



## Effect of B-success herbal supplement on the accessory sex organs of male albino rats

Obi EJEATULUCHUKWU <sup>1\*</sup>, Agbasi Patrick UGOCHUKWU <sup>2</sup> and  
Orish Ebere ORISAKWE <sup>3</sup>

<sup>1</sup> Toxicology Unit, Department of Pharmacology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

<sup>2</sup> Federal University of Technology, Owerri, Imo State, Nigeria.

<sup>3</sup> Toxicology Unit, Faculty of Pharmacy, University of Port Harcourt, Rivers State, Nigeria.

\*Corresponding author; Email: [ejeatuluobi@yahoo.com](mailto:ejeatuluobi@yahoo.com)

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### ABSTRACT

This work investigated the effect of B-success herbal supplement on the accessory sex organs of male albino rats. Twenty weights matched male albino rats were divided into four groups of five rats and were given 0.00, 315,630,945 mg/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, seminal vesicle and prostate were excised and weighed, protein, DNA. The epididymal tissues were also used for the determination of acid phosphatase and alkaline phosphatase. The result shows that there was significant ( $P \leq 0.05$ ) decrease in both absolute and relative weights of seminal vesicle, prostate and epididymis in all the treated animals when compared with the control. The epididymal acid and alkaline phosphatase contents and semen count decreased significantly ( $p \leq 0.05$ ) in all the treated animals when compared with the control. The DNA and protein contents of seminal vesicle (SV), prostate (P) and epididymis (E) of albino rats treated with the B-success herbal supplement decreased significantly ( $p \leq 0.05$ ) in all the treated animals when compared with the control. B-success herbal supplement may have toxic effect on the accessory sex organs of male albino rats.

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**Keywords:** B–success herbal supplement, seminal vesicle (SV), prostate (P) and epididymis (E), male albino rats.

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### INTRODUCTION

Male reproductive toxicology has recently become a rapidly extending area of research and testing. In the last decades, there has been growing concern over the effects of either synthetic or natural products on the male reproductive health (US EPA,

1996). Various plants like *Vinca rosea*, *Solanum xanthocarpum*, *Bambusa amandinacea*, *Ocimum sanctum*, *Dolichos biflorus*, *Amaranathus spinosus*, *Carica papaya* and *Spirulina plantensis* have been reported to possess antifertility activity (Murugavel et al., 1998; Rao, 1998; Chinoy et

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DOI : <http://dx.doi.org/10.4314/ijbcs.v6i5.12>

al., 1999). Treatment with such plants materials result in reducing the sperm count, motility, fertility, and viability, as well as increasing the amount of abnormal sperm. It has been suggested that the extract causes androgen depletion at the target level in the cauda epididymis, thereby affecting the physiological maturation of sperm (Chinoy et al., 1999). U and Dee Sweet bitter, a registered herbal supplement marketed in Nigeria has been reported to be toxic for the accessory sex organs of male albino rats (Obi and Orisakwe, 2011). Operation Sweep (OPS) de rheumatism powder, a registered Nigerian poly-herbal medicine has been reported to cause epididymal toxicity in male albino rats (Orisakwe et al., 2010). B-success herbal supplement has been reported to have testicular toxicity in male albino rats (Obi et al., 2011). *Azadirachta indica* has been reported to have anti-fertility, anti-spermatic activity, and a reversible anti-androgenic effect (Anjali et al., 1996).

The downturn in the Nigerian economy in the early eighties heightened the use of herbal remedies. Even the efforts made by the present government to provide a meaningful health care have not discouraged the people from seeing herbal medication as a popular choice. This resurgence of undying interest in herbal medication has prompted the National Agency Food Drug Administration Control (NAFDAC) to enforce a regulation of the manufacturing, sales and usage of herbal remedies in Nigeria. To this effect many of the herbal preparations available in the country have been registered in a bid to ensure standardization in labelling requirements, contents, precautions/contraindications, place of manufacture, date of manufacture, expiry date, etc. Again other preliminary investigations in our laboratory here in Nigeria have tended to suggest that some of these Nigerian traditional remedies possess

testicular toxicity (Orisakwe et al., 2002, 2004b, 2004a).

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 as is obtained in the developing world (Orisakwe et al., 1996). Since these registered 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 (Giwa-Osagie, 2003). Although there is a general documented belief that the most common cause of infertility in Nigeria is infection (Gates et al., 1995), cases abound where infection have been treated without correction of infertility (Giwa-Osagie, 2003). 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 (Osegbe and Amaka, 1985). Of all 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 (Giwa-Osagie, 2003). It will be worthwhile investigating the possible toxicological implication and consequence of the heavy metals or any other adulterants/contaminants in these herbal remedies. B-success herbal supplement is a powdered herbal preparation registered and marketed in Nigeria with its acclaimed efficacy for treatment of infertility. The present study was undertaken to evaluate the effect of B-success herbal supplement on the accessory sex organs of male albino rats.

## MATERIALS AND METHODS

### Preparation of the extract of B-success herbal supplement

Four hundred grams of the powdered B-success herbal supplement were macerated in one litre of ethanol for 24 hours. The solution was filtered and the filtrate was later concentrated with a vacuum evaporator Centre of Department of Pharmacognosy University of Nigeria, Nsukka, supplied the animals. The animals were fed *ad libitum* with standard rat chow from Pfizer Pharmaceuticals Plc, Ikeja, Nigeria. Water was also supplied along continuously.

### Subchronic toxicity study

The lethal dose (LD<sub>50</sub>) of the B-success herbal supplement has been determined to be 1260 mg/kg. Twenty weight-matched male rats were shared into four groups of five rats each. The first group received 315 mg/kg (25% of the LD<sub>50</sub>), second group 630mg/kg (50% of the LD<sub>50</sub>), and third group 945 mg/kg (75% of LD<sub>50</sub>) of the B-success herbal supplement orally for 90 days. The control group received no herbal drug 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 their body weights were measured weekly.

### Preparation of animals for sacrifice

At the end of the study period (90 days), the animals were anaesthetized with chloroform (Fission & Scientific England) in absorbent cotton wool (Eao Pharmaceuticals, Benin City, Nigeria) and kept in a desiccator (Mermort Instruments, Germany) to prevent evaporation. Each animal was in turn put into the desiccator and the lid firmly put in place. The animals were later sacrificed; the prostate, seminal vesicles and epididymis

(Model 249/2, Corning Ltd, England) for 8 hours.

### Animals study

Adult male albino rats (165-250 g) and Swiss albino mice (18-35 g) of both sexes were used for the study. The Animal Facility

were harvested and their weights were measured. Epididymal sperm count was determined by the WHO Laboratory protocol (1992). The prostate, seminal vesicles, and epididymal tissues were used for determination of protein (Sandermann and Stromiger, 1972) and DNA content (Slim and Stanford, 1976). The epididymal tissues were also used for the determination of acid phosphatase and alkaline phosphatase (Kind and King, 1954).

## RESULTS

Table 1 shows the absolute and relative weights of seminal vesicle (SV), prostate (P), and epididymis (EP) of albino rats treated with the extract of B-Success. The results shows significant ( $P \leq 0.05$ ) decrease in both absolute and relative weights of seminal vesicle, prostate and epididymis in all the treated animals when compared with the control. Also epididymal (E) acid and alkaline phosphatase contents and semen count of treated groups (Table 2) decreased significantly ( $p \leq 0.05$ ) in all the treated animals when compared with the control.

Table 3 shows that DNA and protein contents of seminal vesicle (SV), prostate (P) and epididymis (E) of treated groups decreased significantly ( $p \leq 0.05$ ) in all the treated animals when compared with the control.

**Table 1:** Absolute and relative weights of seminal vesicle (SV), prostate (P), epididymis (E) and final body weight of albino rats treated with the extract of B-success drug and the control.

Dose (mg/kg)	Absolute weight (SV) (g)	Relative weight (SV) (%)	Absolute weight (P) (g)	Relative weight (P) (%)	Absolute weight (E) (g)	Relative weight (E) (%)	Final body weight (g)
0.00 <sup>a</sup>	1.52 ± 0.44	0.67 ± 0.22	0.88 ± 0.19	0.38 ± 0.10	0.98 ± 0.13	0.42 ± 0.03	231.56 ± 9.36
315	0.66 ± 0.11*	0.28 ± 0.08*	0.50 ± 0.10*	0.21 ± 0.04*	0.48 ± 0.08*	0.20 ± 0.02*	245.80 ± 13.47
630	0.52 ± 0.13*	0.23 ± 0.06*	0.50 ± 0.07*	0.22 ± 0.03*	0.44 ± 0.05*	0.19 ± 0.03*	231.78 ± 3.57
945	0.50 ± 0.44*	0.20 ± 0.07*	0.38 ± 0.08*	0.15 ± 0.03*	0.28 ± 0.04*	0.11 ± 0.02*	256.66 ± 11.66

Values are expressed as mean ± SEM for n = 5; \* significantly different from control P ≤ 0.05; a = deionized water.

**Table 2:** Epididymal (E) acid and alkaline phosphatase contents and semen count of albino rats treated with the extract of B-success.

Dose (mg/kg)	Epididymal semen count	Acid phosphatase (IU/L)	Alkaline Phosphatase (iu/L)
0.00 <sup>a</sup>	131.22 ± 4.3	11.86 ± 0.51	39.70 ± 1.23
315	111.43 ± 4.3*	7.54 ± 15*	30.84 ± 2.27*
630	51.32 ± 5.47*	5.08 ± 0.68*	27.38 ± 1.86*
945	29.85 ± 1.75*	3.96 ± 0.86*	21.62 ± 2.09*

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 (SV), prostate (P) and epididymis (E) of albino rats treated with the B-success herbal supplement.

Dose (mg/kg)	DNA ( $\mu\text{g/ml}$ ) (SV)	Protein (mg/gm tissue) (SV)	DNA ( $\mu\text{g/gm}$ tissue) (P)	Protein (mg/gm tissue) (P)	DNA ( $\mu\text{g/gm}$ tissue) (E)	Protein (mg/gm tissue) (E)
0.00 <sup>a</sup>	5.84 $\pm$ 0.43	6.60 $\pm$ 0.16	5.19 $\pm$ 0.02	9.34 $\pm$ 0.56	6.39 $\pm$ 0.06	6.78 $\pm$ 0.08
315	4.69 $\pm$ 0.02*	3.94 $\pm$ 0.20*	3.11 $\pm$ 0.08*	5.18 $\pm$ 0.18*	4.19 $\pm$ 0.12*	2.96 $\pm$ 0.30*
630	3.63 $\pm$ 0.02*	3.46 $\pm$ 0.09*	2.46 $\pm$ 0.09*	4.20 $\pm$ 0.19*	3.12 $\pm$ 0.07*	2.54 $\pm$ 0.17*
945	3.08 $\pm$ 0.03*	2.88 $\pm$ 0.16*	2.05 $\pm$ 0.03*	3.04 $\pm$ 0.15*	2.76 $\pm$ 0.23*	2.12 $\pm$ 0.13*

Values are expressed as mean  $\pm$  SEM for n = 5; \* significantly different from control  $P \leq 0.05$ ; a = Deionized water

## DISCUSSION

The herbal medicines are being used by up to 80% of the population in developing countries. Despite widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. The present study was undertaken to evaluate the effect of B-success herbal supplement on the accessory sex organs of male albino rats.

There was significant decrease in both the absolute and relative weights of the prostate, epididymis, and seminal vesicle in all the animals treated with B-success. The toxic effects of plant extracts were analyzed by monitoring alterations in body and organ weight of animals. In the male reproductive system, weight loss of the gonads, epididymides and accessory sex organs are considered standard criteria for the characterization of toxic agents that may cause fertility problems in the treated subject (Mishra and Singh, 2009). It has also been reported that ethanolic extract of *Tecoma stans*, a herbal medicine reduced significantly the relative weight of testes, epididymis, vas deferens, prostate and seminal vesicle in male albino rats (Nidhi et al., 2010). Operation Sweep (OPS) de rheumatism powder, a registered Nigerian poly-herbal medicine, has been reported to cause a significant reduction of the relative weight of the epididymis in male albino rats

(Orisakwe et al., 2010). U and Dee Sweet bitter a registered herbal supplement marketed in Nigeria has also been reported to be toxic to the accessory sex organs of male albino rats (Obi and Orisakwe, 2011).

From previous study, the administration of B-success herbal supplement was found to cause a significant decrease in epididymal semen number (ESN) (Obi et al., 2011) and this decrease must have brought about a highly significant loss in accessory sex organ weights, which are known to be mostly related to the number of spermatids and spermatozoa in the tissue. The decreasing weight of the reproductive organs in the extract-treated male rats clearly indicated that the extract caused structural and functional alteration in the testes, epididymis, seminal vesicle, ventral prostate and vas deferens (Sarkar et al., 2000). Reduction in the weight of accessory sex organs might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories (Singh and Singh, 2009). Operation Sweep (OPS) de rheumatism powder has also been reported to cause epididymal toxicity in male albino rats (Orisakwe et al., 2010).

This study revealed that there was significant decrease ( $P < 0.05$ ) in epididymal acid phosphatase (ACP) and epididymal alkaline phosphatase (ALP) levels in all the animals treated with the aqueous extracts of

B-success. The epididymides are known to provide a suitable environment for morphological and biochemical changes in spermatozoa (Manonayagi et al., 1987). Acid phosphatase and alkaline phosphatase serve as reliable markers for androgen action in the accessory organs of male animals and their levels are directly correlated with sperm counts (Orgebin-Crist, 1996). The observed reduction in the activities of these enzymes and reduction in ESN by these herbal remedies suggest a decreased androgen supply to the epididymides. This is in agreement with the work of Manonayagi et al., 1989 that reported reduction in epididymal ACP and ALP by the ethanolic extract of *Bambusa arundinaceae*. *Amalakyadi churna*, has also been reported to cause reduction in the activity of ACP in male albino mice (Setharam et al., 2003). The seed extract of *Carica papaya* another common plant also caused reduction in the epididymal ACP in male albino rats (Verma and Chinoy, 2001). U and Dee Sweet bitter, a registered herbal supplement in Nigeria, has been reported to decrease the epididymal ACP and ALP in male albino rats (Obi and Orisakwe, 2011). Similarly B-success also significantly decreased ( $p<0.05$ ) the DNA and protein contents of the accessory sex organs which have been found to be decreased by the aforementioned herbal remedies. U and Dee Sweet bitter has been reported to decrease the DNA and protein contents of the accessory sex organs of male albino rats (Obi and Orisakwe, 2011).

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 (Zenick and Clegg, 1989). Standard toxicity studies that are limited to fertility evaluations provide insufficient information to conclude that synthetic or natural product possesses no reproductive hazards to humans (Dalsenter et al., 1997). 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 (Toppari et al., 1996). 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 human males have a relatively low sperm count; the number of sperm per ejaculate is typically only 2- and 4- fold higher than that at which fertility is significantly impaired. In contrast the number of sperm in a rat or rabbit ejaculate is many times (up to 1000-fold) that which will produce maximum fertility. The epididymal sperm count can be reduced by as much as 90% in the rat without significantly affecting fertility. Thus, a reduction in sperm concentration that did not alter rat fertility might have an important effect on human fertility.

## Conclusion

B-success herbal supplement may have toxic effect on the accessory sex organs of male albino rats. This study forms a basis for studies in man, and involving yet lower concentrations, to determine at what concentrations B-success may be said to be non-toxic to the accessory sex organs. Further studies are necessary to clarify the mechanisms of the various phyto-constituents found in herbal supplements and to study their effects on the reproductive/ endocrine function and on various hormone parameters.

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