International Journal of Health Sciences and Research
Vol.13; Issue: 9; September 2023
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Evaluation of the Hepatotoxic Effect of some Commonly Sold Male Herbal Fertility Supplements in Port Harcourt on Male Albino Rats

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DOI: https://doi.org/10.52403/ijhsr.20230928

ABSTRACT

Infertility is one of the most challenging social problems facing developed and undeveloped nations. The liver is the primary organ involved in the bio-transformation of food and drugs as such hepatic disorders is common especially following prolonged exposure to drug metabolites. This study evaluated the hepatotoxic effect of three different male herbal fertility supplements (Libron Herbal, Mascum Herbal Pride and Energy 3000) in male albino rats. Doses of 9 mg/kg, 18 mg/kg, 30 mg/kg and 60 mg/kg of lead acetate were administered for three weeks to determine the concentration that would bring about the lowest sperm concentration. Sixty (60) mg/kg gave the lowest sperm concentration and testosterone levels. Eighty (80) male rats weighing 150 – 250 g were divided into five groups, twenty in a group: A (negative control), B (positive control), C (Libron-treated, LT), D (Mascum-treated, MT) and E (Energy 3000-treated, ET). The rats in the treatment groups were administered 60 mg/kg of lead acetate for three weeks before treatment with the various herbal supplements for eight weeks. After every two weeks, four rats from each group were anaesthetized with chloroform and sacrificed. Blood samples were collected via cardiac puncture. Liver aspartate amino transferase (AST), alanine amino transferase (ALT), total protein and albumin were determined using colorimetric method. Data was analyzed using statistical package for social sciences (SPSS) version 23 and results were considered significant at $P < 0.05$. At week 2, mean value for AST in Mascum group was significantly ($P = 0.086$) higher than in all the other groups. Mean value for ALT in Energy 3000 treated group was seen to be significantly ($P < 0.05$) lower than the other groups ($P = 0.055$). There was no significant ($P < 0.05$) difference in the mean values of AST, ALT and total protein in all the groups. As the week progressed, mean value of AST in the positive control group was significantly ($P < 0.05$) different from the other groups but not significantly ($P < 0.05$) different from the Mascum treated group. Total protein in the positive control group was significantly ($P < 0.05$) lower than in all the other groups. Mean values for albumin ($P<0.001$) were significantly ($P < 0.05$) lower in the negative control but not significantly ($P < 0.05$) different from all the three herbal supplements. Prolonged exposure to some herbal supplements can bring about inflammation of the liver enzymes.

Keywords: Male Infertility, Hepatotoxic, Herbal supplements.

1. INTRODUCTION

Infertility is one of the most challenging social problems facing developed and undeveloped nations and is defined as the reproductive disease that prevents a healthy woman from conceiving after 12 months of regular unprotected sexual intercourse (WHO, 2022). There is a surge in male factor infertility and they appear to contribute approximately 20% - 50% of the

population and is attributed with abnormal semen analysis (Agarwal *et al.,* 2015). Men generally do not want to be associated with sampling process as infertility and incompetence are likened together in a western society like ours.

It is estimated that 10 - 15 % of the general population is affected by infertility (Ahmed *et al.,* 2023). About 15 % of couples find it difficult to achieve pregnancy within one year and so seek medical treatment. Three percentages (3 %) of these remain childless, six percentage (6 %) cannot bear as much children as they want while one in six (1:6) couple trying to impregnate their wives encounter difficulties. Studies show that men have a greater number of cases of primary than secondary infertility as such it is now a reality that male factor infertility is as important as the female factor (Malik *et al.,* 2022).

According to statistics, annually 60 - 80 million couples around the world suffer from infertility (Sudha & Reddy, 2013). The number of couples affected by infertility has increased from 42 million people in 1990 to 48.5 million people in 2010 (Mascarenhas *et al.,* 2012). Ten percent (10 %) to 12 % of couples around the world are suffering from infertility of which half of the infertile cases are caused by men. Statistics show that at least 30 million men worldwide are infertile with the highest rates in Africa and Eastern Europe (Ashok *et al.,* 2015). Herbal supplement is viewed as a moderate approach to healing as such huge amount of money is spent on it globally, this has indeed boosted its sales (WHO, 2002b). Increase usage by the public has raised concerns on its safety as many of them in the open market remain untested and there is lack of suitable quality controls and appropriate patient information as to proper usage of these supplements (Raynor *et al.,* 2011).

The liver is the primary organ involved in bio-transformation of food and drugs as such hepatic disorders are possible especially following prolonged exposure to hepatotoxic drug metabolite. This can occur due to release of toxic chemicals, heavy metals and biological agents into the blood stream.

From the year 2004- 2012, liver disease due to use of herbal supplements has been on the rise, this is especially seen in Chinese herbal products (Frenzel & Teschke, 2016). Reports in China reveals incidence of acute liver failure as a result of herbal medicine usage. Liver injury due to herbal supplements accounts for 20 % of cases of hepatoxicity. Study in other parts of Nigeria highlights correlations between use of traditional herbal medicine and liver disease. Since these herbal products are composed of complex mixtures, the exact components are generally not known (Nwokeiuko *et al.,* 2013).

In Nigeria especially in the South East and south region, herbal supplements is gradually taking over contemporary medicines as the main source of health care management with a strong conviction that it cures all manner of illnesses due to the supposed presence of natural phytochemicals in these plants (Singh *et al.,* 2013). Therefore, evaluation of this herbal supplements will help protect the populace against organ damage.

2. MATERIALS AND METHODS

2.1 Experimental Animals:

Eighty (80) male albino rats, weighing 150 g - 250g were purchased from the Pharmacology Department of the University of Port Harcourt, Rivers State. The rats were housed in compartmentalized cage and allowed to acclimatize for two weeks. The rats were allowed access to standard feed (finisher) manufactured by Top Feeds Nig. Ltd and water *ad libitum.*

2.2 Experimental Drugs

Lead acetate, a product of Kermel Pharmaceuticals, China, was purchased from Joechem ventures, 170 Uniport Road Choba, Port Harcourt, Rivers State.The three herbal supplements used in this study, (Libron herbal capsule, Mascum herbal pride and Energy 3000), were purchased

from herbal stores in Rumueme area of Port Harcourt, Nigeria.

2.3 Determination of Therapeutic Doses

Doses of 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 (1964) conversion table. Therein, the daily dose of all three herbal supplements were determined using the OECD's guidelines (OECD, 2001).

2.4 Determination of LD 50 of Lead Acetate

This was done using the two stages of Lorke's method. Upon calculation, 60 mg/kg was obtained similar to studies by Ibrahim *et al.* (2012) & Offor *et al.* (2019).

2.5 Induction of infertility using Lead Acetate

Two rats were used in four different groups of A, B, C and D to induce infertility in the male albino rat. Group A was given 9 mg/kg, group B, 18 mg/kg, group C, 30 mg/kg and group D, 60 mg/kg of lead acetate respectively to induce infertility according to the method of Ibrahim *et al.* (2012) and Offor *et al.* (2019). After 3 weeks, 60 mg/kg of lead acetate gave the lowest concentration of spermatozoa and testosterone levels.

2.6 Experimental Design

Eighty (80) male albino rats were put into five (5) groups of sixteen (16) rats each as follows:

Group A- (Negative control) – Normal diet and water.

Group B- (Positive control) –Lead acetate, normal diet and water.

Group C –Eight weeks administration of Libron herbal after inducement with 60 mg/kg of lead acetate for three weeks.

Group D –Eight weeks administration of Mascum herbal after inducement with 60 mg/kg of lead acetate for three weeks.

Group E –Eight weeks administration of Energy 3000 herbal after inducement with 60 mg/kg of lead acetate for three weeks.

Four rats in each group were sacrificed every two weeks.

2.6 Treatment of the Rats

The rats were treated with lead acetate for 3 weeks to reduce their fertility. Thereafter treatment using the three different herbal supplements was given by oral gavage once daily for eight weeks.

2.7 Sample Collection

After every two weeks, twenty rats, four in each group, were anaesthetized with chloroform and sacrificed and blood samples were collected via cardiac puncture into heparin containers for biochemical analysis.

2.8 Laboratory Analysis

Analysis of aspartate amino transferase (AST), alanine amino transferase (ALT), total protein and albumin was estimated quantitatively by the colorimetric method of Reitman and Frankel, (1957).

2.9 Data Analysis

All data generated from this study were analyzed using statistical package for social sciences (SPSS) version 23. Comparison of mean and standard deviation values was done for the various parameters using the "student" t- test and one-way analysis of variance (ANOVA). Results were considered statistically significant at 95% confidence interval ($p < 0.05$).

3. RESULTS

In the first two weeks of administration of the supplements, no significant ($p > 0.05$) variation in the mean ESD of AST was seen in the albino rats of negative control when compared to the positive control. However, significant ($p > 0.05$) reduction in the value of AST following administration of Libron herbal and Energy 3000 herbal supplements from week 4 to week 8 was observed. No therapeutic response was observed in AST

level for albino rats treated with Mascum herbal supplement as shown on table 1.

ALT level was also seen to be unaffected by treatment with the three herbal supplements at week 2 of administration. However, significant ($p > 0.05$) reduction of ALT levels was seen in the treatment with all the three herbal supplements for weeks 4, 6 and 8 when compared with the positive control as shown on table 2. In the same vein, while the values of total protein and albumin were not significantly ($p > 0.05$) affected with treatments with the three herbal supplements at week 2, significant ($p > 0.05$) increase in total protein and albumin levels were observed at week 4, 6 and 8 respectively (tables 3 and 4).

Results for Herbal therapies on Liver parameters

Table 1: Mean ESD of Aspartate Amino Transference (AST) levels of Albino Rats in the study.

	2 Weeks	4 weeks	6 weeks	8 weeks
PC	66.50+11.84	119.25±17.25[a]	112.00±6.48[a]	118.00±6.68[a]
NC	68.25±2.99	58.00±17.01[b]	59.75±10.05[b]	44.50±13.63[b]
LT	20.00±2.58	46.25±6.18[c]	53.75±6.34 [b]	85.75±8.22[c]
MT	109.50±2.65	118.25±5.74[a]	110.00±8.68[a]	113.50±9.40[a]
ET	77.25±3.59	86.00± 5.48[d]	89.00±1.82 [b]	97.25±4.99[e]
F-Value	2.513	32.763	28.980	72.832
P-Value	0.086	<0.001	<0.001	<0.001
Remarks	NS	S	S	S

PC = Positive Control, NC = Negative Control, WK = Week, AST-aspartase amino transaminase. Values with different superscripts differ significantly at P<0.05, S- significant, NS- Not- significant

Table 2: Mean ESD of Alanine Amino Transference (ALT) levels of Albino Rats in the study.

	2 Weeks	4 weeks	6 weeks	8 weeks
PC	95.75±3.20	130.50±14.20[a]	141.25±16.66[a]	162.75±4.35[a]
NC	89.75±5.32	64.00±26.96[b]	68.50±21.46[b]	83.25±9.00[b]
LT	56.75±7.89	64.50±6.61[b]	57.25±9.74[b]	62.00±20.31[c]
MT	41.25±6.24	39.00±11.92[b]	32.00±4.97[b]	37.25±10.21[d]
ET	16.00±2.58	48.25±5.19[b]	54.75±4.65 [b]	59.75±5.44 [c]
F-Value	2.948	22.604	28.980	72.832
P-Value	0.055	<0.001	<0.001	<0.001
Remarks	NS	S	S	S

PC = Positive Control, NC = Negative Control, WK = Week, ALT- alanine amino transaminase. Values with different superscripts differ significantly at P<0.05, S- significant, NS- Not- significant

Table 3: Mean ESD of Total Protein (TP) levels of Albino Rats in the study.

	2 Weeks	4 weeks	6 weeks	8 weeks
PC	46.50±10.34	35.75±5.91[a]	26.75±4.03 [a]	23.50±4.65[a]
NC	57.50±4.80	62.50±8.74[b]	57.00±5.16[b]	64.75±5.85[b]
LT	42.75±8.54	56.75±7.50[b]	49.75±9.03[b]	57.25±9.95[c]
MT	52.00±2.45	61.75±2.50[b]	59.00±4.97[b]	55.00±3.37[c]
ET	47.25±7.89	56.50±5.00[b]	55.00±5.48[b]	59.75±10.81[c]
F-Value	2.386	12.002	16.308	20.779
P-Value	0.097	<0.001	<0.001	<0.001
Remarks	NS	S	S	S

PC = Positive Control, NC = Negative Control, WK = Week, TP- total protein. Values with different superscripts differ significantly at P<0.05, S- significant, NS- Not- significant

Table 4: Mean ESD of Albumin (ALB) levels of Albino Rats in the study.

	2 Weeks	4 weeks	6 weeks	8 weeks
PC	23.25±3.86`	28.25±3.59[a]	23.75±2.50 [a]	27.50±4.03[a]
NC	40.25±4.03[b]	41.50±2.65[b]	43.00±4.97[b]	27.50±4.03[a]
LT	21.00±5.77[a]	27.25±5.77[a]	26.25±3.86[a]	32.25±2.50[a]
MT	22.25±1.26[a]	24.50±2.89[a]	22.00±3.16[a]	30.25±1.71[a]
ET	21.75±5.32[a]	26.00±2.58[a]	25.25±2.50[a]	29.00±3.37[a]
F-Value	14.169	15.192	23.330	26.578
P-Value	<0.001	<0.001	<0.001	<0.001
Remarks	S	S	S	S

PC = Positive Control, NC = Negative Control, WK = Week, ALB- albumin. Values with different superscripts differ significantly at P<0.05, S- significant, NS- Not- significant

4. DISCUSSION

The study evaluated the effect of herbal supplements on the liver after treatment with lead acetate. The lead control group had significantly higher AST and ALT values compared to the negative control group as the week progresses. Significantly lower total protein and albumin values were obtained in the positive control group compared to the negative control which is indicative of inflammation caused by lead acetate, thus, raising the activities of the enzymes. Such report was seen in a study by Ibrahim *et al.* (2012) whereby ingestion of lead acetate raised liver enzymes significantly. AST and ALT play significant role in the synthesis of active mediators of inflammation especially in the presence of a toxicant. The reduction of total protein and albumin is as a result inflammation produced by hydroxyl radicals which releases reactive oxygen species. These ROS further acts on membrane lipids by reducing saturated lipids and increasing unsaturated lipids. In addition, ROS is highly reactive to membrane protein and DNA thereby bringing about a reduction in protein and albumin levels as observed in the study.

At week two of treatment, hepatic parameters of the three herbal supplements reveals that Mascum herbal raises liver AST at considerably high levels compared to the other two herbal supplements. Thus, Mascum herbal is likely to be composed of chemical compounds that were unable to reverse the inflammation caused by lead acetate. It is obvious from this study that the anti-inflammatory potentials of the other two herbal supplement was able to reverse the inflammation caused by lead acetate. This result is comparable to results derived from El-Tantawy, (2015) in which Spirulina supplement was able to suppress the effect of lead acetate exposure thereby producing no significant ($P>0.05$) changes in the status of the liver components. Some herbal supplements are capable of damaging the functional capabilities of the liver. So many people are very comfortable taking herbs than conventional medicine and are of the opinion that they are natural and safe. This could be due to numerous testimonies and internet loading of its safety with very little clinical trials on its toxicological effects since the liver play ample role in detoxification of drugs and harmful substances. A review by Assis *et al.* (2022) stated that of the 428 clinical cases of liver induced toxicity, 209 were associated with the consumption of herbal supplement. Individuals who consume dietary supplement presented with more complains of abdominal and liver injury than the negative control group. As the week progresses in week 4, week 6 and 8 we see higher levels of AST and corresponding lower levels of ALT in Mascum herbal group as compared to the other herbal supplements but total protein was not significantly ($P> 0.05$) different from the negative control group. Albumin levels was significantly ($P> 0.05$) lower than the negative control but not significantly ($P> 0.05$) different from the positive control at week 2. We observe a steady rise in albumin levels as the week progresses. Navarro *et al.* (2014) in his study revealed liver damage due to intake of herbal drug supplements. In this study, we observe an elevation of AST levels which is not peculiar to the liver. Interaction of chemicals in herbal supplements can actually lead to inflammation of the liver thereby raising liver enzymes (El-Beltagi *et al.*, 2011). Mascum herbal supplement was seen to be composed of different chemical compounds discovered in the GC/MS analysis of the herbal supplements carried out from this study such as benzene, cyclohexasiloxane, cycloheptasiloxane and diisooctyl phthalate which could be linked as a causative agent of inflammation of the liver. Studies by Michael *et al.* (1999) shows that siloxane compound is harmful to the liver. Not much study has been done on cyclohexane on the liver. A study by Fitri *et al.* (2021) shows that duration of exposure of benzene had no relationship with SGOT (AST) and SGPT (ALT) levels in car painting workers. This is

in constrast to findings by Fayed *et al*. (2017) in which they observed that benzene significantly increased the activities of liver enzymes. Similar results were reported by D'Andrea & Reddy, (2014) in which they were able to prove that workers exposed to benzene had alteration in haematological and liver markers. Benzene is a harmful and toxic substance that can cause severe health problem (Aksoy, 2017). The same is true for siloxane and diisooctyl phthalate compounds as such their presence in considerable amount can alter liver enzyme activity.

In this study we observed the ability of these herbal supplements to reverse the hepatological parameters in the treated groups to levels that were significantly ($P > 0.05$) lower than the negative control group except in the Mascum herbal supplement treated groups.

5. CONCLUSION

From this study, it is concluded that Libron and Energy 3000 herbal supplements exhibited the potentials of ameliorating liver inflammation caused by lead acetate apart from Mascum herbal supplement.

Declaration by Authors

Ethical Approval: Approved

Acknowledgement: Department of Pharmacognosy and Drug development, faculty of pharmaceutical sciences, Ahmadu Bello University, Zaria, Kaduna State for GC/MS studies.

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Arit Okechukwu Nwogu, Davies G. Tamuno-Emine, Adline E. Ben-Chioma, Ebirien-Agana Samuel Bartimaeus. Evaluation of the hepatotoxic effect of some commonly sold male herbal fertility supplements in Port Harcourt on male albino rats. *Int J Health Sci Res.* 2023; 13(9):194-200. DOI: *https://doi.org/10.52403/ijhsr.20230928*

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