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Afr. J. Biomed. Res. Vol. 27 (May 2024); 309- 316

Research Article

Effects of a Local Aphrodisiac on Body Weight Changes, LD50, Lipid Profile, Liver Enzymes and Antioxidant Activities in Male Wistar rats

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ABSTRACT

Rejuvenator is a local commercial libido enhancing drink extensively consumed in Nigeria as 'over-the-counter product'. Following reports from the users, it is alleged to improve libido and fertility in males. Listed as ingredients are *Tribulus terrestris, Capsasine, Cilantrosied, Myristica fragrans, Zingiber officinale, Rhodiola rosea, Ocinum bacilicum*, honey and cinnamon, which are natural products believed to treat erectile dysfunction in males and other ailments. In this study we aimed at investigating the effect of this aphrodisiac on LD50, body weight changes and some other biochemical parameters to ascertain the implications if any of the drink on the living system. In the experimentation, fractions of the drink (100, 200 and 400 mg/kg bodyweight) and Viagra (100 mg/kg bodyweight) were administered to Wistar rats for 21 days. Toxicity evaluation of the drink revealed that its LD50 is above 5000 mg/kg body weight. A favorable lipid profile was observed in the test groups and no significant negative impact was seen on the antioxidant enzymes. The drink has the tendency of increasing body weight and may impact the liver as both ALT and ALP were significantly high ($p < 0.05$) in group 3. The study has revealed that the drink has no toxicity challenge (LD50 > 5000 mg/kg bw) nor the tendency to increase free radical activity in the system as well as triggering lipid peroxidation. The animals possessed favorable lipid profile, though; some liver enzymes increased which suggests that the drink could impact on the liver and may be greater after a long-term use. Finally, the drink has the tendency to increase weight.

Keywords: *Rejuvenator, aphrodisiac, antioxidants, liver enzymes, lipid profile*.

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Received: January 2024; Accepted: May 2024

DOI: https://doi.org/10.4314/ajbr.v27i2.15

INTRODUCTION

Rejuvenator is a local commercial libido enhancing drink extensively consumed in Nigeria as 'over-the-counter product'. It is a branded product although not registered by National Agency for Food and Drug Administration and Control (NAFDAC), an agency of Nigerian government that monitors and controls drug and food production and use. Following reports from the users, it is alleged to improve libido and fertility in males. Listed as ingredients are *Tribulus terrestris, Capsasine, Cilantrosied, Myristica fragrans, Zingiber officinale, Rhodiola rosea, Ocinum bacilicum*, honey and cinnamon, which are natural products believed to treat erectile dysfunction in males and other ailments. (Tajuddin *et al*, 2003; Tajuddin *et al*, 2005; Qureshi *et al*, 2014; Santos *et al*, 2014; Akhtari *et al*, 2014; Garma *et al*, 2014; Goranova *et al*, 2015; Chuan *et al*, 2015; Ehab *et al*, 2016; Kamenoz *et al*, 2017; Yonghong *et al.*, 2017; Yevgeniya *et al*, 2017; Borelli *et al*, 2018 and Gamal El Din *et al*, 2019). According to the manufacturers, a single dose of half tumbler daily, taken consecutively for 7 days will correct any form of erectile dysfunction in males and infertility in both males and females, a claim that is yet to be verified scientifically.

Libido refers to overall sexual drive of an individual, which can be influenced by biological, psychological and social factors. Physiologically, libido is governed primarily by the activities of the mesolimbic dopamine pathway. Compounds that enhance arousal and sexual desire also strive to address psychological issues such as stress, anxiety and mood swings, which affect sexual desire. In similar vein, compounds that enhance fertility equally target sperm count, motility and morphology. Sexual arousal/drive in males is a vital aspect of life necessary for procreation and sexual enjoyment. Disorders of sexual behavior and sexual sensation may include impaired sexual psychology and physical reaction (Chen *et al*, 2019). It has been shown that the endothelial cells play an essential role in penile erection (Bivalacqua *et al*, 2003) as it secretes nitric oxide (NO), a neurotransmitter involved in non-adrenergic and non-cholinergic vasodilatory activity on the vascular wall. Reports have implicated lack of NO activity as the reason for most sexual dysfunctions in males (Bivalacqua *et al*, 2003 and Toda *et al*, 2005). Sexual dysfunction may be acute or chronic. In acute sexual dysfunction, it may be due to environmental factors, loss of loved ones, poverty, loss of job, anxiety etc. (Adindu *et al*, 2023). On the other hand, chronic sexual dysfunction otherwise known as erectile dysfunction is persistent due to underlying diseases and metabolic syndromes. The main factors that may cause decrease in conception are congenital, immunological, oligozoospermia, iatrogenic etc. (Adindu *et al*, 2023). In a quest to solving this debilitating challenge that causes low esteem, man has over the centuries sought assistance through the use of both orthodox and traditional concoctions, which are cautiously and limitedly used due to high costs and attendant side effects (Stella *et al*, 2019). This has led to the development of various aphrodisiacs, substances that may be in form of scent, drug, food, device, etc. that are capable of improving libido in both males and females (Sharma *et al*, 2019). Also, numerous efforts are ongoing in order to develop novel drugs that will permanently address and resolve this ugly and demeaning condition that robs man of his ego and pride. Such approach is computational bio-simulation method (Thirunavukkarasu *et al*, 2018; Sharma, *et al*, 2021; Kenny *et al*, 2022; Dege *et al*, 2022; Benjamin *et al*, 2022; Agwamba *et al*, 2022; Asogwa *et al*, 2022; Eno *et al*, 2022; Benjamin *et al*, 2022; Palanichamy *et al*, 2022; Louis *et al*, 2022; Adindu *et al*, 2023 and Grau *et al*, 2023), which has launched insights into drug design and discoveries through various ligand interactions. Infidelity with its consequences results among other factors due to lack of sexual satisfaction and many shy away from medical attention and this regrettably has led to the breakdown of many marriages. On the other hand, many deaths have been recorded due to over use (in most cases without prescription) of these libido enhancing agents (Chen *et al*, 2019; Stella *et al*, 2019 and Sharma *et al*, 2019). Therefore, this study x-rays the acute toxicity of this heavily consumed concoction by investigating the LD_{50}, body weight changes and some other biochemical parameters to ascertain how safe this product is over short and long term periods of use. These indices will help to understand the various implications if any of the use of this product and help advise the users accordingly. We also intend to compare the outcome with that of known standard drug.

MATERIALS AND METHODS

Determination of LD_{50}: Lethal dose was estimated according to the method described by (Chinedu *et al*, 2013).

Body weight measurements: The body weights of the animals were measured and recorded weekly in triplicates using digital weighing balance.

Animal grouping and treatment: Twenty-five (25) male Wistar rats weighing 50 – 80 g were obtained from the Animal Facility of a University. All rats were housed in step-B plastic cages (temperature, 25°C, relative humidity, 50 – 60% and 12/12-hour light/dark cycle) and allowed to acclimatize to a standard diet and water *ad libitum* for three weeks before being used for the experiment after growing to a substantial weight range between 150 – 200 g. The rats were divided into 5 groups of 5 animals each and the treatments were as shown in Table 1.

Table 1:
Experimental Design and animal groups treated with Standard drug (Viagra) and Extract

Groups	Administered Dosage	n
1. (Normal control)	Placebo	5
2. (Drug control)	Viagra (100 mg/kg bodyweight)	5
3 (Extract group)	Extract (100 mg/kg bodyweight)	5
4 (Extract group)	Extract (200 mg/kg bodyweight)	5
5 (Extract group)	Extract (400 mg/kg bodyweight)	5

Administration of extract: The standard drug (Viagra) and the extract were administered orally twice a day to each assigned group via the use of an oral gavage for 21 days.

Sample collection: After the 21-day experimental period, the animals were fasted overnight by removing food with constant access to water. Before sacrifice, the test animal was anaesthetized with chloroform via inhalation. Whole blood was gotten through the heart with sterilized syringes and needles. The blood sample was emptied into plain test tube bottles and left standing for 2 hours at 4°C. The blood sample was centrifuged at 3000 rpm for 10 minutes to obtain serum from the cells. The serum was separated into simple test tubes using sterilized syringes and needles and stored frozen until required for biochemical analysis. Liver tissues were removed and blotted with Whatman No 1 filter paper to clean the excess blood on the organs. The remaining tissue was used for biochemical analysis. Ethical approval for this study was obtained from Faculty of Basic Medical Sciences Committee on animal use for research with the approval code: 193ANA1023.

Oxidative stress assay

Determination of superoxide dismutase (SOD) activity: This was done according to the method described by (Alici and Arabaci, 2016).

Determination of catalase activity: Catalase activity was determined according to the method described by (Sinha, 1972).

Lipid peroxidation: Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method described by (Buege and Aust, 1978).

Assay of Glutathione peroxidase (GPx): This was determined according to the method described by (Rotruck *et al*, 1973).

Estimation of Lipid Profile: The lipid profile was estimated by method described by (Peters *et al*, 2016).

Estimation of Liver Enzymes

Estimation of alanine amino transaminase (ALT) concentration: This was determined using the method described by (Peters *et al*, 2016).

Estimation of aspartate amino transferase (AST) concentration: This was determined using the method described by (Sanyal *et al*, 2015)**.**

Determination of alkaline phosphatase (ALP) Activity: This was determined using the method described by (Sanyal *et al*, 2015)**.**

Statistical Analysis

All data are presented as the mean ± standard error of mean (SEM). The data obtained in the study was analyzed using one-way analysis of variance (ANOVA), followed by Tukey post-hoc test ($p < 0.05$) and Prism Graphpad 8 (GraphPad Software, La Jolla, USA)

RESULTS

Result showed progressive significant increases ($P < 0.05$) in body weights of the animals until the second week (Day 14) of treatment after which the body weights decreased as presented in Table 2. The LD_{50} result of the extract affirms that no mortality was recorded at 5000 mg/kg within 24 hours of administration (figure 1). There was a significant decrease ($p < 0.05$) in group 2 for SOD (figure 2) and CAT (figure 3) while there was no significant difference ($p > 0.05$) for MDA and GPx for all the test groups as presented in figure 4 and 5. Lipid profile results showed favorable lipid profile for all test groups with respect to serum total cholesterol (figure 6), serum high density lipoprotein cholesterol (HDL-C) (figure 7), serum triglyceride (figure 8), serum low density lipoprotein cholesterol (LDL-C) (figure 9) and serum very low density lipoprotein cholesterol (VLDL-C) (figure 10) when compared with the normal control. Result of the liver enzyme parameters showed that AST and ALP were the same in groups 2, and 3 (refer to figures 11 and 13) while ALT was significantly high ($p < 0.05$) in group 3 (figure 12).

Table 2:
Body weight changes of experimental groups (%)

Groups	Bodyweight (g)				% Bodyweight Change
	Day 0	Day 7	Day 14	Day 21	
Control	166.7 ± 3.40	182.6 ± 4.28	198.2 ± 2.77	177.6 ± 2.89	7.86 ± 0.82
2	162.2 ± 7.65	178.7 ± 7.60	189.6 ± 3.79	172.9 ± 2.49	8.43 ± 0.96
3	163.7 ± 5.50	176.3 ± 8.17	195.3 ± 14.80	186.8 ± 2.97	9.76 ± 0.64
4	155.8 ± 5.66	167.9 ± 5.75	183.9 ± 11.19	174.3 ± 12.97	14.46 ± 0.45^{*,a,b}
5	160.7 ± 3.40	180.0 ± 5.37	205.5 ± 9.34	178.1 ± 9.19	9.03 ± 0.35^{c}

Values are presented as mean ± SEM, n = 3
** = significantly different from Control at p < 0.05; a = significantly different from group 2 at p < 0.05*
b = significantly different from 3 at p < 0.05; c = significantly different from 4 at p < 0.05

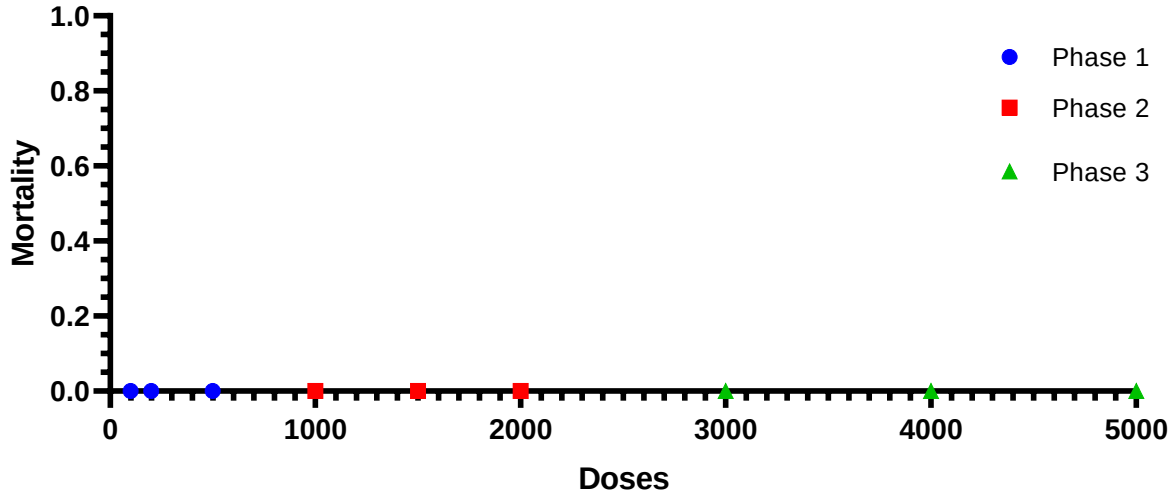

Figure 1:
Lethal Dose of Extract
No mortality was recorded at 5000 mg/kg bw

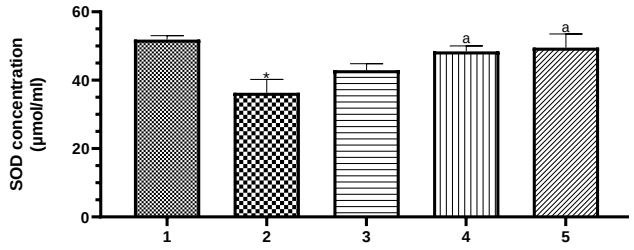

Figure 2:
SOD concentrations in experimental groups;
Values are presented as mean ± SEM, n = 3;
** = significantly different from group 1 at p < 0.05;*
a = significantly different from 2 at p < 0.05

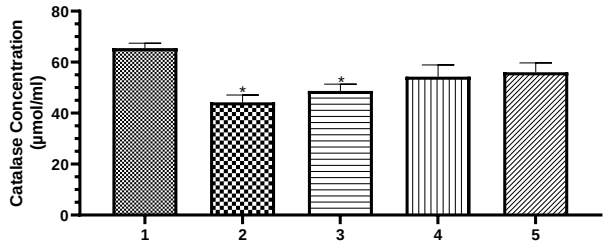

Figure 3:
Catalase concentrations in experimental groups
Values are presented as mean ± SEM, n = 3;
**= significantly different from group 1 at p < 0.05;*
a= significantly different from group 2 at p < 0.05

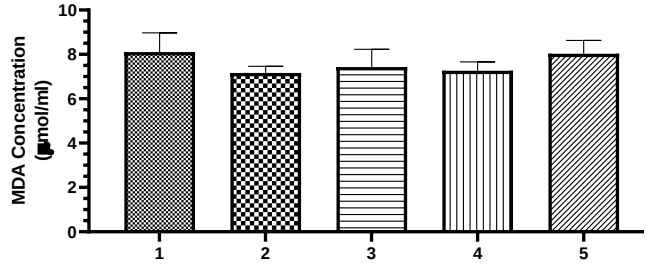

Figure 4:
Malondialdehyde concentrations in experimental groups
Values are presented as mean ± SEM, n = 3;
No significant difference among experimental groups

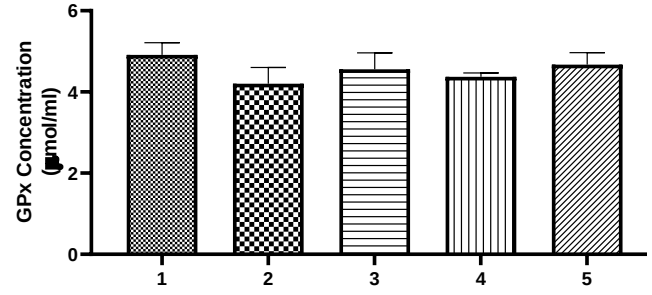

Figure 5:
Glutathione peroxidase concentrations in experimental groups
Values are presented as mean ± SEM, n = 3;
No significant difference among experimental groups

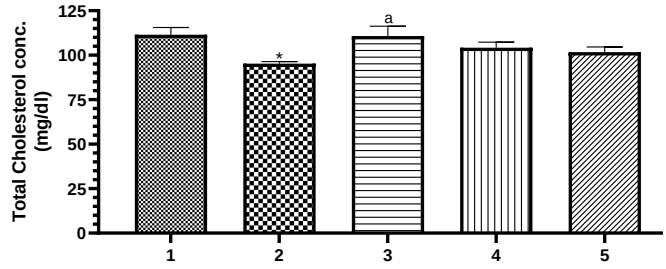

Figure 6:
Total cholesterol concentration in experimental groups
Values are presented as mean ± SEM, n = 3
* = significantly different from group 1 at p < 0.05
a = significantly different from group 2 at p < 0.05

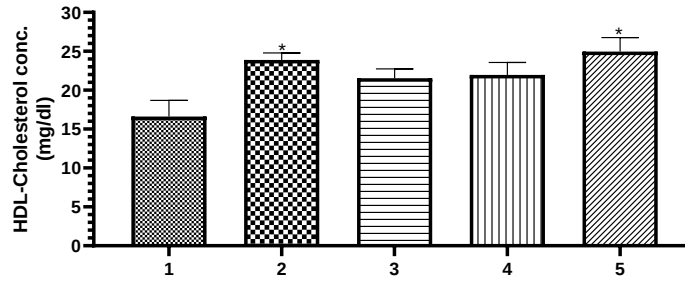

Figure 7:
HDL-cholesterol concentration in experimental groups
Values are presented as mean ± SEM, n = 3;
** = significantly different from group 1 at p < 0.05*

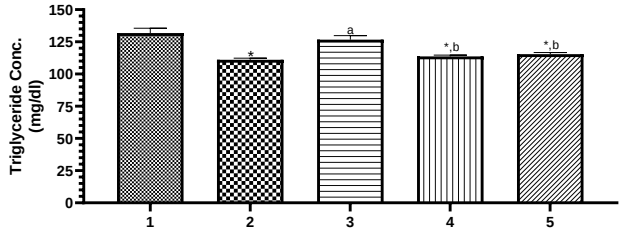

Figure 8:
Triglyceride concentration in experimental groups
Values are presented as mean ± SEM, n = 3
* = significantly different from group 1 at p < 0.05
a = significantly different from group 2 at p < 0.05
b= significantly different from group 3 at p < 0.05

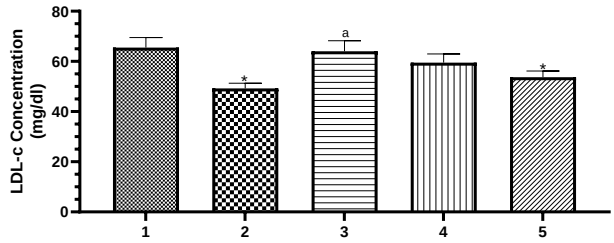

Figure 9:
LDL-cholesterol concentration in experimental groups
Values are presented as mean ± SEM, n = 3
* = significantly different from group 1 at p < 0.05
a = significantly different from group 2 at p < 0.05

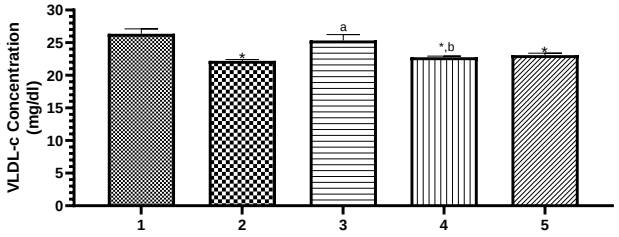

Figure 10:
VLDL-cholesterol concentration in experimental groups
Values are presented as mean ± SEM, n = 3
* = significantly different from group 1 at $p < 0.05$
a = significantly different from group 2 at $p < 0.05$
b = significantly different from group 3 at $p < 0.05$

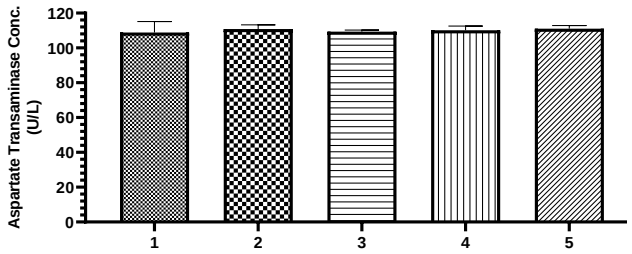

Figure 11:
Aspartate Transaminase concentrations in experimental groups
Values are presented as mean ± SEM, n = 3
No significant difference among experimental groups

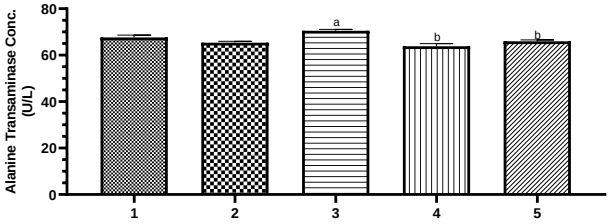

Figure 12:
Alanine Transaminase concentrations in experimental groups
Values are presented as mean ± SEM, n = 3
a = significantly different from group 2 at $p < 0.05$;
b = significantly different from group 3 at $p < 0.05$

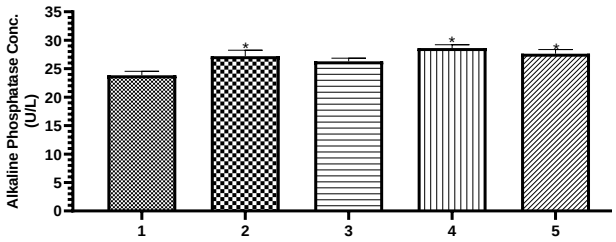

Figure 13:
Alkaline Phosphatase concentrations in experimental groups
Values are presented as mean ± SEM, n = 3
* = significantly different from group 1 at $p < 0.05$

DISCUSSION

The LD_{50} is a remarkable test to ascertain the level of toxicity of a compound in order to determine how safe it is in vivo. It is a general indicator of acute toxicity and a lower value is indicative of higher toxicity. At 5000 mg/kg body weight, if no mortality is observed within 24 hours, the compound is adjudged to be safe (Chinedu *et al*, 2013). The result affirms that no mortality was recorded at 5000 mg/kg within 24 hours of administration, hence rejuvenator energy drink is therefore adjudged safe. Body weight changes serve as an indicator of physiological and metabolic reactions occurring within an organism (Eric and Luc, 1999). Weight gain is believed to be associated with increase in both anabolic and metabolic processes while in most cases, weight loss is believed to be associated with disease condition. Body weight changes can be influenced but not limited to feed consumption rate, proximate composition of the extract and disease conditions (metabolic disorders). In all the studied groups, there were progressive significant increases in body weights until the second week after which the body weights started decreasing significantly. The initial increase may be associated with increased appetite and nutritive value of rejuvenator (Sharma *et al*, 2001). However, the later decrease may be associated with decrease in appetite and physiological changes associated with the consumption of rejuvenator. Increase in body weight may lead to obesity, which in turn leads to poor male reproductive health (Sharma *et al*, 2001).

The lipid profile of the studied animals was analyzed and compared with the normal and positive control. In this study, there was a favorable lipid profile for all test groups when compared with the normal control which suggests that the weight gain the rats experienced within the period of experiment may not be as a result of body fat accumulation which is contrary to some studies that have implicated dietary fat as promoters of weight gain (Prachi *et al*, 2012 and Peter *et al*, 2018 and Xihua and Hong, 2021). However, studies have shown that the side effects of certain drugs include weight gain (Domecq *et al*, 2015 and Verhaegen and Van Gaal, 2019). Therefore, the weight gain in this study could be as a result of underlying factor s which may not be detected by our study design. On the other hand, a favorable cholesterol level is ideal for the production of several physiologically important compounds like steroid hormones, bile acids, and vitamin D, hence can improve testosterone levels and generally enhance libido and reproductive function (Institute of Medicine (US) Subcommittee on Military Weight Management, 2004). Therefore under normal dosage consumption, Viagra and rejuvenator are not likely to raise cholesterol levels. In figures 4 and 5, there was no significant difference ($P<0.05$) for MDA and GPx for all the test groups while in figures 2 and 3, there was a significant decrease ($p < 0.05$) in group 2 for SOD while having significantly decreased CAT level but no difference with group 3 suggesting that the drink is a good antioxidant and not likely going to cause lipid peroxidation hence, the reduced SOD and CAT levels. The works of (Yasar *et al*, 2008; and Contreras *et al*, 2018) have revealed that elevated antioxidant enzyme concentrations are suggestive of free radical activities. This is because these enzymes especially SOD are inducible (Wong *et al*, 1989), meaning that their

concentration tend to increase via gene expression in the presence of free radicals. For the liver enzymes, AST and ALP were the same in groups 2, and 3 while, ALT was significantly high ($p < 0.05$) in group 3. Increased dose of the drink at 200 mg/kg was significant for ALP while no significance was recorded when it was increased to 400 mg/kg suggesting that the increase in group 4 is not dose specific but could be as a result of other underlying factors. Furthermore, this outcome suggests that this drink should not be abused to avoid any potential liver related injuries.

In conclusion, this study has revealed the effects of rejuvenator on the parameters analyzed and has shown that the drink has no toxicity challenge (LD50 > 5000 mg/kg bw) and has no negative impact on the lipid profile of the animals. It does not also possess the tendency to increase free radical activity in the system. The lipid peroxidation analysis revealed that the drink is not likely to trigger lipid peroxidation. However, since some liver enzymes were elevated, caution needs to be employed while consuming the drink especially in terms of long term consumption. Finally, there is the tendency of weight gain from its consumption.

In conclusion, this study has revealed the effects of rejuvenator on the parameters analyzed and has shown that the drink has no toxicity challenge (LD50 > 5000 mg/kg bw) and has no negative impact on the lipid profile of the animals. It does not also possess the tendency to increase free radical activity in the system. The lipid peroxidation analysis revealed that the drink is not likely to trigger lipid peroxidation. However, since some liver enzymes were elevated, caution needs to be employed while consuming the drink especially in terms of long term consumption. Finally, there is the tendency of weight gain from its consumption.

Authors' contribution

Eze A. Adindu contributed to the conceptualization supervision, methodology, validation and investigation; Obinna C. Godfrey contributed to resources, formal analysis and roles/writing—original draft. Michael O. Odey contributed to formal analysis and roles/writing—original draft. Ikenna O. Okoro contributed to formal analysis and roles/writing—original draft. Destiny A. Bisong contributed to formal analysis and roles/writing—original draft.

Acknowledgement

The authors acknowledge the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar for providing the facilities that facilitated the success of this project

REFERENCES

Adindu, E. A., Godfrey, O. C., Agwupuye, E. I., Ekpong, B. O., Agurokpon, D. C., Ogbodo, S. E., ... & Louis, H. (2023). Structural analysis, reactivity descriptors (HOMO-LUMO, ELF, NBO), effect of polar (DMSO, EtOH, H2O) solvation, and libido-enhancing potential of resveratrol by molecular docking. *Chemical Physics Impact*, *7*, 100296.

Agwamba E.C., I. Benjamin, H. Louis, A.D. Udoikono, A.T. (2022): Igbalagh, T. C. Egemonye, A.S. Adeyinka, Antitubercolusic potential of amino-(formylphenyl) diazenyl-hydroxyl and nitro-substituted naphthalene-sulfonic acid derivatives: experimental and theoretical investigations, Chem. Afr. 5 (5) (2022) 1451–1467.

Akhtari, E., Raisi, F. and Keshavarz, M., (2014). *Tribulus terrestris for the treatment of sexual dysfunction in women: radomized double blind placebo-controlled study. Daru 22: 40*

Alici, E. H., & Arabaci, G. (2016). Determination of SOD, POD, PPO and cat enzyme activities in Rumex obtusifolius L. *Annual Research & Review in Biology*, *11*(3), 1-7.

Asogwa F.C., E.C. Agwamba, H. Louis, M.C. Muozie, I. Benjamin, T.E. Gber, A. I. Ikeuba (2022): Structural benchmarking, density functional theory simulation, spectroscopic investigation and molecular docking of N-(1H-pyrrol-2-yl) methylene)-4-methylaniline as castration-resistant prostate cancer chemotherapeutic agent, Chem. Phys. Impact 5 (2022), 100091.

Benjamin I., A.D. Udoikono, H. Louis, E.C. Agwamba, T.O. Unimuke, A.E. Owen, A. S. Adeyinka (2022): Antimalarial potential of naphthalene-sulfonic acid derivatives: molecular electronic properties, vibrational assignments, and in-silico molecular docking studies, J. Mol. Struct. 1264 (2022), 133298.

Benjamin I., T.E. Gber, H. Louis, T.N. Ntui, E.I. Oyo-Ita, T.O. Unimuke, A. S. Adeyinka, (2022): Modelling of aminothiophene-carbonitrile derivatives as potential drug candidates for hepatitis B and C, Iran. J. Sci. Technol. Trans. A Sci. 46 (5) 1399–1412.

Bivalacqua, T. J., Usta, M. F., Champion, H. C., Kadowitz, P. J., & Hellstrom, W. J. (2003). Endothelial dysfunction in erectile dysfunction: role of the endothelium in erectile physiology and disease. *Journal of andrology*, *24*(S6), S17-S37.

Borelli, F., Colalto, C., Delfino, D.F., Iriti, M. and Izzo, A.A., (2018). Herbal dietary supplements for erectile dysfunction: A systematic review and Meta- analysis. *Drugs 78(6): 643-673.*

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. In *Methods in enzymology* (Vol. 52, pp. 302-310). Academic press.

Chen, L., Shi, G. R., Huang, D. D., Li, Y., Ma, C. C., Shi, M., ... & Shi, G. J. (2019). Male sexual dysfunction: A review of literature on its pathological mechanisms, potential risk factors, and herbal drug intervention. *Biomedicine & Pharmacotherapy*, *112*, 108585.

Chinedu, E., Arome, D., & Ameh, F. S. (2013). A new method for determining acute toxicity in animal models. *Toxicology international*, *20*(3), 224.

Chuan, R.Z., Ettannil, J., Paramasivam, S.K. and Muraleedaran, G.N., (2015). Antioxidant and anti-inlammatory compounds in nutmeg (*Myristica fragrans*) pericarp as determined by invitro assays. *Nat. Prod. Commun. 10(8): 1399-1402.*

Contreras R.A., Pizarro M., Köhler H., Sáez C.A., Zúñiga G.E (2018). Copper stress induces antioxidant responses and accumulation of sugars and phytochelatins in Antarctic *Colobanthus quitensis* (Kunth) Bartl. *Biol. Res.* 2018;51:48. doi: 10.1186/s40659-018-0197-0.

Dege N., H. G¨okce, O.E. Do˘gan, G. Alpaslan, T. A˘gar, S. Muthu, Y. Sert, (2022): Quantum computational, spectroscopic investigations on N-(2-((2-chloro-4, 5-

dicyanophenyl) amino) ethyl)-4-methylbenzenesulfonamide by DFT/TD-DFT with different solvents, molecular docking and drug-likeness researches, Colloids Surf. A 638 128311.

Domecq, J. P., Prutsky, G., Leppin, A., Sonbol, M. B., Altayar, O., Undavalli, C., … Murad, M. H. (2015). *Drugs Commonly Associated With Weight Change: A Systematic Review and Meta-analysis. The Journal of Clinical Endocrinology & Metabolism, 100(2), 363–370.* doi:10.1210/jc.2014-3421

Ehab, A.A. and Abir, T, E., (2016). Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (*Myristica fragrans*). *Phytochem. Rev. 15(6): 1035-1056.*

Eno E.A., J.I. Mbonu, H. Louis, F.S. Patrick-Inezi, T.E. Gber, T.O. Unimuke, O. E. Offiong, (2022): Antimicrobial activities of 1-phenyl-3-methyl-4-trichloroacetyl-pyrazolone: experimental, DFT studies, and molecular docking investigation, J. Indian Chem. Soc. 99 (7) (2022), 100524.

Eric, J. & Luc T. (1999). Regulation of body weight in humans. Physiology Reviews. https://doi.org/10.1152/physrev.1999.79.2.451

GamalEl Din, S. F., Abdel Salam, M. A., Mohamed, M. S., Ahmed, A. R., Motawaa, A. T., Saadeldin, O. A., & Elnabarway, R. R. (2019). Tribulus terrestris versus placebo in the treatment of erectile dysfunction and lower urinary tract symptoms in patients with late-onset hypogonadism: a placebo-controlled study. *Urologia Journal*, *86*(2), 74-78.

Garma, C.R., Lasmar, R. and Gama, G.F., (2014). Clinical assessment of *Tribulus terrestris* extract in the treatment of female sexual dysfunction. *Clin.Med. Insights Women's Health 7:45-50.*

Goranova, T.E., Bozhanoz, S.S., Lozanoz, V.S.,Mitev, V.I, Kaneva, R.P. and Georgieva, E.I (). Changes in gene expression of CXCR4, CCR7and BCL2 after treatment of breast cancer cells with saponin extract from *Tribulus terrestris. Neoplasma 62(1): 27-33.*

Grau L., R. Soucek, M.D. Pujol (2023): Resveratrol derivatives: synthesis and their biological activities, Eur. J. Med. Chem. 246, 114962.

Institute of Medicine (US) (2004): Subcommittee on Military Weight Management. Weight Management: State of the Science and Opportunities for Military Programs. Washington (DC): National Academies Press (US); 2004. 3, Factors That Influence Body Weight. Available from: https://www.ncbi.nlm.nih.gov/books/NBK221834/Wrzosek, M., Woźniak, J., & Włodarek, D. (2020). The causes of adverse changes of testosterone levels in men. *Expert Review of Endocrinology & Metabolism*, *15*(5), 355-362.

Kamenoz, Z., Fileva, S., Kalinov, K. and Jannini, E.A., (2017). Evaluation of the efficacy and safety of *Tribulus terrestris* in male sexual dysfunction- A prospective, randomnized, double blind, placebo controlled clinical trial. *Maturitas 99: 20-26.*

Kenny P.W., Hydrogen-bond donors in drug design, J. Med. Chem. 65 (21) (2022) 14261–14275.

Louis H., G.E. Mathias, T.O. Unimuke, W. Emori, L. Ling, A.E. Owen, C.R. Cheng (2022): Isolation, characterization, molecular electronic structure investigation, and in-silico modeling of the anti-inflammatory potency of trihydroxystilbene, J. Mol. Struct. 1266 133418.

Palanichamy C., P. Pavadai, T. Panneerselvam, S. Arunachalam, E. Babkiewicz, S. Ram Kumar Pandian, S. Kunjiappan, (2022): Aphrodisiac performance of bioactive compounds from mimosa pudica Linn.: *in silico* molecular docking and dynamics simulation approach, Molecules 27 (12) 3799.

Peter Arner, Daniel P. Andersson, Jesper Bäckdahl, Ingrid \], Mikael Rydén, (2018): Weight Gain and Impaired Glucose Metabolism in Women Are Predicted by Inefficient Subcutaneous Fat Cell Lipolysis, Cell Metabolism, Volume 28, Issue 1, , Pages 45-54.e3, ISSN 1550-4131, https://doi.org/10.1016/j.cmet.2018.05.004.

Peters, S. A., Singhateh, Y., Mackay, D., Huxley, R. R., & Woodward, M. (2016). Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: A systematic review and meta-analysis. *Atherosclerosis*, *248*, 123-131.

Prachi, S., Virend, K. S., Abel, R., Fatima, H. S., Snigdha, P., Diane, E. D., and Michael, D. J. (2012). Effects of weight gain and weight loss on regional fat distribution. Am. J. Clin. Nutr., 96(2), 229 – 233. 10.3945/ajcn.111.033829

Qureshi, A., Naughton, D. P., & Petroczi, A. (2014). A systematic review on the herbal extract Tribulus terrestris and the roots of its putative aphrodisiac and performance enhancing effect. *Journal of dietary supplements*, *11*(1), 64-79.

Rotruck, J. T. ., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W., (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science*, *179*(4073), 588-590.

Santos Jr, C. A., Reis, L. O., Destro-Saade, R., Luiza-Reis, A., & Fregonesi, A. (2014). Tribulus terrestris versus placebo in the treatment of erectile dysfunction: a prospective, randomized, double-blind study. *Actas Urológicas Españolas (English Edition)*, *38*(4), 244-248.

Sanyal, D., Mukherjee, P., Raychaudhuri, M., Ghosh, S., Mukherjee, S., & Chowdhury, S. (2015). Profile of liver enzymes in non-alcoholic fatty liver disease in patients with impaired glucose tolerance and newly detected untreated type 2 diabetes. *Indian journal of endocrinology and metabolism*, *19*(5), 597-601.

Sharma V., N.S. Chauhan, U.K. Patil, V.K. Dixit (2019): Effect of Vajikaran Rasayana herbs in sexual behavior of old age rats, Indian J. Nat. Prod. 33 (1) (2019). [21] A.S. Bolla, A.R. Patel, R. Priefer. The silent development of counterfeit medications in developing countries–a systematic review of detection technologies, Int. J. Pharm. 587 (2020), 119702.

Sharma, A. M., Pischon, T., Hardt, S., Kunz, I., & Luft, F. C. (2001). Hypothesis: β-adrenergic receptor blockers and weight gain: a systematic analysis. *Hypertension*, *37*(2), 250-254.

Sharma, S., Sharma, A., & Gupta, U. (2021). Molecular docking studies on the anti- fungal activity of Allium sativum (Garlic) against Mucormycosis (black fungus) by BIOVIA discovery studio visualizer 21.1. 0.0.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical biochemistry*, *47*(2), 389-394

Stella A., S. Marinelli, P. Berretta, R. Pacifici, A. Del Rio, (2019): Sex enhancers: challenges, threats and the need for targeted measures, Clin. Ter. 170 (3) (2019) e181–e183

Tajuddin, A., Shamshad, A., Abdul, L. and Iqbal, A.Q., (2003). Aphrodisiac activity of 50% ethanolic extracts of *Myristica fragrans* and *Syzygium aromaticum* in male mice: a comparative study. *BMC Complement Altern. Med. 3:6*

Tajuddin, A., Shamshad, A., Abdul, L., Iqbal, A.Q. and Kunwa, M.Y.A., (2005). An experimental study of sexual function improving effect of *Myristica fragrans. BMC Complement Altern. Med. 5: 16.*

Thirunavukkarasu K., P. Rajkumar, S. Selvaraj, R. Suganya, M. Kesavan, S. Gunasekaran, S. Kumaresan, (2018): Vibrational (FT-IR and FT-Raman), electronic (UV–Vis), NMR (1H and 13C) spectra and molecular docking analyses of anticancer molecule 4-hydroxy-3-methoxycinnamaldehyde, J. Mol. Struct. 1173 (2018) 307–320.

Toda, N., Ayajiki, K., & Okamura, T. (2005). Nitric oxide and penile erectile function. *Pharmacology & therapeutics*, *106*(2), 233-266.

Verhaegen AA, Van Gaal LF. (2019) Drugs That Affect Body Weight, Body Fat Distribution, and Metabolism. [Updated 2019 Feb 11]. In: Feingold KR, Anawalt B, Blackman MR, *et al*., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537590/

Wong HWG, Elwell JH, Oberley LW, Goeddel DV. (**1989**): Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell.* ;58:923–931.

Xihua, L. & Hong, L. (2021). Obesity: Epidemiology, pathophysiology, and therapeutics. Front. Endocrinol. (Lausanne). 12, 706978. 10.3389/fendo.2021.706978

Yasar F., Ellialtioglu S., Yildiz K (2008):Effect of salt stress on antioxidant defense systems, lipid peroxidation, and chlorophyll content in green bean. *Russ. J. Plant Physiol.* ;55:782–786. doi: 10.1134/S1021443708060071

Yasar F., Ellialtioglu S., Yildiz K. **(2008):** Effect of salt stress on antioxidant defense systems, lipid peroxidation, and chlorophyll content in green bean. *Russ. J. Plant Physiol.* ;55:782–786. doi: 10.1134/S1021443708060071

Yevgeniya, L., Irina, Z. and Anna, W., (2017). *Rhodiola rosea* in subjects with prolonged or chronic fatigue symptoms: Results of an open-label clinical trial. *Complement Med. Res. 24(1): 46-52.*

Yonghong, L., Victor, P. Michelle, B., Liankun, S., Chunli, W.,Arman, W.,Edward, U., Feng. S.L. and Xiaolin, Z., (2017). *Rhodiola rosea L.:* an herb with anti-stress, anti-aging and immuno stimulating properties for cancer chemoprevention. *Curr. Pharmacol. Rep.3(6):384-395.*