterone compared to Group 2 (phenyl hydrazine induced toxicity) rats ($p<0.05$): Suggesting a possible modulatory function of the extract.Significantly dose dependent higher values of sperm volume, viability, count, normal and active sperm was observed amongst groups 3-5 rats following the administration of graded doses of the extract compared to Group 2 (phenyl hydrazine induced toxicity) rats ($p<0.05$). Suggesting a possible amelioration of the toxic effect of phenyl hydrazine.

Conclusion: This study reports that administration of hydromethanol extract of C*raterispermumschweinfurthi*caused a significant and dose dependent improvement in the concentration of male reproductive hormones: resulting in a predictable increase in sperm indices in male Wistar rats. The actual mechanism of action is presently unclear and would require further studies

Keywords: C*raterispermumschweinfurthi*, Phenyl hydrazine, toxicity, hypothalamic-pituitary-gonadal axis

INTRODUCTION

A challenging global phenomenon affecting mankind lies in adequate understanding, prevention, management and treatment of the ever-increasing male infertility, infertility is defined as the inability to conceive after about a year of unprotected regular sexual intercourse[1]. With about 12% prevalence rate, male infertility impacts over 30 million people globally.Male infertility is amajor contributing factor toabout 30% ormore reported cases of infertility worldwide[2] [3]. Male fertility depends largely on the serum concentration of male conceptive hormones: Luteinizing hormone, Follicle stimulating hormone &Testosterone and sperm characteristic: sperm count, quality, motility, viability, morphology, defects in any of these factors can cause infertility[4]About 90% of all reported infertile cases have a direct association with hormonal and sperm indices[5].Elevated scrotal temperature, endocrine disorders, lifestyle, environmental and nutritional factors have all been reported to negatively impact sperm parameters resulting in male infertility[6].Most of the aforementioned factors responsible for male infertility can be reversed surgically or therapeutically using drugs[7]. However, treatment options solely depend on the possible cause of male infertility, financial status, facilities available in a designated hospital, the patient's age and expertise[8].

In recent years, complementary therapies for infertility have received growing attention, and various nutritional approaches, and medicinal plants have been explored for the treatment of male reproductive disorders[9]. Several local medicinal plants with fertility boosting effects have been traditionally used globally[10] [11]. Fertility-related properties of plants are also of interest in modern day scientific research[12]. Specific important compounds identified in most medicinal plants are effective in the treatment, management, and prevention of disease conditions[13]. Contemporary approaches to infertility treatment have received growing attention following

men's increasing interest and reliance on effective herbal supplementation[14]. The European Association of Urology and the World Health Organization (WHO) have recently reported the use of traditional medicine as a multidimensional integrative approach to infertility treatment[15] [16]. Such a growing interest in medicinal plants has inspired scientists to clarify their effects in fertility studies. *Craterispermumschweinfurthi*species are shrubs with axillary paired at the nodes and often condensed. Its applications in traditional medicine are numerous. In traditional folklore medicine the seed, leaves, and inner bark have been described to have beneficial effects in stomach afflictions, ulcer, infertility, anemia, diabetes and fever[17]. Despite the wide use of *Craterispermumschweinfurthi* in folklore medicine in our environment, scientific studies on its fertility properties arerelatively scanty.

Hence, on account of its many described anecdotal benefits, the present study attempts an evaluation of the potential modulatory functions of thehydromethanol leaf extract of *Craterispermumschweinfurthi*on the hypothalamic-pituitary-gonadal axis of male wistar rats in phenyl hydrazine induced testicular toxicity.With the view of evaluating the traditional application of the leaves of C*raterispermumschweinfurthi*as an enhancer of reproductive functions in our environment. Also, an attempt was made to compare the effects of phytosterol, a major inherent bioactive compound identified in C*raterispermumschweinfurthi*leaves[13].

MATERIALS AND METHODS

Collection, Identification and Extraction of Plant Materials

Fresh leaves of *Craterispermumschweinfurthii*were obtained from the University of Port Harcourt Botanical Garden. Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Nigeria identified and authenticated the specimen and assigned a reference code; UPH/V/296. Voucher specimen was subsequently deposited in

the University Herbarium for future reference. The plant leaves were gathered, and all extraneous materials carefully removed. The leaves were air dried at room temperature for a minimum of 7 days after which it was pulverized into powder and the weighed quantity of 670.6g dissolved using Soxhlet device in 390ml of water-methanol mixture (25:75% v/v BDH) for three days in a jar. It was filtered and concentrated using a rotary evaporator at 40°C and the yield was 73%. Obtained extract was preserved in airtight containers and stocked at room temperature prior administration.

Procurement and Handling of Experimental Animals

Wistar rats weighing between 100-250g were used for the study. Animals were acquired from the Department of Physiology Animal House, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. Rats were placed in different compartments, one for each experimental group and cared for under standard laboratory conditions. Wood shavings and beddings were changed on a daily basis to prevent any infection due to unkept beddings. The animals were acclimatized for two weeks and subsequently grouped for the study.

Ethical Approval and Acute Toxicity Studies

Ethical approval was sought and obtained from the University of Port Harcourt Ethical Committee vide a communication referenced: UPH/CEREMAD/REC/MM82/024 and dated 23rd November 2021. The acute toxicity of the hydromethanol extract of C*raterispermumschweinfurthi*leaves was determined using Karber's method as modified by Aliu and Nwude, (1982)[18]. Lethal dose (LD50) of the extract was found to be 3968mg/kg body

weight. The study was conducted in accordance with the guidelines for the care and use of laboratory animals][19].

Phenyl hydrazine (PHZ), Drug and Phytosterol Purchase

Phenyl hydrazine (PHZ) was purchased from JHD Co., LTD, 618, Qingshan Road, Licang Dist., Qingdao, Shandong, China; Bioferonprocured from Biopharm Quality and Tradition, 12 Klemenova Dacha Street, Apt. 11, Kharkiv, 61033, Ukraine while Phytosterol was obtained from Wakunaga of America Co., LTD. Mission Viejo, CA92691 U.S.A.

Experimental Design

40 Wistar rats weighing between 100-250g were used for the study. After 14 days of aclimatisation, the rats were randomly divided into 8 groups of 5 rats each: designated Groups 1-8. Phenyl hydrazine testicular toxicity was induced intraperitoneally following 40mg/kg body weight of phenyl hydrazine administration on day 0 and two additional injections at 9am and 6pm on day 1 in all rat groups except groups 1 and 8 as was described previously[20] [21]. And were treated as follows for 14 days;

Group 1: Control group; rats in this group received extract vehicle only

Group 2: Untreated Phenyl hydrazine toxicity rats

Group 3: Low extract dose group; rats in this group received 250mg/kg of the leaf extract of C*raterispermumschweinfurthi*

Group 4: Medium extract dose group; rats in this group received 500mg/kg of the leaf extract of C*raterispermumschweinfurthi*

Group 5: High extract dose group; rats in this group were given 750mg/kg of the leaf extract of C*raterispermumschweinfurthi*

Group 6: Bioferon group; rats in this group were administered 0.23ml/kg of Bioferon[22] [21].

Group7: Phenyl hydrazine toxicity + Phytosterol (2000mg/kg)

Group 8: Phytosterol only (2000mg/kg)

24 hours after the last administration, the rats were anaesthetizedusing 3.5% chloroform soaked in cotton wool and blood samples collected through direct cardiac puncture and immediately transferred into plain sample tubes for the estimation of serum concentration of reproductive hormones: Luteinizing hormone, Follicle stimulating hormone and Testosterone.Also, rats caudal epididymides containing sperm were excised for the determination of sperm indices: Count, Viable, Active, Normal, Abnormal, Volume, Appearance/Morphology etc. The samples were immediately used for the estimation of the above variables.

Determination of Reproductive Hormones and Sperm Indices

Serum level of luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone was estimated using enzyme immunoabsorbent assay kits (Accu-Bind ELISA Microwells, California, USA). Procedure was as specified in the available manual.

Semen indices was analyzed using the computer-assisted semen analysis (CASA) version 11 (Hamilton Thorne Bioscience). The testes were excised and caudal epididymis carefully isolated and placed in a petri dish containing 3 ml of sodium bicarbonate (NaHCO3) buffered tyrodes' solution. 1 mm incisions were made on them and sperm carefully drawn into a plastic pipette which was subsequently transferred into 5 ml test tubes and shaken for homogeneity/dispersal of sperm cells. The following Sperm indices were evaluated: sperm motility, morphology, count, viability, activeness, sluggishness, dead rate etc.

Statistical Analysis

Results are as presented in Tables 1 and 2 as Mean ± Standard Error of Mean (SEM). Significant differences were determined using one-way ANOVA and LSD Post Hoc test. A p value of less than 0.05 was considered statistically significant.

RESULTS

Values of male reproductive hormones in phenyl hydrazine induced toxicity treated with extract and phytosterol

Table 1 shows that significantly lower values of Luteinizing hormone, Follicle stimulating hormone and Testosterone were observed amongst Group 2 rats following the administration of 40mg/kg body weight of Phenyl hydrazine compared to Group 1 (Control) rats ($p<0.05$). Suggesting a possible harmful reproductive effect of phenyl hydrazine in male Wistar rat. However, upon the administration of graded doses (250mg/kg, 500mg/kg and 750mg/kg) of the extract of C*raterispermumschweinfurthi*to rats in Groups 3, 4 and 5, significantly higher values of luteinizing hormone, Follicle stimulating hormone and Testosterone were observed compared to Group 2 (Untreated phenyl hydrazine) rats($p<0.05$).Indicating a possible modulatory function of the extract in male Wistar rats. Surprisingly, the values of these hormoneswere significantly increased ($p<0.05$) in a dose dependent manner with the administration of the extract**.** Similarly, Bioferon administration to Group 6 rats shows a significant improvement in the serum concentration of luteinizing hormone, follicle stimulating hormone and testosterone compared to Group 2 rats($p<0.05$). At a dose of 750mg/kg, the extract exhibited an increase of 0.36±0.004, 0.20±0.003 and 0.62±0.003 respectively in luteinizing hormone, Follicle stimulating hormone and Testosterone compared to Bioferon with an increase of 0.32±0.003, 0.18±0.005 and

0.61±0.003 in luteinizing hormone, Follicle stimulating hormone and Testosterone. Suggesting a possible greater potency of the extract at 750mg/kg body weight.

Also, Groups 7 and 8 rats administered 2000mg/kg body weight of phytosterol shows significantly higher values of luteinizing hormone, Follicle stimulating hormone and Testosterone compared to Group 2 rats($p<0.05$).

Values of sperm indices in phenyl hydrazine induced toxicity treated with extract and phytosterol

Table 2 shows significant reduction in sperm volume, viability, count, active and normal sperms amongst Group 2 rats following phenyl hydrazine administration compared to Group 1 (Control)rats. Also, there was a corresponding and significant increase in the population of sperms that were, abnormal, sluggish and dead compared to Group 1 rats.Indicating a possible harmful effect of phenyl hydrazine on sperm indices in male Wistar rats. Administration of graded dosesof the extract of C*raterispermumschweinfurthii*to Groups 3-5 rats demonstrated a dose dependent significant improvement in sperm volume, viability, count, normal and active sperms compared to Group 2 (phenyl hydrazine induced toxicity) rats ($p<0.05$). However, sperm viscosity and appearance remained unchanged thought out the duration of the study. Population of abnormal, sluggish and dead sperms were significantly decreased amongst Groups 3-5 rats compared to Group 2 ($p<0.05$). These findings indicate a likely beneficial effects of the extract on sperm parameters. Significant increases in sperm viability, count, active and normal sperms were also observed following Bioferon administration to rats in group 6 compared to Group 2 rats ($p<0.05$). Similarly, phytosterol administration to Groups 7 and 8 rats caused a significant increase in sperm viability, count, normal and active sperm while the population of abnormal,

sluggish and dead sperms were significantly decreased compared to Group 2 rats: suggesting a possible reversal of the deleterious effect of phenyl hydrazine in male Wistar rats.

DISCUSSION

Recent researches on medicinal plants have assumed an incredible global recognition in past years. The use of some identified plant constituents in pharmaceutical supplementation and intervention have come a long way in the elevation of the status of traditional medicine in West Africa[23]. The need for fertility modulation and enhancement in men cannot be overemphasized. In the present study, obtained results showed a significant improvement and elevation in the serum concentration of luteinizing hormone, follicle stimulating hormone and testosterone in male Wistar rats following the administration of leaf extract of C*raterispermumschweinfurthi*compared with the control. This suggest probably that C*raterispermumschweinfurthi*extract plays an important role in the modulation and improvement of hormonal level which confers pro-fertility functions. Luteinizing hormone stimulates the production of testosterone by the Leydig cells, which causes the Sertoli and peritubular cells of the seminiferous tubules to initiate spermatogenesis[24] [25]. Increased secretion of Follicle stimulating hormone aids spermatogenesis, fertility and gonadal development. LH and FSH secreted by the pituitary gland are of major importance in male reproduction. Increased testosterone concentration indicates that the extract improves libido: Testosterone concentration is associated with the gonadotropins such that an increased secretion would predictably induce relative increase in testosterone secretion[26]. Our findings are consistent with Allouh*et al.* (2015)[27], who earlier reported an elevation in serum reproductive hormones concentration following the administration of medicinal plants with aphrodisiac properties in male Wistar rats.

The modulatory functions of the leaf extract of C*raterispermumschweinfurthi*on sperm indices was examined in the present study. Nowadays, medicinal plant extracts have been given due recognition and their effects on various organs and tissues of the body identified and documented. Reproductive tissues like testis and epididymaltissues are major target tissues of plant extracts with aphrodisiac properties. Sperm motility, viability, count etc. are important factors in natural or experimental reproductive functions. In fertile men, sperm indices especially motility, viability and count are directly associated with copulatory potentials[28].Scientists believe that free radicals in the testicular region are largely responsible for dysfunction in sperm characteristics and sperm cell membrane fluidity, which destroys cytoplasmic bridges and ultimately decrease sperm count and motility[29] [30].Apparently, the antioxidant properties of C*raterispermumschweinfurthi*improved the quality of sperm by increasing the expression of sperm indices and cell membrane stabilization[31]. Findings from this study are in line with Wong *et al*.,2006[32] andOyeyemi, 2008 [4]in which extracts of plants improved sperm indices and oxidative stress.

CONCLUSION

This study reports that administration of hydromethanol extract of C*raterispermumschweinfurthi*caused a significant and dose dependent increase in the concentration of male reproductive hormones: resulting in a predictable improvement in sperm indices in male Wistar rats. The actual mechanism of action is presently unclear and would require further studies.

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Table 1: Values of male reproductive hormones in phenyl hydrazine induced toxicity treated with extract and phytosterol

	Groups	Luteinizing Hormone (miu/ml)	Follicle Stimulating Hormone (miu/ml)	Testosterone (ng/ml)
1	Control	0.30 ± 0.005^{b}	0.15 ± 0.003^{b}	0.58 ± 0.003^{b}
2	Untreated Phenyl hydrazine toxicity rats	0.25 ± 0.004^{a}	0.10 ± 0.000^{a}	0.45 ± 0.004^{a}
3	Phenyl hydrazine + 250mg/kg Extract	0.29 ± 0.002^{b}	0.14 ± 0.003^{ab}	0.54 ± 0.003^{ab}
4	Phenyl hydrazine + 500mg/kg Extract	0.34 ± 0.003^{ab}	0.19 ± 0.003^{ab}	0.60 ± 0.003^{ab}
5	Phenyl hydrazine + 750mg/kg Extract	0.36 ± 0.004^{ab}	0.20 ± 0.003^{ab}	0.62 ± 0.003^{ab}
6	Phenyl hydrazine	0.32 ± 0.003^{ab}	0.18 ± 0.005^{ab}	0.61 ± 0.003

	+ Bioferon			ab
7	Phenyl hydrazine + 2000mg/kg Phytosterol	0.28±0.003[ab]	0.14±0.003[ab]	0.50±0.003 ab
8	2000mg/kg Phytosterol only	0.33±0.003[ab]	0.17±0.003[ab]	0.60±0.003 ab

Values are shown as Mean ± SEM; n=5; [a] Significant at P<0.05 compared with Group 1 (control). [b] Significant at p<0.05 compared with Group 2 (untreated phenyl hydrazine induced toxicity).

Table 2: Values of sperm indices in phenyl hydrazine induced toxicity treated with extract and phytosterol

	Control	Untreated Phenyl hydrazine toxicity rats	Phenyl hydrazine + 250mgkg Extract	Phenyl hydrazine + 500mg/kg Extract	Phenyl hydrazine + 750mg/kg Extract	Phenyl hydrazine + Bioferon	Phenyl hydrazine + 2000mg/kg Phytosterol	2000mg/kg Phytosterol only
Volume (ul)	0.2.00±0.001[b]	0.1.00±0.002[a]	0.2.00±0.001[b]	0.2.00±0.000[b]	0.3.00±0.008[ab]	0.2.00±0.000[b]	0.2.00±0.001[b]	0.2.00±0.000[b]
Ph	8.00±0.002	8.00±0.001	8.00±0.000	8.00±0.000	8.00±0.001	8.00±0.000	8.00±0.002	8.00±0.002

Viability (%)	90.00±0.002 [b]	70.00±0.007 [a]	70.00±0.001 [a]	80.00±0.008 [ab]	85.00±0.00 [ab]	80.00±0.002 [ab]	70.00±0.00 [a]	80.00±0.001 [ab]
Count	600.00±0.001 [b]	400.00±0.00 [a]	500.00±0.002 [ab]	600.00±0.005 [b]	700.00±0.003 [ab]	600.00±0.001 [b]	500.00±0.001 [ab]	500.00±0.005 [ab]
Normal (%)	80.00±0.000	60.00±0.001 [a]	70.00±0.002 [ab]	75.00±0.001 [ab]	80.00±0.00 [b]	75.00±0.000 [ab]	70.00±0.005 [ab]	80.00±0.002 [b]
Abnormal (%)	20.00±0.001 [b]	35.00±0.002 [a]	30.00±0.001 [ab]	25.00±0.004 [ab]	20.00±0.002 [b]	25.00±0.000 [ab]	30.00±0.000 [ab]	20.00±0.000 [b]
Active (%)	80.00±0.002 [b]	60.00±0.001 [a]	70.00±0.003 [ab]	80.00±0.000 [b]	85.00±0.001 [ab]	80.00±0.001 [b]	70.00±0.007 [ab]	80.00±0.006 [b]
Sluggish (%)	5.00±0.001 [b]	10.00±0.001 [a]	10.00±0.000 [a]	10.00±0.002 [a]	5.00±0.000 [b]	10.00±0.001 [a]	10.00±0.002 [a]	10.00±0.001 [a]
Dead	10.00±0.005 [b]	25.00±0.003 [a]	20.00±0.000 [ab]	10.00±0.002 [b]	10.00±0.001 [b]	10.00±0.002 [b]	20.00±0.003 [ab]	10.00±0.002 [b]
Appearance	Milky	Milky	Milky	Milky	Milky	Milky	Milky	Milky
Viscosity	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Values are shown as Mean ± SEM; n=5; [a] Significant at P<0.05 compared with Group 1 (control). [b] Significant at p<0.05 compared with Group 2 (untreated phenyl hydrazine induced toxicity).