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Effect of Sub-Chronic Administration of Uand Dee Sweet Bitter Herbal Supplement on the Accessory Sex Organs of Male Wistar Rats

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Abstract: The study investigated the effects of sub-chronic administration of UandDee Sweet bitter herbal supplement on the accessory sex organs of male wistar rats. Twenty male wistar rats were allocated to four dose groups of five rats each namely 0.00, 539, 1077, 1616mg/kg of the herbal product orally for 90 days. Animals had access to deionized water and were fed *ad libitum* with rat chow for 90 days. The feed and fluid consumption of the animals were measured on daily basis while the body weight was measured weekly. Animals were anaesthetized after 90 days, bled-sacrificed; epididymis (E), seminal vesicle (SV) and prostate (P) were excised and weighed, protein, DNA, epididymal sperm counts (ESC) were also determined. There was non-significant increase ($p > 0.05$) in feed and fluid intake and a significant decrease ($p<0.05$) in absolute and relative weights of the E, SV and P in all the treated animals when compared with the control. There were significant ($p \leq 0.05$) decreases in ESC, DNA and protein levels in all the treated animals when compared with the control. The present work suggests that UandD sweet bitter herbal supplement may be toxic to the accessory sex organs of male wistar rats.

Key words: Epididymal sperm count · UandDee Sweet bitter · prostate · Seminal vesicle · Epididymis

INTRODUCTION

The downturn in the Nigerian economy in the early 80's heightened the use of herbal remedies. At least 80% of the population use herbal remedies. This resurgence of interest has made the National Agency Food Drug Administration Control to enforce a regulation of the manufacture, sale and use of herbal remedies in Nigeria. Previous investigations in our laboratory suggest that some Nigerian herbal remedies contain heavy metals [1] and show testicular toxicities [2, 3].

Cases of reproductive failure after prolonged intake of herbal preparations have been anecdotally reported in Nigeria. An increasing number of cases remain undocumented due to poor record keeping in the developing world. Since both registered and unregistered herbal remedies are widely used in Nigeria because of their acclaimed pharmacological properties, it is feared that high doses and chronic intake may be implicated in some undocumented cases of reproductive failure in men. Nigeria has about 12 million infertile persons [4]. Although there are previous records mentioned that the most common cause of infertility in Nigeria is infection [5], cases abound where infection have been treated without correction of infertility [4]. In Nigeria there are higher rates of irreversible oligospermia or azoospermia than most other causes of infertility and less resources for the management of infertility[6]. Of adult couples in African countries, it is estimated that 10-25% are sub-fertile and of these sub-fertile couples female factors account for about 55% and male factors for about 30-40% of causes, while 5-15% of causes are unexplained [4].

U andDee Sweet bitter is a registered herbal preparation marketed in Nigeria for the treatment of various ailments. This herbal supplement is in liquid form and it is acclaimed to have efficacy for treatment of infertility. The aim of the present work is to investigate the effect of UandDee Sweet bitter on the accessory sex organs of male wistar rats namely prostate, seminal vesicles and epididymis.

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MATERIALS AND METHODS

This study was approved by the Toxicology Unit of the Department of Pharmacology, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.

Preparation of the Extract of U and Dee Sweet Bitter: About 5000mls of the liquid herbal supplement was concentrated using a vacuum evaporator for 8 hours. 120g of the extract were recovered.

Phytochemical Studies: Phytochemical studies were done using the extract [7].

Animal Study: Adult male wistar rats (165-250g) supplied by the Animal Facility Centre of Department of Pharmacognosy, University of Nigeria, Nsukka were used. The animals were fed *ad libitum* with water and standard rat chow (Pfizer Pharmaceuticals Plc, Ikeja Nigeria).

Twenty male wistar rats were shared into four groups of five rats each. The first group received 539mg/kg (25% of the LD_{50}), second group 1077mg/kg (50% of the LD_{50}) and third group 1616mg/kg (75% of LD_{50}) of the herbal product orally for 90 days. Doses of LD_{50} based on Lorke [8]. The (fourth) control group received no UandDee sweet bitter herbal supplement but had access to deionized water and were fed *ad libitum* with rat chow for 90 days. The feed and fluid consumption of the animals were measured on daily basis while the body weight was measured weekly.

Animals were sacrificed under ether anaesthesia. The prostrate, seminal vesicles and epididymis were harvested and their weights were measured. Epididymal sperm count was determined by the WHO Laboratory protocol [9]. The prostrate, seminal vesicles and epididymal tissues were used for determination of protein [10] and DNA content [11]. The epididymal tissues were also used for the determination of acid phosphatise (ACP) and alkaline phosphatise (ALP) [12].

Statistical Analysis: Fluid and feed intake, animal weights and ESC were analyzed using the Student's t-test and comparison was done with the Duncan's multiple-range test.

The values were expressed as means±standard error mean (Mean±SEM). All differences were considered significant at 5% level, therefore a $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the absolute and relative weights of seminal vesicle (SV), prostate (P) and epididymis (EP) of wistar rats treated with the extract of U and Dee Sweet bitter**.** The result showed significant decrease ($p<0.05$) in both absolute and relative weights of SV,P and EP in all the treated animals when compared with the control group.

Table 1: Absolute and relative weights of seminal vesicle (SV), prostate (P) and epididymis (EP) of the wistar rats treated with the U and Dee Sweet bitter herbal supplement and the control

Dose (mg/kg)	Absolute weight (SV) (g)	Relative weight (SV) (%)	Absolute weight (P) (g)	Relative weight (P) (%)	Absolute weight (EP) (g)	Relative weight (EP) (%)
0.00[a]	1.52±0.44	0.67±0.22	0.88±0.19	0.38±0.10	0.98±0.13	0.42±0.03
539	0.84±0.23*	0.35±0.13*	0.48±0.05*	0.23±0.06*	0.52±0.08*	0.25±0.07*
1077	0.74±0.28*	0.35±0.11*	0.46±0.05*	0.20±0.03*	0.40±0.07*	0.17±0.04*
1616	0.54±0.23*	0.67±0.22*	0.34±0.05*	0.18±0.03*	0.30±0.07*	0.15±0.04*

Values are expressed as mean±SEM for n = 5

* Significantly different from control $p<0.05$

a = deionized water

Table 2: Epididymal (EP) ACP and ALP contents and sperm count of wistar rats treated with the U and Dee Sweet bitter herbal supplement

Dose (mg/kg)	Epididymal sperm count	Acid phosphatase (iμ/L)	Alkaline Phosphatase (iμ/L)
0.00[a]	131.22±4.31	11.86±0.51	39.70±1.23
539	114.06±2.39*	7.64±0.98*	28.64±1.39*
1077	48.70±1.11*	5.50±0.48*	24.74±1.06
1616	41.54±1.34*	4.06±0.56*	20.22±0.76*

Values are expressed as mean±SEM for n = 5

* Significantly different from control $p<0.05$

a = Deionized water

Table 3: DNA and protein contents of seminal vesicle, prostate and epididymis of wistar rats treated with the U and Dee Sweet bitter herbal supplement

Dose (mg/kg)	DNA (mg/ml) (SV)	Protein (mg/gm tissue) (SV)	DNA (mg/ml) (P)	Protein (mg/gm tissue) (P)	DNA (mg/ml) (EP)	Protein (mg/gm tissue) (EP)
0.00[a]	5.84±0.43	6.60±0.16	5.19±0.02	9.34±0.56	6.30±0.06	6.78±0.08
539	4.15±0.27*	3.54±0.17*	4.35±0.67*	3.90±0.32*	4.49±0.02*	3.74±0.11*
1077	3.76.70±0.36*	3.12±0.13	3.83.70±0.42*	3.48±0.76	3.36±0.09	3.30±0.10
1616	3.62±0.23*	2.20±0.14*	3.06±0.04*	1.96±0.53*	3.06±0.02*	3.18±0.13*

Values are expressed as mean±SEM for n = 5

* Significantly different from control p<0.05

a = Deionized water

Table 4: Feed and fluid intake of wistar rats treated with the extract of UandDEE Sweet Bitter herbal supplement and the control

Dose (mg/kg)	Feed (g)	Fluid (ml)
0.00[a]	118.15±27.98	172.99±32.39
539	119.90±32.79	183.74±46.83
1077	122.08±25.96	182.77±52.35
1616	120.98±30.55	180.81±52.35

Values are expressed as mean±SEM for n = 5

* Significantly different from control p<0.05

a = Deionized water

Also EP acid and alkaline phosphatase contents and sperm count of treated groups (Table, 2) decreased significantly ($p<0.05$) when compared with the control. Table (3) showed that DNA and protein contents of SV,P and EP of treated groups decreased significantly ($p<0.05$).

Table (4) showed the feed and fluid intake of treated animals. The result showed non-significant increase in feed and fluid intake if compared to the control group.

DISCUSSION

The Phytochemical analysis of the U and Dee Sweet bitter herbal supplement revealed the presence of alkaloids, saponins, terpenoids, glycosides and tannins. Terpenoids have been shown to decrease epididymal sperm count [13, 14].

The significant decrease in both the absolute and relative weights of the prostate, epididymis and seminal vesicle in all the animals treated with UandDee sweet bitter showed the harmful effect of this herbal supplement on male reproductive performance as the weight loss of the epididymides and accessory sex organs are considered standard criteria for the characterization of toxic agents that may cause infertility problems in the treated subject. In this respect earlier studies showed that the ethanolic extract of *Bambusa arundinaceae,* an Indian medicinal plant, decreased epididymal weight of male albino rats [15]. Also the ethanolic extract of Tecoma strans reduced significantly the relative weight of testes, epididymis, vas deferens, prostate and seminal vesicle in male albino rats [16]. The methanol extract of Momordica dioica reduced the weight of reproductive organs of male albino rats [17].

The current study revealed significant decrease in epididymal sperm count (ESC). In this respect earlier studies traced similar results when tried the extracts of *Hibiscus sabdariffa* calyx, *Bambusa arundinaceae and Azadirachta indica* in rats [3, 15, 18].

A major strength in conducting sperm evaluations in test animals is that similar data can be obtained from humans, enhancing the ability to confirm effects seen in test species and vice versa [19]. Standard toxicity studies that are limited to fertility evaluations provide insufficient information to conclude that a synthetic or natural product posses no reproductive hazards in humans [20]. Unlike humans, normal males of most animal models produce sperm in numbers that greatly exceed the minimum requirements for fertility. In some strains of rats and mice, sperm production can be reduced by 90% without compromising fertility. However, less severe reductions can have dramatic consequences for human males who are close to the threshold for the number of sperm needed to ensure reproductive competence [21]. This situation reinforces the importance of determining possible adverse effects of either synthetic or natural products on the male reproductive system as a part of safety evaluation.

The study revealed that there was significant ($p<0.05$) decrease in epididymal acid phosphatase (ACP) and epididymal alkaline phosphatase (ALP) levels in all the animals treated with U and Dee sweet bitter. The epididymides are known to provide a suitable environment for morphological and biochemical changes in spermatozoa [22]. Acid phosphatase and alkaline phosphatase serve as reliable markers for androgen action in the accessory organs of male animals and are directly correlated with sperm count [23]. The observed

reduction in the activities of these enzymes and reduction in ESN by U andDee Sweet bitter suggest a decreased androgen supply to the epididymides. These results are in agreement with the work of Manonayagi et al who reported reduction in epididymal ACP and ALP by *Bambusa arundinaceae*[15] and also the work of Kachhawa et al who reported reduction in ACP and ALP content of testes by Momordica dioica [17].

U and Dee sweet bitter markedly lowered the DNA and protein contents of the accessory sex organs. In this respect Amalakyadi churna, *Carica papaya and Momordica dioica* are reported to have the same effect in male albino rats [17, 24, 25].

The observed reduction in absolute and relative weights of the accessory sex organs, epididymal sperm count, epididymal ACP and ALP, DNA and protein contents of wistar rats treated with UandDee sweet bitter could be attributed to one or more of the constituents of this herbal supplement.

Taken together it is concluded that UandDee sweet bitter may have toxic effect on the male reproductive system of wistar rats. This study forms a basis for studies in man and involving yet lower concentrations, to determine at what concentrations UandDee sweet bitter may be said to be non-toxic to the testis. Further studies are necessary to clarify the mechanisms of action of the various phyto-constituents found in herbal supplements and to study their effects on reproductive/endocrine function and on various hormone parameters.

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