

Full Length Research Paper

Testicular toxicity of B-success herbal supplement in male albino rats

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The effects of B success, a herbal supplement on the reproductive organs of male albino rats were investigated. The herbal product was given orally to three dose groups of five male rats each, namely 315, 630, 945mg/kg/body weight (b.w) for 90 days and were fed *AD LIBITUM* with rat chow, the control group received only deionised water. Afterwards, the rats were bled-sacrificed. The testes were excised and weighed, and their protein and DNA determined. Also epididymal sperm count (ESC), testosterone and lead in blood were determined. There was a significant decrease ($p \leq 0.05$) in absolute and relative weights, DNA and protein levels of the testes, and in ESC and testosterone in all the treated animals when compared with the control. Testicular tissues of all the treated animals showed dose dependent spermatocytic arrest. The present work suggests that the high lead content of B Success herbal supplement may be responsible for the testicular toxicity of this herbal supplement.

Key words: Testis, lead, B Success, herbal supplement, spermatocyte, testicular toxicity.

INTRODUCTION

It was reported that 80% of the world's population relies on "alternative" plant-based medicines as their primary medical intervention (Kroll and Shaw, 2003). Herbal medications are claimed and widely believed to be beneficial; however, some reports indicated that the use of herbal medicines may results in acute and chronic intoxications resulting from their use (Garvey et al., 2001). The popularity and availability of the traditional remedies has generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies (Chan, 1995).

Heavy metals are a known contaminant or adulterant of many traditional remedies. The Asian and Indian traditional remedies have been reported to contain high levels of arsenic, lead and mercury (Garvey et al., 2001), and high levels of lead (Ernest, 2002), respectively. The clinical manifestations of metal poisoning have been well characterized (Harbison, 1998). Heavy metal poisoning has decreased because of improved industrial hygiene and environmental

controls so that the signs and symptoms of such poisoning are likely to go unrecognized. If metal poisoning is identified, the true source may be wrongly associated with environmental occupational exposures, and not with medicaments (Smitherman and Harber, 1991). Failure to establish the true cause of exposure means that the patient continues taking the metal-containing medication. Thus the screening of traditional remedies for efficacy, and safety has been recommended to protect public health (Chan, 1995).

The downturn in the Nigerian economy in the early eighties heightened the use of herbal remedies. An estimated 80% of the population use herbal remedies. This resurgence of interest has made the National Agency for Food Drug Administration and Control to enforce a regulation of the manufacture, sale and use of herbal remedies in Nigeria. Some of our previous studies have shown the presence of some heavy metals in herbal remedies and reduction in the sperm count of male albino rats that were exposed to them (Obi, 2006, Orisakwe et al., 2002, Orisakwe et al., 2004a, Orisakwe et al., 2004b, Obi et al., 2006).

Cases of reproductive failure after prolonged intake of herbal preparations have been anecdotally reported in

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Nigeria. 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 (Cates et al., 1985) 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 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 cases are unexplained (Giwa-Osagie, 2003).

B-success herbal supplement is a powdered herbal preparation, registered and marketed in Nigeria with its acclaimed efficacy for treatment of infertility. This herbal supplement has been reported to be contaminated with heavy metals (Obi et al., 2006). It will be worthwhile to investigate a possible toxicological implication of heavy metals or any other adulterants in these herbal remedies. Also, the present work aims at investigating whether heavy metal contamination of herbal supplement can cause testicular toxicity.

MATERIALS AND METHODS

A preparation of the extract of B-success herbal supplement with high lead level as observed from a previous study (Obi, 2006) was used. Four hundred grams of the powdered herbal supplements were macerated in one litre of ethanol for 24 hours. The filtrate was concentrated with a vacuum evaporator for 8 hours. Seventy two grams of the extract were recovered.

Phytochemical studies

Phytochemical studies were conducted using the extract of the herbal product following the method of Trease and Evan, 1978 for the presence of alkaloids, saponins, glycosides, carbohydrate, flavonoids, tannins, proteins, terpenoids, resins, oils.

Animal Study

Animals

Adult male albino rats (165-250g) were supplied by the Animal Facility Centre of Department of Pharmacognosy University of Nigeria, Nsukka. The

animals were fed *ad libitum* with water and standard rat chow Pfizer Pharmaceuticals Plc, Ikeja Nigeria.

Subchronic toxicity study

Twenty male rats were allocated to four dose groups of (5 rats/group). The first group, the control group received no herbal extract but had access to deionized water, second group 315mg/kg body weight (25% of the LD₅₀), third group 630mg/kg body weight (50% of the LD₅₀), and fourth group 945mg/kg body weight (75% of LD₅₀) of aqueous extract of B-success herbal supplement by oral gavage for 90 days. Doses were selected based on previous LD₅₀ determination using the modified method of Lorke, (1983). The feed and fluid consumption of the animals were measured on daily basis while the body weight measured weekly. After the end of treatment period (i.e. day 91), blood samples were collected from all the animals by sub-scleral puncture under ether anaesthesia for the determination of testosterone using ELISA kits and lead. The animals were thereafter sacrificed; the testes and epididymis were collected and weighted. Epididymal sperm count was determined by the WHO Laboratory protocol (WHO, 1992). The left testes were immediately fixed in 10% buffered formal saline and subsequently dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Transverse sections of 5µm thick were stained with haematoxylin and eosin for histopathological studies (Druny and Wallington, 1980). Whereas, the right testis was used for determination of protein (Sandermann and Stromiger, 1972) and DNA content (Slin and Standford, 1976).

Determination of lead in blood samples

Two millilitres of the blood sample was measured into 50ml beaker, 3ml of nitric acid was added and later made up to 10ml with deionized water. The solution was heated gently on kjeldatherm digestion machine at 45°C for 1 hour, then cooled at a room temperature, filtered and made up to 10ml with deionized water. The digested filtrate was used for the determination of lead using Atomic Absorption Spectrophotometry Uni-cam Model 929 at a wavelength of 217.0nm with a detection limit of 0.001. Reference blanks and spiked samples were used for quality control.

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

Table 1. Intake (feed and fluid), testis weights (Absolute and relative) and final body weight of albino rats treated with the extract of B-Success for 90 days.

Dose (mg/kg/body weight)	Feed (g/group/day)	Fluid (ml/group/day)	Absolute weight of testis (g)	Relative weight of testis (%)	Final body weight (g/group)
0.00 ^a	118.15± 27.98	172.97 ± 32.39	1.30 ± 0.07	0.57 ± 0.06	231.56 ±9.36
315	119.14 ± 29.16	207.46 ± 33.19*	0.52 ± 0.08*	0.22 ± 0.04*	245.80 ± 13.47
630	118.32 ± 31.57	240.74 ± 25.798	0.50 ± 0.06*	0.21 ± 0.02*	231.78 ± 3.57
945	121.96 ± 27.60	199.31 ± 39.59	0.36 ± 0.11*	0.14 ± 0.05*	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 semen counts, testosterone, DNA, protein and blood lead levels in albino rats treated with the extract of B-success herbal supplement.

Dose (mg/kg/day)	Epididymal sperm count x10 ⁶	Testosterone (nmol/L)	DNA tissue (µg/g)	Protein (mg/g tissue)	Bloodlead (µg/ml)
0.00 ^a	131.22 ± 4.3	13.02 ± 2.14	5.91 ± 0.31	7.42 ± 0.28	4.34 ± 1.21
315	111.43 ± 6.43*	9.03 ± 0.52*	3.21 ± 0.09*	4.68 ± 0.34*	11.64 ± 1.06*
630	51.32 ± 5.47*	2.73 ± 0.53*	2.80 ± 0.21*	3.84 ± 0.33*	12.20 ± 1.36*
945	29.85 ± 1.75*	1.40 ± 0.42*	2.32 ± 0.14	3.24 ± 0.17*	13.19 ± 1.30*

Values are expressed as mean ± SEM for n = 5

* Significantly different from control p≤ 0.05

a = deionized water

significant at p <0.05.

RESULTS

Phytochemical analysis of B-Success

The B-success herbal supplement contained high concentration of tannins, glycoside, alkaloids and terpenoids.

The sub-chronic toxicity study of B-Success

Table (1) shows the feed/fluid intake and testes weights (absolute and relative) of rats treated with extract of B-Success. The result shows that there was no significant increase in feed intake in the treated animals when compared with the control. There was a significant increase (p< 0.05) in the fluid intake of the animals treated with 315 and 630mg/kg body weight of the B-success herbal drug extract when compared with the control but this was not observed in the higher dose-treated group (945mg/kg body weight). Moreover, there was a significant decrease (P≤ 0.05) in absolute and relative weights of the testes in all the treated animals when compared with the control (Table 1).

Data presented in Table (2) shows the epididymal sperm count, testosterone, DNA, protein and lead levels

in albino rats treated with the extract of B-success herbal supplement. There was a significant (P≤ 0.05) decrease in testosterone level and epididymal semen counts in all treated groups when compared with the control. The DNA and protein content of the testes were decreased significantly (p =0.05) in all treated groups when compared with the control. The blood lead content of all groups treated with the extract of the B-Success was significantly increased (p=0.05) when compared with the control.

The level of lead in B-Success herbal product is shown in Table 3. B-Success contains 4200µg of lead representing about 3.26% of the heavy metal content. Figure 1 shows the testosterone levels of albino rats treated with the extract of B-success herbal supplement.

The histological examination of the testicular tissues of the control animals show normal testicular structure with seminiferous tubules filled with germinal cells, which are at different levels of normal maturation as shown in figure (2a). However the microscopic examination of the extract-treated animals showed graded spermatocytic arrest associated with equally graded germ cells sloughing into lumina of the tubules. These changes are in proportion to the level of exposure to the extract with the highest dose (945 mg/kg body weight) which showed complete spermatocytic arrest, producing relatively empty lumina which contain sloughed-off remnants of germ cells as shown in figures (2b, c, d).

Table 3. Heavy metal levels (∞ g) in B-Success herbal remedy showing the lead content.

Iron	3650
Nickel	8650
Cadmium	1150
Copper	46500
Lead	4200
Mercury	31
Selenium	6250
Zinc	25,500

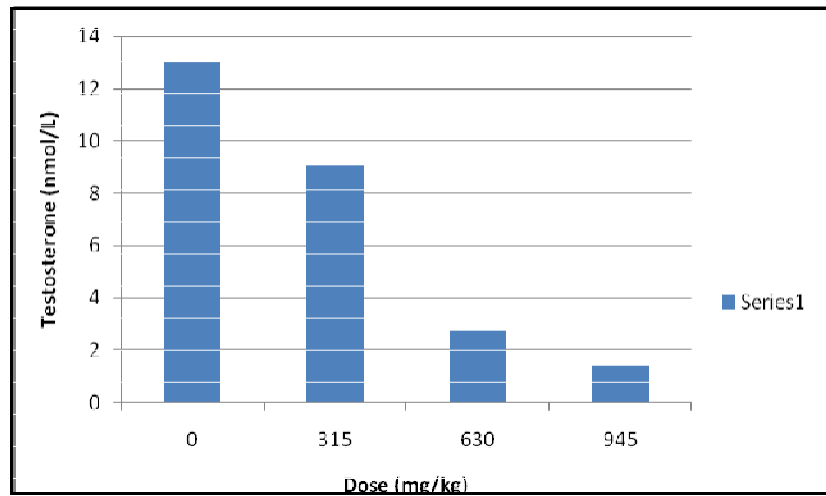
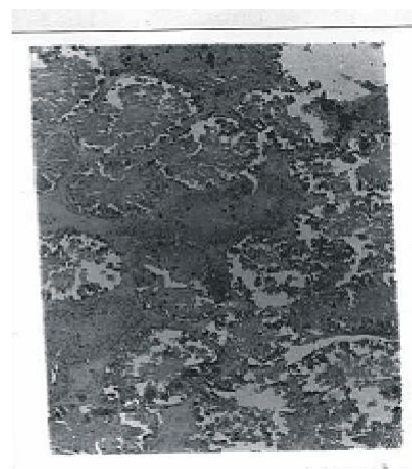


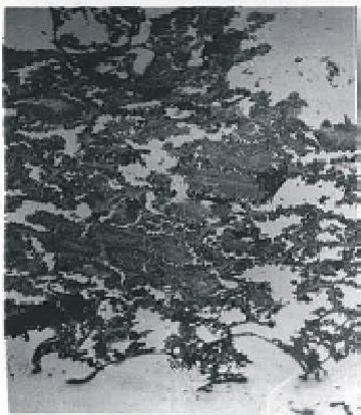
Figure 1. Testosterone level of albino rat treated with B-success extract



Figure 2. Photomicrograph of albino rats testis stained by H and E
 2a. CONTROL Showing normal testicular structure with seminiferous tubules filled with germinal cells.



2b. LOW DOSE group (315 mg/kg) rat testis showing mild spermatogenic arrest



2c. MEDIUM DOSE (630 mg/kg) showing moderate spermatocytic arrest and moderate amount of germinal cells sloughing in their lumina.



2d. HIGH DOSE (945 mg/kg) showing complete spermatocytic arrest and relatively empty lumina, Magnification x 70.

DISCUSSION

The phytochemical analysis of the B-success extract showed the presence of tannins, glycosides, alkaloids and terpenoids. Triptolide, a diterpene triepoxide isolated from a medicinal plant has been reported to decrease epididymal sperm number. It is well known that terpenoids decreased epididymal sperm count (Huynh et al., 2000). S Vindesine, a semi-synthetic vinca alkaloid has been reported to cause a decrease in testicular weight of adult male Swiss albino mice (Jagetia et al., 1992). Quinine an alkaloid derived from cinchona bark has been reported to induce testicular toxicity in rats by affecting the cytoarchitecture of seminiferous tubules (Osinubi et al., 2005). Theobromine, a methylxanthine alkaloid which is used in coffee and cocoa beverages and as an ingredient in

many prescriptions and over the counter medications as stimulants, analgesics and diuretics has been reported to cause seminiferous tubular lesions in male rats (Eteng et al., 2005). The presence of these compounds in B-success herbal supplement may have contributed to the observed testicular toxicity seen in this study.

There was significant ($p < 0.05$) increase in blood lead level of the albino rats treated with the B-success. This increase could be as a result of high lead content of this B-success. The United States Centres for Disease Control and Prevention (CDC) had defined an elevated blood lead level as $10 \mu\text{g/dl}$. The blood lead level of albino rats treated with B-success extract ranged from $9.6 \mu\text{g/dl}$ to $13.9 \mu\text{g/dl}$. A decrease in various parameters of semen quality and a possible modest effect on the endocrine profile has been observed at blood lead concentrations of $40 \mu\text{g/dl}$ (Apostoli et al., 1998). There are a number of mechanisms by which lead may affect male reproductive health, although direct toxic effects on sperm and gonads have been observed in animal testes (Sallmen, 2001).

Both animal experiments and human studies suggest that the sperm chromatin structure is altered at low exposure to lead. A biological rationale for this finding is that lead and other cations (mercury, copper) may cause a partial replacement of zinc, which is essential for sperm head chromatin stabilization (Sallmen, 2001). Failure of or delay in sperm chromatin decondensation may lead to decreased fertility or different kinds of DNA damage in the fertilization process (Johansson and Pellicciari, 1988).

Treatment of the animals with the extract of B-success herbal supplement did not result in any changes in feed intake. This effect was similar to that of Rinbacin, a local Nigerian herbal remedy which did not produce any significant increase in feed and fluid intake of albino rats (Orisakwe et al., 2002). Moreover there was no significant difference in body weight between the treated and untreated animals. The B-success did not produce a generalized toxic effect to the animals; rather the toxicity was probably organ specific. Ingestion and inhalation of lead has been reported to cause semen parameter abnormalities and decrease in testicular weight in albino rats (Kempinas et al., 1994). It has been reported that lead acetate decreased the testicular weight of albino rats exposed for a period of eight weeks (Salawu et al., 2006). The ability of lead to reduce organ weight can be linked to the less-efficient metabolic processes associated with lead toxicity (Struzypska et al., 1997). The decrease in absolute and relative weights of the testes may be as a result of the high lead content of this B-success herbal supplement.

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 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.

The B-Success extract decreased significantly ($p < 0.05$) the testosterone level in all the treated animals. It has been reported that ethanolic extract of *Tecoma stans* leaves a herbal medicine reduced significantly testosterone level in male albino rats when compared with the control (Nidhi et al., 2010). Extract of *Carica papaya* caused androgen depletion at the target level, particularly in the cauda epididymis, thereby affecting the physiological maturation of semen (Lohia and Goyal, 1992). Testosterone is very important in spermatogenesis; therefore decreased level of this hormone by B-Success herbal supplement must have contributed in the reduction of ESN. Testosterone is produced by Leydig cells in the testes and decreased number of Leydig cells and their nuclear diameter diminished the production of testosterone (Bhatt et al., 2007). Testosterone level is depleted in serum of animals treated with B-Success herbal supplement.

B-Success extract decreased significantly ($P < 0.05$) the DNA and protein contents of the testes. Reduced testicular and epididymal protein content could be correlated with absence of spermatozoa in the lumen (Zhen et al., 1995). In this concern, *Amalakyadi churna* an Indian medicinal plant caused similar significant decrease in protein and DNA content of the testes in male albino rats (Seetharam et al., 2003). Lead intoxication also causes significant decrease in total protein of the testes (Corpas et al., 2002).

The histopathological examination of the testicular tissues of the animals treated with the B-Success extract show graded spermatocytic arrest associated with equally graded germ cells sloughing into lumina of the tubules. The negative impact of *B-Success extract* on the male structural and functional integrity of testicular tissues was evidenced by the histopathological data highlighting the seminiferous tubules appear reduced in size. Lead acetate has been reported to cause alteration of histopathologic pattern and reduction in the size of seminiferous tubules, decrease in the cell content of the germinal layer, lesions of the spermatocytes and spermatids in albino rats (Ghelberg and Bordas, 2006). These changes could be due to the level of exposure to the herbal drugs with the highest dose (945mg/kg body weight for B-success) showing complete spermatocytic arrest. Our work suggests that administration of B-Success gave rise to lead loading in the animals. We opine therefore that the evidence of lead in blood may implicate in testicular toxicity of this supplement i.e lead may be one of the testicular toxicants.

CONCLUSION

This study forms a basis for studies in man and involving yet lower concentrations, to determine at what

concentrations B-Success extract may be said to be non-toxic to the testis. Further studies are necessary to quantify the concentration of the various phyto-constituents found in the herbal supplement and also study the mechanisms of their effects on reproductive endocrine function and on various hormone parameters. B-success extract herbal supplement may have toxic effect on the male reproductive system of albino rats probably as a result of high lead. The public should be cautioned on the inherent danger in consuming some of these herbal remedies. We recommend further studies in a placebo controlled clinical trial to test the hypothesis that all the affected testicular parameters seen in albino rats can be observed in men who are on B-success herbalsupplement.

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