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Evaluation of Heavy Metal Contamination and Antioxidant Efficacy of Polyherbal Male Fertility Supplements in Lead-Acetate-Induced Albino Rats

Arit Okechukwu Nwogu*, Onengiyeofori Ibama and Ebirien-Agana Samuel Bartimaeus

Department of Clinical Chemistry, Faculty of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
Author for Correspondence*: arietta457@gmail.com. https://dx.doi.org/10.4314/sokjmls.v10i2.16

Abstract

Male infertility is a major public health concern in sub-Saharan Africa, with increasing reliance on herbal supplements for treatment. While these supplements are believed to enhance fertility due to their antioxidant properties, concerns about their heavy metal content and long-term safety persist. The aim of this study was to evaluate the heavy metal content and antioxidant potential of three polyherbal male fertility supplements commonly sold in Port Harcourt, Nigeria. A total of 120 male albino rats were acclimatized, weighed, and divided into five groups (positive control, negative control, Libron, Mascum, and Energy 3000; n=16 per group). Lead acetate (60 mg/kg) was used to induce infertility over three weeks, followed by oral administration of herbal supplements over eight weeks. Doses were extrapolated from human therapeutic doses using OECD guidelines. Rats were sacrificed at two-week intervals after overnight fasting, and blood samples were collected via cardiac puncture under chloroform anesthesia. Serum levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were analyzed using ELISA, while heavy metal content in supplements was determined using Atomic Absorption Spectrophotometry. Data were analyzed using SPSS (version 23), ANOVA, and Tukey's post hoc test at $p<0.05$. All supplements demonstrated antioxidant potential, with Libron and Energy 3000 showing sustained SOD elevation and MDA reduction. However, cadmium levels in Libron (0.514 mg/kg) and Mascum (0.912 mg/kg) exceeded WHO limits (0.3 mg/kg). The antioxidant effect of Mascum declined over time, specifically by week 8, likely due to heavy metal interference. Thus, Energy 3000 had the safest heavy metal profile. The findings from this study infer that these polyherbal supplements may offer antioxidant benefits in male infertility but also pose potential risks due to heavy metal contamination, particularly cadmium. Therefore, long-term usage without regulation could lead to tissue damage.

Keywords: *Polyherbal supplements, Male infertility, Heavy metal toxicity, Superoxide dismutase, Malondialdehyde, Antioxidant activity, Lead acetate model*

Introduction

Infertility is a serious socio-economic problem in Nigeria and is defined as the disease of the reproductive system and recognized as a global public health challenge that affects one in four couples (WHO, 2013). Infertility is associated with an increased economic burden and is sadly poorly researched as couples are being left to handle their issues themselves (Agarwal *et al*., 2015).

The rate of infertility is alarming in Africa, which has the largest burden with an incredible rate of 10-15% in developed countries while in the sub-Saharan region it ranges between 20-46% (Boitrelle *et al*., 2021). Studies by Martinez *et al.* (2012) shows that the rate of infertility was highest in Africa and Central Eastern Europe whereas the percentage of infertile men globally ranged from 2.5 % to 12 % while in North America it ranges from 4.5 % - 6 % and Australia it is about 9 %. In the UK and USA, it is estimated to be 6 % and 10 % respectively while in sub–Saharan Africa its about 2.5%-4.8% and in

Nigeria 40–50% of men are infertile (Ebdrup *et al*., 2022, Agarwal *et al*., 2015). A recent study by Cox *et al*. (2022), shows that the rate of infertility was highest in Africa (18.1%), America had a percentage of 11.2% while Europe had an infertility rate of 12.6%. A study by Abarikwu (2013) in Nigeria on 17 males whose female partners were diagnosed infertile proves that eight of the men (47 %) had severe semen abnormalities and could be responsible for the infertility in their female partners.

Due to this alarming rate in Nigeria, lots of couples seek medical attention to remedy this situation and coupled with the economic situation, many seek cheaper means of treatment hence, the search for herbal supplements. As of 2008, 80 % of the world's population was using herbal medicine for one form of primary health care or another and its health risk such as damage to vital organs after prolonged usage posed a major concern (Aware *et al.,* 2022). This upsurge has prompted WHO to integrate herbal supplements into the national health care system and to set out policy to regulate their usage in some countries (WHO, 2013). World Health Organization (WHO) recognizes that herbal medicines are highly lucrative in international market such that annual revenue in Europe were estimated at $ 320 billion in 2020 and will rise to 352.92 billion in 2021 and is expected to increase to $ 658.11 billion in 2028 (Lordan, 2021) and in Nigeria it was estimated at N145 trillion in 2021 to hit N 356 trillion by 2030. As such there is a great demand for herbal supplements.

Herbal supplements, poly herbal supplements or botanical medicines possess two special characteristics which distinguish them from chemical drugs; use of crude herbs and prolonged usage and preparations containing part of plants such as their flowers, stem, roots, bark, leaves or seeds (Ehrlich, 2010). These plants contain active ingredients which are being used in producing conventional drugs for example salicylic acid, a precursor of aspirin was originally derived from white willow bark and meadow sweet plant (*Filipendula ulmaria*) (Klessig *et al.,* 2016). Some polyherbal supplements are seen to contain various phytochemicals as seen in a study by Nwogu *et al*. (2023) which gives them their pharmacological activity for the enhancement of fertility in the male users.

Reports also show that there may be the presence of heavy metals in these supplements which can alter antioxidants levels in the body system. This has attracted the interest of health sectors, researchers, scientists and the public at large due to the uncertainty of its safety.

Antioxidants are substances that are present in low concentrations; they delay or inhibit oxidation of substrate and play a vital role in body defense system against reactive oxidative stress (ROS). Antioxidants inhibit the process of oxidation at every minute hence they play a diverse physiological role. It is a molecule capable of preventing or slowing down oxidation and they are compounds that interact with and act as radical scavengers in converting Reactive oxygen species (ROS) to less reactive species.

Reactive oxygen species attack polyunsaturated fatty acid chain reactions (lipid peroxidation) on cell membrane thereby destroying fatty acids upon which hydrocarbons gases (pentane or ethane) are released and malondialdehydes (MDA) (an aldehyde) are formed. This is the most abundant enzymatic product that is gotten from lipid peroxidation. It is colorless and a highly reactive liquid that serves as a maker for oxidative stress, causing toxic stress in cells. Antioxidants intervene by terminating this chain reaction, this they do by removing free radical intermediate and inhibit other oxidation reaction by being oxidized themselves as such antioxidant are reducing agent (Landete, 2013). Hence, this study aims to evaluate the heavy metal content and the antioxidant potential in polyherbal supplement sold in Port Harcourt, Rivers State, Nigeria.

Materials and Methods

Experimental Animals

One hundred and twenty (120) male albino rats, weighing 150g – 250g were purchased from the Pharmacology Department of the University of Port Harcourt, Rivers State. The rats were allowed access to standard feed and water at *ad libitum*. They were housed in a

compartmentalized cage and allowed to acclimatize for two weeks.

Herbal Supplement
Three commonly used fertility enhancing herbal drugs in Port Harcourt were included in this study namely Libron Herbal capsule, Mascum Herbal Pride and Energy 3000 and were purchased from commercially available herbal stores in mile III and mile IV in Port Harcourt, Nigeria.

Experimental Design
The design of this study involves an acute toxicity pilot study.

Pilot study
Eight male albino rats were used and grouped into four groups of two rats each. Lead acetate was administered at the following doses: 9mg/kg was given to group A, 18mg/kg to group B, 30mg/kg to group C and 60mg/kg to group D for three weeks to determine the concentration that would bring about the lowest sperm concentration. Sixty (60) mg/kg gave the lowest sperm concentration of semen parameters.

Determination of LD_{50} of Lead Acetate (Acute Toxicity Study)
This was done using the two stages of Lorke's method with 32 albino rats. Upon calculation, 49mg/kg was obtained, and this is similar to the procedures reported by Ibrahim *et al.* (2012) & Offor *et al.* (2019).

Chronic Study
All the albino rats for the study were weighed and grouped into 5 groups, positive control (A), Negative control (B), Libron herbal (C), Mascum herbal (D) and Energy 3000 (E). All groups had sixteen (16) rats each. This study lasted for 11 weeks. Lead acetate was given by gavage to all the rats for three weeks to alter sperm parameters except rats in the negative control group. Then treatment was administered and after every two weeks, four rats were sacrificed from each group. Four sets of rats from the positive and negative controls were sacrificed alongside after every two-week treatment. This was repeated for Libron, Mascum herbal and Energy 3000 herbal supplements.

Induction of Infertility
All the rats in groups were given 60mg/kg of lead acetate apart from the negative control group for 3 weeks to induce infertility based on the pilot study result obtained. This is similar to the method of Ibrahim *et al.* (2012) and Offor *et al.* (2019).

Determination of Doses of Herbal Drugs
The dose of the herbal supplements to be given to the rats were extrapolated from the human therapeutic doses which is based on the body surface area ratio, derived by Paget and Barnes and developed by Janhavi *et al.* (2019) conversion table. The daily dose of all three herbal supplements were determined using the OECD's guidelines (OECD, 2001).

Sample Collection and Laboratory Analysis
At the expiration of 77 days, the rats were anaesthetized with chloroform and sacrificed on the 78th day. Blood samples were collected via cardiac puncture into plain containers and allowed to clot for the collection of serum for superoxide dismutase and malondialdehyde estimation quantitatively by the sandwich enzyme linked immunosorbent assay (ELISA) technique using Auto Elisa P microplate reader (Labtech). Heavy metals were analyzed using atomic absorption spectrometry.

Statistical Analysis
All data generated from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 23. Results were expressed as mean ± standard deviation. Comparison of means was made for the various parameters using the one-way analysis of variance (ANOVA). Post- Hoc was done with Tukey's multiple comparison analysis test. Results were considered statistically significant at 95% confidence interval ($p<0.05$).

Results
Table 1 presents the quantitative analysis of heavy metals in the herbal supplements. While all measured heavy metals were within the recommended safety limits, cadmium levels in Libron Herbal (0.514 mg/kg) and Mascum Herbal Pride (0.912 mg/kg) exceeded the World Health Organization's permissible limit of 0.3 mg/kg.

Table 1: Quantitative Result of the Heavy Metal Analysis of Libron Herbal, Mascum Herbal Pride and Energy 3000

S/N	Sample ID	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)	Arsenic (As)	Nickel (Ni)
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
1	Libron Herbal	0.124	0.514	< 0.001	0.002	0.081
2	Mascum Herbal pride	0.102	0.912	< 0.001	0.001	0.081
3	Energy 3000	0.201	0.213	< 0.001	0.943	0.091
	*Mean $\pm_{SD}$	0.14±0.05	0.54± 0.35	0.013±0.05	0.315±0.001	0.084±0.004

The one-way Anova of the herbal supplement concentration shows a p value of 0.4720 and f -value of 0.9561, indicating non-significant difference.

Table 2 summarizes the SOD and MDA levels during the treatment period with Libron Herbal. The results demonstrate that Libron Herbal exerted a notable antioxidant effect, as evidenced by a sustained increase in SOD levels and a significant reduction in lipid peroxidation products (MDA) throughout the treatment duration.

Table 2: Comparative Mean Values of Antioxidants Parameters of Lead Acetate Induced Male Albino Rats Throughout the Duration of Treatment with Libron Herbal

Groups	SOD (µmol/L)	MDA (µmol/L)
PC	16.44 ± 0.83^{a}	1.36 ± 0.06^{a}
NC	24.16 ± 1.54^{b}	0.74 ± 0.05^{b}
LT WK 2	28.93 ± 1.21^{c}	0.93 ± 0.02^{c}
LT WK 4	25.60 ± 1.44^{c}	0.89 ± 0.08^{c}
LT WK 6	27.43 ± 1.08^{c}	0.69 ± 0.05^{b}
LT WK 8	29.79 ± 0.76^{c}	0.79 ± 0.05^{b}
F-Value	67.523	99.152
P-Value	<0.0001	<0.0001
Remarks	S	S

Keys: PC=Positive Control, C = Negative Control, WK = Week, SOD- superoxide dismutase, MDA- malondialdehyde, LT- Libron treatment. Post Hoc: Values with different superscripts differ significantly at $P<0.05$, S- significant, NS- Not- significant

Table 3 presents the SOD and MDA levels observed during the treatment period with Mascum Herbal Pride. The findings reveal that Mascum Herbal Pride exhibited antioxidant activity, particularly within the first two weeks of treatment, alongside a significant reduction in lipid peroxidation (MDA) levels throughout the treatment period when compared to the positive control group.

Table 3: Comparative Mean Values of Antioxidant Parameters of Lead Acetate Induced Male Albino Rats Throughout the Duration of Treatment with Mascum Herbal

Variable	SOD (µmol/L)	MDA (µmol/L)
PC	16.44±0.83[a]	1.36±0.06[a]
NC	24.16±1.54[b]	0.74±0.0[b]
MT WK 2	27.91±1.58[c]	0.87±0.1[c]
MT WK 4	24.45±0.35[b]	0.79±0.08[b]
MT WK 6	25.29±0.83[b]	0.53±0.04[b]
MT WK 8	22.48±0.72[b]	0.89±0.06[b]
F-Value	52.162	59.121
P-Value	<0.0001	<0.0001
Remarks	S	S

Keys: PC = Positive Control, C = Negative Control, MT- Mascum treatment, WK = Week, SOD- superoxide dismutase, MDA- malondialdehyde. Post Hoc: Values with different superscripts differ significantly at $p<0.05$, S- significant, NS- Not- significant

Table 4 presents the SOD and MDA values recorded during the treatment period with Energy 3000. The results indicate that while the antioxidant effect of Energy 3000 was comparable to the negative control group, there was a significant reduction in lipid peroxidation (MDA) levels throughout the treatment duration relative to the positive control group.

Table 4: Comparative Mean Values of Antioxidant Parameters of Lead Acetate Induced Male Albino Rats Throughout the Duration of Treatment with Energy 3000 Herbal

Variable	SOD (µmol/L)	MDA (µmol/L)
PC	16.44±0.83[a]	1.36±0.06[a]
NC	24.16±1.54[b]	0.74±0.0[b]
MT WK 2	25.31±0.93[b]	0.84 ±0.04[b]
MT WK 4	24.69 ±0.47[b]	0.65 ±0.03[c]
MT WK 6	24.67 ±0.87[b]	0.65 ±0.04[c]
MT WK 8	24.43±0.91[b]	0.70 ±0.11[b]
F-Value	9.422	93.597
P-Value	0.001	<0.0001
Remarks	S	S

Keys: PC= positive control, NC= negative control, ET=Energy Treatment, WK= week, SOD= superoxide dismutase, MDA- malondialdehyde. Post Hoc: Values with different superscripts differ significantly at $P<0.05$, S- significant, NS- Not- significant.

Discussion

The findings of this study reveal that the quantitative heavy metal analysis of Libron herbal contains lead 0.124 mg/kg, cadmium 0.541mg/kg, mercury <0.001mg/kg, arsenic 0.002 mg/kg and nickel 1.81 mg/kg with cadmium being higher than the required acceptable standards (0.3 mg/kg) (WHO, 2006). All other heavy metals assayed were at acceptable levels to the minimal required standard except cadmium. Cadmium is known to possess very long biological half-life of 10 – 30 years and can generate reactive oxygen species which damages cells and tissues and displaces essential metals such as zinc and iron from their binding sites thereby disrupting important cellular processes such as alteration in DNA pattern. This may bring about alteration in cellular membrane, testicular tissue, sperm production and hormone production (Ali *et al*., 2022). Several studies prove that cadmium affect reproductive development in man and animals, decreasing libido, fertility and testosterone levels (Pizent *et al*., 2012; Chandel et al., 2014). Libron herbal and Mascum herbal pride is seen to contain cadmium coupled with lead acetate used in pretreatment of these laboratory rats, this amounts to significant exposure to two hazardous metals. A combined effect of lead and cadmium in a study by Li *et at*. (2018) acts as an endocrine disruptor thereby altering the endocrine system by bringing about inflammation due to release of reactive oxygen species, this in turn can affect the production of LH and testosterone. So, we should expect an altered system in these treated rats. In this study we observe an increase in SOD with similar reduction in MDA as the week progresses and so we will expect appropriate maintenance in cell membrane and tissue repair as the week progresses. Also, for Mascum herbal pride, our study shows that this herbal supplement contains 0.102 mg/kg of lead, 0.912 mg/kg of cadmium, <0.001 mg/kg of mercury, 0.001 mg/kg of arsenic and 0.081 mg/kg of nickel. Cadmium is the only metal that is higher in concentration than the required minimal level. Rani *et al*. (2014) in his study shows that this metal affects cell differentiation, proliferation and apoptosis thus generating ROS which results in oxidative stress thereby leading to cell death. It also has the potential to affect the kidney, liver and can accumulate in the bone, placenta and gut (Satarug, 2018). The level of cadmium coupled with an additional level of lead previously exposed may be the reason we see masked reduction of SOD and increase in MDA towards the eight weeks of administration. These herbal supplements are plant derived as such contain phytochemicals which may be capable of minimizing the effect of induced chemical and heavy metal present as such, we observe an increase on SOD levels even at the first two weeks of treatment with slight reduction as we approach the 11th week. A reduction in SOD is seen to bring about increase MDA which further explains the fact that free radical production reduces when there are antioxidants to mop them up and vice versa. So, cell membrane, testicular tissue and other tissues in the body will stay protected in the presence of adequate antioxidant which is not overwhelmed by excess production of peroxidation products. The balance between these two is paramount.

For Energy 3000 herbal supplement, the concentration of lead was 0.20 mg/kg, cadmium (0.213 mg/kg), mercury (<0.001 mg/kg), arsenic (0.943 mg/kg) and nickel (0.091mg.kg). All metals were lower than the required minimal standard in this herbal preparation, thus we see masked increase in SOD upon administration even at the first two weeks of treatment compared to the positive control.

Conclusion

The findings in this study reveal that lead acetate was capable of reducing SOD and increasing MDA. Libron herbal supplements were seen to contain cadmium at higher levels beyond acceptable limit and could enhance antioxidant potential hence was able to curb up free radical released upon peroxidation. Mascum herbal supplement was observed to contain the highest concentration of cadmium, much higher than the acceptable limit. Antioxidants parameters were at adequate concentration because Mascum herbal pride contains numerous phytochemicals hence its ability to curb up excess free radicals. Cadmium levels in Energy 3000 were seen to be at the lowest concentration compared to the other herbal supplements and possess good

antioxidant potentials. Hence, the intake of these herbal supplements will expose users to heavy metal contamination. Though the phytochemicals may protect from deleterious effect, prolong usage is sure going to expose its users to deleterious effect.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved of the final manuscript.

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