

Review Article

EFFECTS OF TSUMIN MARKE(TM) AND MI JIAN FEN(MJF) APHRODISIACS ON SOME BIOCHEMICAL PARAMETERS IN FEMALE WISTAR ALBINO RATS

Comment [u1]: The title should be modified to:
Evaluation of different aphrodisiac therapy effect by using some Biochemical parameters in female wistar albino rats

ABSTRACT

This research aimed to study the effect of Mijian fen (MJF) (a foreign aphrodisiac) and Tsuminmarke (TM) (a local aphrodisiac) on female libido. The research also aimed at determining the possible effect of MJF and TM on some toxicological parameters. Eighteen Female wistar albino rats were assigned into six groups of three animals each. Group A served as the control group. Group B served as the standard rats in this group were given 0.2mg/kg dose of Clomid. Groups C and D were given low (0.2mg/kg) and high (0.4mg/kg) doses of MJF respectively. Groups E and F were given low (0.2mg/kg) and high (0.4mg/kg) doses of TM, respectively. All treatments were administered for a period of thirty days after which rats were sacrificed and blood samples collected. FSH, LH, Estrogen, ALT, AST, ALP, Urea, Creatinine and Hematological parameters were assayed using standard methods. Levels of LH increased significantly ($p < 0.05$) in all the groups that were treated with high and low doses of MJF and TM, likewise FSH and estrogen. AST and ALT were slightly increased in all test groups while ALP was increased significantly ($p < 0.05$) in all test groups. Urea increased significantly ($p < 0.05$) in all test groups while creatinine increased slightly in all test groups. The results obtained confirmed MJF and TM to be potent aphrodisiacs but with some toxicity in the albino rats. Long term usage of these substances could lead to clinical complications among human users.

Comment [u2]: 1. The abstract is very shallow. There is no sufficient information.
2. It should include the background, methodology, result and conclusions.
3. Scientific words should be italicized throughout the paper
4. What do you say about risk and benefit of analysis of this research?
5. Is the benefit greater than the risk? Why?

Keywords: Aphrodisiac, TsuminMarke (TM), Mijian fen (MJF)

Comment [u3]: Keywords itself had better to be bold and put keywords in alphabetical order and italicize it.

INTRODUCTION

The increasing widespread use of traditional medicine has prompted the WHO to promote the integration of traditional medicine and complementary or alternative

medicine into the national health care systems of some countries and to encourage the development of national policy and regulations as essential indicators of the level of integration of such medicine within a national health care system (WHO, 2011). The plant materials include seeds, berries, roots, leaves, bark or flowers (Oreagbaet *al.*, 2011)

An aphrodisiac is a substance that increases sexual desire (Raskin, 1992). Many foods, drinks, and behaviors have had a reputation for making sex more attainable and/or pleasurable (Garbaet *al.*, 2013). The name comes from *Aphrodite*, the Greek goddess of sexuality and love, and substances are derived from plant, animal or mineral and since the time immemorial they have been the passion of man. Men and women alike have continued to use aphrodisiacs whether or not these drugs have any scientific basis of truly improving sexual satisfaction without regards to their composition (Garbaet *al.*, 2013). For centuries men and women have attempted to enhance their sexual experiences with a variety of chemicals (Garbaet *al.*, 2013). There is a rich history in all cultures of using substances derived from plants and animals, as well as synthetic materials, to change the sexual experience. Aphrodisiac can be classified by their mode of action into three types, those that can increase libido, potency or sexual pleasure (Garbaet *al.*, 2013).

Ficussycomorus (*F. sycomorus*), also known as fig-mulberry belonging to the family of moraceae is a semi-deciduous tree that grows up to 20 -21 m tall, not exceeding 46 m (Okparaet *al.*, 2017). Aqueous extract of *F. sycomorus* stem bark and other parts of the plant have been reportedly used traditionally to treat infertility and sterility in humans and animals in parts of Africa (Pakia and Cookie, 2003; Kone and Atindehou, 2008).

Its common names in English include: Stranglerfig, Sycamore, sycamore fig, and bush fig. Locally, it is called Baure in Hausa, "Epin" (Yoruba) and Tarmur in Kanuri (Wakiliet *al.*, 2019).

Anogeissus leiocarpus locally known as 'Marke' in Hausa language and commonly called African birch or axle-wood (Victor, 2013) is a deciduous tree species that can grow up to 15–18 m of height and measure up to 1m diameter. Bark greyish, scaly. Many traditional uses have been reported for the plant. In Sudanese traditional medicine the decoction of the barks is used against cough (El-Ghazaliet *al.*, 2003). Rural populations of Nigeria use sticks for orodental hygiene, the end of the sticks are chewed into fibrous brush which is rubbed against teeth and gum (Rotimi, 1988). Ivory Coast traditional practitioners use the plant for parasitic disease such as Malaria, Trypanosomiasis, Helminthiasis and dysenteric syndrome (Okpekon, 2004). In Togolese traditional medicine it used against fungal infections such as dermatitis and Mycosis, also the decoction of leaves is used against stomach infections (Batawila, 2005). The plant is also used for the treatment of diabetic ulcers general body pain, blood clots, asthma, coughing and tuberculosis (Victor, 2013).

Mi Jian Fen Power Female Products Description Spanish Female Sex Powder Mijianfen is the latest female aphrodisiac imported in Spain, also known as "MiJianFen". The product is a kind of powder containing powerful formula, is the most popular product at present.

Comment [u4]: 1. Use recent reference
The reference citation should be based on the guide line of international journal of biochemistry

Comment [u5]: 2. Use recent reference
3. The reference citation should be based on the guide line of international journal of biochemistry.

Comment [u6]: No citation or reference

It will work in 5 minutes before sex and bring you a strong sexual desire instantly, as well as breast inflating, fully activating the strong sex desire women to man. The product does not contain any hormones and mental paralysis composition, long-term use, non-toxic non-addictive, safe and reliable.

Clomiphene citrate (CC), a selective oestrogen-receptor modulator, used for treatment for infertile women (Abu Hashim, 2012). It works to induce ovulation by inhibiting negative, endogenous, oestrogen-feedback on the hypothalamic-pituitary axis, resulting in increased FSH secretion, follicular growth, and ovulation (Emily *et al.*, 2010). On the other hand, uses of clomiphene citrate was accompanied with many adverse effects, such as ovarian enlargement, vasomotor flashes, nausea, vomiting, breast discomfort, headache, abnormal vaginal bleeding, visual symptoms, weight gain and shortness of breath (Sherbahn, 2015). Keskinet *al.*, (2007), reported that CC induced acute pancreatitis. It also caused myocardial infarction (Duran and Raja, 2007), hypertriglyceridemia (Yasar and Ertugrul, 2009), deep vein thrombosis (Benshushanet *al.*, 1995) and pulmonary embolism (Chamberlain and Cumming, 1986). Nagao and Yoshimura (2001) reported that Clomiphene citrate has been shown to cause ovarian and uterine abnormalities.

Open discussions concerning sex and sexual activities are considered as taboo on private and as such, aphrodisiac usage is something that is talked about in low tones especially among women in our society (Garba *et al.*, 2013). In the course of improving sexual performance, some married men chose to use aphrodisiac herbs as a source of improving sexual pleasure and activeness, culturally referred to as fixing their marriage. The aphrodisiac is getting increasingly popular amongst young adults and sexually active men to enhance their sexual ability (Iwuozor, 2019). Aphrodisiac herbs are increasing in our society because every woman expects that their men are “capable” sexually (Kaadaaga *et al.*, 2014). The herbs are prepared in different forms. There are local variants such as a mixture of local gin and herbs (AgboGbogbonise, Sepe or Paraga), Tsumi (a local concoction prepared in different ways using plant stem bark and other ingredients). There are also well packaged industrially made variants in packets of pills, or tablets such as “Spanish fly, Enpulse, Vimax, Virillis, M-Energex, High T, Male X” and those in liquid forms such as Alomo bitters among others. Due to the high utilization of local aphrodisiacs among northern women, questionnaires were issued to married women in Keffi community to have an overview of the most popular and active aphrodisiac. Majority of the women confessed to the fact that the tsumi concoction is the most active widely used aphrodisiac as a result Hajjiya Sa’iha Abdullahi (Personal contact, 2022) who is a user and a dealer of aphrodisiac was interviewed and the method for the preparation of TM was obtained.

General Objective:

Specific Objective

MATERIALS AND METHOD

Samples Collection and Preparation.

Fresh stem barks that of *Ficussycomorus* and of *Anogeissus leiocarpus* were purchased from Keffi market in Nassarawa state, they were identified and authenticated at the Department of Plant Science and Biotechnology of Nasarawa State University, Keffi. The stem barks were thoroughly washed with water to remove the adherent impurities and shade-dried.

Comment [u7]: No citation or references.
The reference should be placed based on guideline of Journal of Biochemistry

Comment [u8]: It should be italicized and do not use old references

Comment [u9]: Old references

Comment [u10]: No references

Comment [u11]: 1. Put clearly the objective of this research at the end of the introduction.
2. There is no clear specific objective

Comment [u12]: 1. Describe study design
2. Sampling method and sample size determination
3. Study methodology (sample collection and preparation).

According to the method described by Mrs. Sa'iha Abdullahi (personal contact, 2022) 50g of the *Ficussycomorus* and 50g of *Anogeissus leiocarpus* was cooked with 1ltr of distilled water for 30 minutes and then allowed to cool. The decoction was filtered after cooling and stored in a clean plastic bottle.

The Clomifene and Mi Jian Fen aphrodisiac were also purchased from Keffi market and identified by specialists.

Experimental Animals

Eighteen (18) adult female Albino rats weighing between 160 – 220g each were purchased from National Veterinary Research Institute (NVRI), Jos, Plateau State. The rats were allowed to acclimatize under standard conditions of humidity and temperature in an animal house of the Department of Biochemistry, Nasarawa State University, Keffi. The animals were housed in clean iron cages lined with wood chip bedding. Standard pellet diet (Livestock Feeds, Sapele Nigeria) and water were given ad libitum.

Body weights

Initial body weight of all the animals will be measured at the beginning of the experiment, and continued weekly throughout the period of the study.

Experimental Design

Following two weeks (14 days) of acclimatization, the rats will be randomly divided into six (6) groups of 3 rats each:

Group 1 (control) received standard diet and water.

Group 2 (standard) received 0.2 mg/kg of clomid.

Group 3 received 0.2 mg/kg of Mijian fen

Group 4 received 0.4 mg/kg of Mijian fen

Group 5 received 0.2 mg/kg TsuminMarke

Group 6 received 0.4 mg/kg TsuminMarke

Biochemical Assay

After the administration for thirty (30) days, the rats were anaesthetized with diethyl ether and blood sample was collected with the aid of capillary tube via an ocular vein puncture into sample containers for biochemical analysis. The blood samples were centrifuged at 2000 rpm for 20 minutes and plasma was separated. The biochemical parameters including Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined using the method of Reitman and Frankel (1957). Alkaline phosphatase was determined using commercial kit (Randox Laboratories, 1997). Urea and Creatinine were determined using the method of Bartels and Bohmer (1972). Serum will be separated and assayed for Follicle stimulating hormone, Luteinizing hormone and Estrogen using enzyme linked immunosorbent assay (ELISA) kits by the method of Tietz (1995) following the manufacturer's manual.

Ethical Clearance

Ethical approval (REF: NSUK-ACUREC/BCH/23/04-13/01/2023) was sought and granted by the Ethical Review Committee Nasarawa State University Keffi.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences Software (SPSS; version 27.0). Differences between groups was examined by one-way

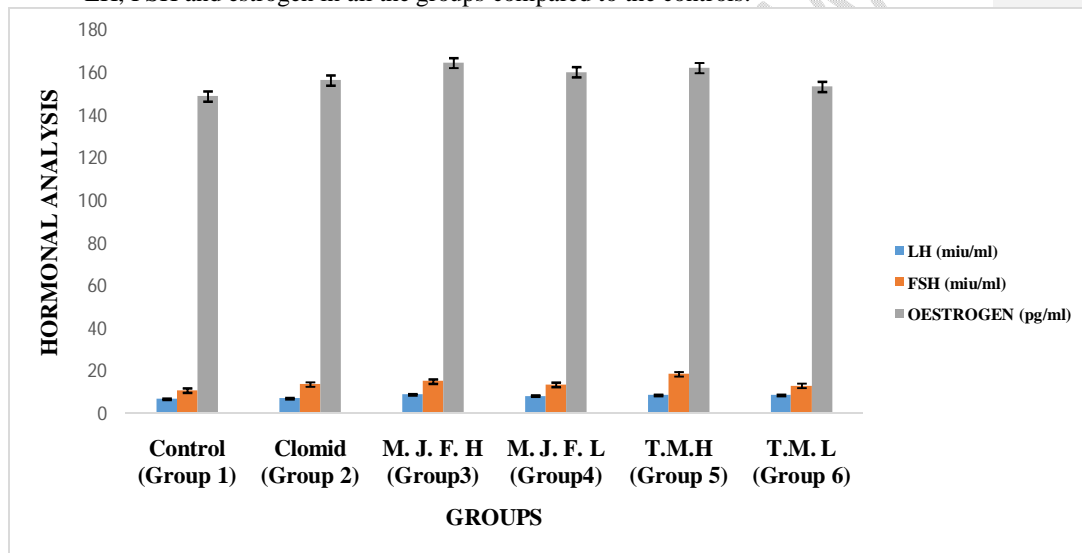
Comment [u13]: The ethical consideration had better to be written before experiment, during experiment and after experiment based on international animal ethics guide line.

ANOVA- test with mean and standard error of mean ($M \pm S.E.M$). P value ≤ 0.05 was considered as statistically significant.

Result

Effect of oral administration of Clomid, Mijian fen and Tsuminmarke on LH, FSH and Estrogen

The mean and standard deviation values of LH, FSH and estrogen for groups that were administered Clomid, Mijian fen and Tsuminmarke and their control are presented in figure 1. There was statistically significant ($P < 0.05$) increase in the mean values of LH, FSH and estrogen in all the groups compared to the controls.



Results are expressed as Mean \pm SD, and are significantly different at $p < 0.05$. **Key:** LH= Leutinizing Hormone, FSH= Follicle stimulating hormone, M.J.F.H= Mijian fen high dose, M.J.F.L= Mijian fen low dose, T.M.H= TsuminMarke high dose, T.M.L= TsuminMarke low dose

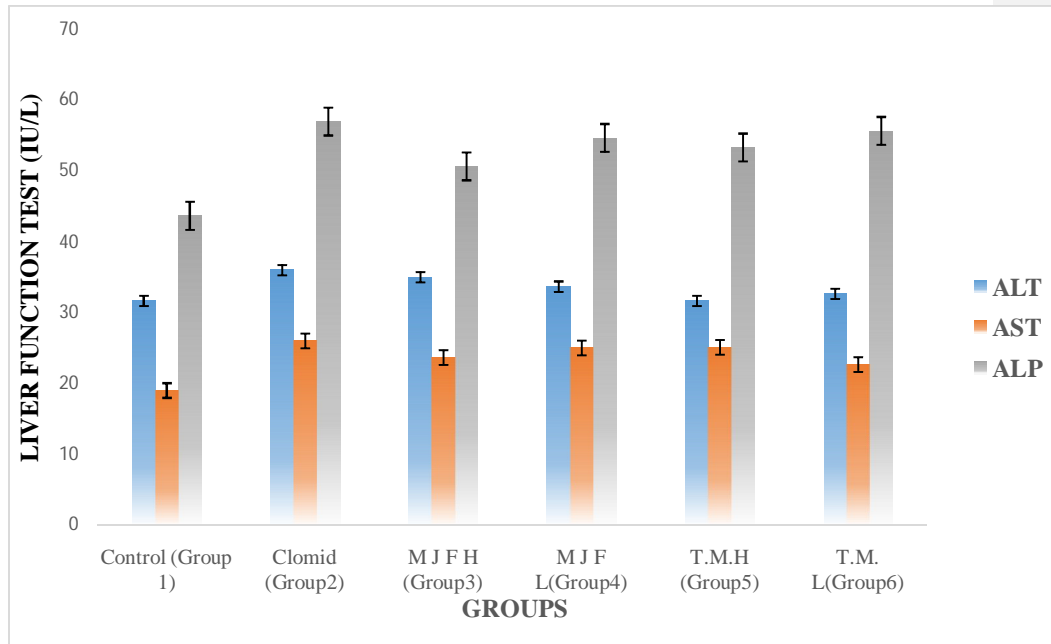
Figure 1: Effects of oral administration of Clomid, Mijian fen and Tsuminmarke on LH, FSH and Estrogen

Comment [u14]: It should not be bold

Effects of oral administration of Clomid, Mijian fen and Tsuminmarke on liver function parameters

The mean and standard deviation values of AST, ALT and ALP of rats that were administered Clomid, Mijian fen and Tsuminmarke and their controls are presented in figure 2. There were slight increases in the mean values of ALT and AST in all test

groups compared to the controls. A statistically significant ($P < 0.05$) increase in the mean values of ALP in all test groups was observed compared to the controls.



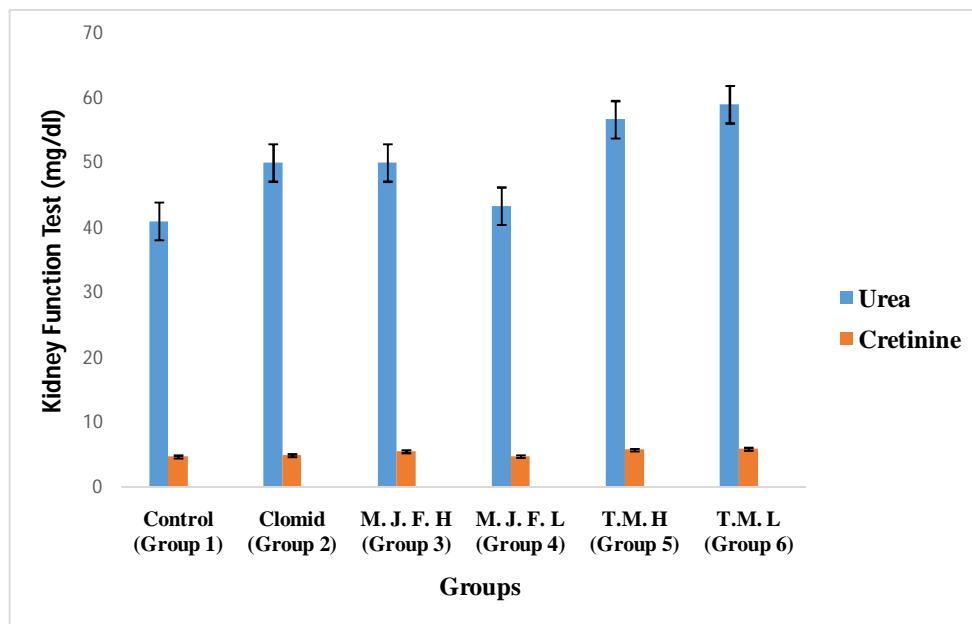
Results are expressed as Mean \pm SD, and are significantly different at $p < 0.05$. **Key:** AST = Aspartate amino transferase, ALT = Alanine amino transferase, ALP = Alkaline phosphate, M.J.F.H= Mijian fen high dose, M.J.F.L= Mijian fen low dose, T.M.H= TsuminMarke high dose, T.M.L= TsuminMarke low dose

Figure:2 Effects of oral administration of Clomid, Mijian fen and Tsuminmarke on liver function parameters

Effect of oral administration of Clomid, Mijian fen and Tsuminmarke on kidney function parameters (urea and creatinine)

Figure 3 showed the mean values of Urea and Creatinine for of rats that were administered Clomid, Mijian fen and Tsuminmarke and their control are presented in figure 3. Statistically significant increase ($P < 0.05$) was observed in the level of urea in

all the test groups compared to the control. Creatinine level slightly increased in the all the test groups compared to the control.



Results are expressed as Mean \pm SD, and are significantly different at $p < 0.05$ Key: M.J.F.H= Mijian fen high dose, M.J.F.L= Mijian fen low dose, T.M.H= TsuminMarke high dose, T.M.L= TsuminMarke low dose.

Figure 3: Effect of oral administration of clomid, mi jian fen and tsuminmarke on kidney Function Parameters (Urea and Creatinine)

DISCUSSION

Although there is an increased acceptance and utilization of medicinal plants worldwide, many are used without reference to any safety (Melanie, 1999). However, they are generally considered to be safe and effective agents (George, 2011). Several researches have indicated that chemical substances including plant extracts could interfere with the concentration and function of sex hormones (Beniet al., 2003), likewise certain physiological fluids.

Estrogen is the main female gonadal hormone produced by the ovaries. Its importance for the maintenance of normal sexual behavior (libido) in females cannot be over emphasized. The neuroanatomical site at which oestrogen acts to facilitate sexual behavior (libido) has been reported to be the ventromedial nucleus of the hypothalamus(Olivier et al., 2011). The increase in the estrogen levels in all the

Comment [u15]: Italyze the rest part also

treatment groups may be linked to the induction of hormone synthesis by the granulosa cells of the growing follicles in the ovary, which enhanced the secretion of the hormone. This implies that the MJF and TM stimulated the mechanism intervening in the process of the hormonal synthesis in the granulosa cells and its secretion into the blood stream (Moundipa et al., 1999). Such increase in the estrogen concentration may account for the enhanced libido in the female rats following the administration of MJF and TM to the animals in the present study. Normal female reproductive functions depend on the secretion of LH and FSH by the pituitary gland under the influence of hypothalamic gonadotropin-releasing hormone (GnRH). In females, LH stimulates the theca cells of the ovaries to secrete testosterone while FSH induces the granulosa cells of the growing follicles to produce estrogen and also aromatase, an enzyme that converts testosterone to estrogen. The testosterone produced is then converted to estrogen by the aromatase. Therefore, the elevated levels of LH and FSH in this study may be ascribed to a stimulatory effect on the hypothalamic-pituitary axis; it suggests a progonadotropic and consequently effect on libido (Yakubu and Afolayan, 2009).

Liver enzymes are well known biomarkers for the prediction of liver toxicity and as such, have been used in scientific reports (Gray and Howorth, 1982; Rahman et al., 2001). Available evidence show that damage to liver cells results in elevations of these enzymes in the serum and the measurement of enzyme activities is of clinical and toxicological significance in determining liver damage by toxicants or in diseased conditions (Wolf et al., 1972; Singh et al., 2001). The level of these enzymes in the blood is directly related to the extent of the tissue damage (Wolf et al., 1972; Singh et al., 2001).

The slight increase in AST and ALT activities in all the test groups compared to controls indicates that the MJF and TM may have capacity to induce liver damage in all the test groups ().

ALP level was observed to increase significantly in the groups that were administered Clomid, low dose of MJF and low dose of TM. Hepatic alkaline phosphatase are most densely represented near the canalicular membrane of the hepatocyte (Essam and Faiha'a, 2012). Obstructive diseases, bile duct obstruction, primary biliary cirrhosis are some examples of diseases in which elevated ALP levels are often predominant over transaminase elevation (Essam and Faiha'a, 2012). This then signifies a possible predisposal of these ailment by the substances users.

Although elevated levels of ALP have been associated with bone diseases, it is also an indicator for obstructive jaundice and intra-hepatic cholestasis (Adebayo et al., 2010). Hence, the observed higher activities of the enzymes in the test groups relative to control, suggests that the Clomid, MJF and TM can induce hepatic cell damage and/or other diseases like osteotoxicity ().

Increasing serum creatinine and urea level is an important indicator of poor glomerular filtration and has been a significant clinical marker for renal dysfunction and loss of renal integrity (Ogbeke and George, 2016). Creatinine as a definitive marker for kidney function, was observed to increase slightly in all test groups. As regards urea being an indicator for kidney disorder, the significant increase observed in the test group implies that the TM may contain some toxic components that are nephrotoxic which, according to Varelyet al. (1987), can be linked to the presence of increased toxic compounds in the blood.

Comment [u16]: No reference

Comment [u17]: No reference

Clomiphene citrate (CC) works to induce ovulation by inhibiting negative, endogenous, oestrogen-feedback on the hypothalamic-pituitary axis, resulting in increased FSH secretion, follicular growth, and ovulation (Emily et al., 2010). As was observed from the result of this research, the substances used also acted in a similar manner as CC which suggest a similar mechanism of action of MJF and TM with CC().

Comment [u18]: No reference

On the other hand, uses of CC was accompanied with many adverse effects, such as ovarian enlargement, vasomotor flashes, nausea, vomiting, breast discomfort, headache, abnormal vaginal bleeding, visual symptoms, weight gain and shortness of breath (Sherbahn,2015). Keskin et al., (2007), reported that CC induced acute pancreatitis. It can also myocardial infarction (Duran and Raja, 2007), hypertriglyceridemia (Yasar and Ertugrul, 2009), deep vein thrombosis (Benshushan et al., 1995) and pulmonary embolism (Chamberlain and Cumming, 1986). Nagao and Yoshimura (2001) reported that Clomiphene citrate has been shown to cause ovarian and uterine abnormalities. These observations can also be observed in the case of MJF and TM in long term users so also an effect on liver and kidney.

Conclusion

In conclusion, the study revealed that Clomid, Mijian fen and Tsuminmarke estrogenic effect and a positive effect on LH and FSH as such revealing their effects on libido. It is also observed from the result of this study that Clomid, Mijian fen and Tsuminmarke have an effect on liver, kidney and possibly the bone.

Comment [u19]: The conclusion section is very shallow. There is no sufficient information. There is no recommendations based on your conclusion.

1. Which aphrodisiacs is most effective to increase libido with minimal side effect.
2. What is your recommendation for your finding?
3. What initiates to do this research?

References

- Abu Hashim H. (2012). Clomiphene citrate alternatives for the initial management of polycystic ovary syndrome: an evidence-based approach. Arch. Gynecol. Obstet., 285(6):1737-1745
- Adebayo AH, Abolaji AO, Opata TK, Adegbenro, IK (2010) Effects of Ethanolic Leaf Extract of *Chrysophyllum albidum* G. on Biochemical and Haematological Parameters of Albino Wistar Rats. African Journal of Biotechnology.9(14):214550
- Batawila K. Antifungal activities of five Combretaceae used in Togolese traditional medicine. (2005) Fitoterapia.;76(2):264-8.
- Bartels, H. and Bohmer, M. (1992) Quantitative Determination of Creatinine. Clinical Chemical Acta, 37, 193.
- Benie, T., Duval, J and Thieulant, M. L. (2003). Effects of some traditional plant extract on rat estrous cycle compared with clomid. Phytotherapy Research, 17(7):748-755.
- Benshushan A, Shushan A, Paltiel O, Mordel N, Laufer N (1995). Ovulation induction with clomiphene citrate complicated by deep vein thrombosis. Eur. J. Obstet. Gynecol. Reprod. Biol., 62(2):261-262.

Chamberlain R.A and Cumming D.C (1986). Pulmonary embolism during clomiphene therapy for infertility in a male: a case report. *Int. J. Fertil.*, 31(3):198199.

Duran, J.R. and Raja, M.L. (2007). Myocardial infarction in pregnancy associated with clomiphene citrate for ovulation induction: a case report. *J. Reprod. Med.*, 52(11):1059-62.

El Ghazali GEB, Abdalla WE, Khalid HE, Khalafalla MM and Hamad AA. (2003) Medicinal Plants of the Sudan, part V, "Medicinal Plants of Ingassana Area. Sudan. National Centre for Research.

Emily, S. Jungheim, M.D, Anthony O. Odibo, M.D. (2010). Fertility treatment in women with polycystic ovary syndrome: a decision analysis of different oral ovulation induction agents. *Fertil. Steril.* 94(7):2659-2664

Essam F. Al-Jumaily and Faiha'a, M. khaleel. The Effect of Chronic Liver Diseases on some Biochemical Parameters in Patients' Serum. *Current Research Journal of Biological Sciences.* 2012;4(5):638-42.

Garba I D, Yakasai I A, Magashi M K, (2013), Use of Aphrodisiacs amongst women in Kano, northern Nigeria *IOSR Journal of Pharmacy*, Vol 3, 01-04

George, P. (2011). Toxicity and Safety Profile of Medicinal Plants. *Journal of Herb Med Pharmacology*, 01(6): 40-44.

Gray C, Howorth PJ (1982). *Clinical Chemical Pathology. The ELBS*, Edward Arnold Publishers Ltd., 9th ed.67-73/263-9.

Iwuzor OK (2019). Heavy metal concentration of aphrodisiac herbs locally sold in the South-Eastern region of Nigeria. *Pharmaceutical Science and Technology.* 2019; 3(1): 22-26. DOI: -10.11648/j.pst.20190301.13.

Kaadaaga HF, Ajean, J, Ononge S, et al. (2014) Prevalence and factors associated with use of herbal medicine among women attending an infertility clinic in Uganda. *BMC Complementary and Alternative Medicine.*14: 27. DOI: <https://doi.org/10.1186/1472-6882-14-27>.

Keskin, M., Songür, Y. and Isler, M. (2007). Clomiphene-induced acute pancreatitis without hypertriglyceridemia. *Am. J. Med. Sci.*, 333(3):194-196.

Kone, W.M and Atindehou, K. K. (2008). Ethnobotanical Inventory of medicine in Northern Cote d' Ivore (West Africa). *Journal. African Journal of Botany*, 74:76-84.

- Nagao, T. and Yoshimura, S. (2001). Oral administration of clomiphene to neonatal rats causes reproductive tract abnormalities. *Teratog. Carcinog. Mutagen.*, 21(3):213-221
- Melanie, J.C. (1999). Herbal Remedies: Adverse Effects and Drug Interactions. *American Academy of Family physicians*, 59(5):1239-1244
- Moundipa FP, Kamtchouing P, Koueta N, Tantchou J, Foyang NP, Mbiapo FT. . (1999) Effects of aqueous extracts of *Hibiscus macranthus* and *Basella alba* in mature rat testis function. *J Ethnopharmacol.* 65(2): 133–139.
- Ogbeke GI, George BO, Ichipi-Ifukor PC. (2016). *Aframomumsceptrum* modulation of renal function in monosodium glutamate (MSG) induced toxicity. *UK J Pharm Biosci* 4:54–60.
- Okpekon T. (2004) Antiparasitic activities of medicinal plants used in Ivory Coast. *J Ethnopharmacol.*;90(1):91-7.
- Olivier B, Chan JS, Snoeren EM, Olivier JD, Veening JG, Vinkers CH, Waldinger MD, Oosting RS (2011). Differences in sexual behavior in male and female rodents: role of serotonin. *Curr Top BehavNeurosci.* 8: 15–36.
- Oreagba A I, Oshikoya A K, Amachre M. (2011), Herbal medicine use among urban residents in Lagos, Nigeria, *BMC Complementary and Alternative Medicine*, www.biomedcentral.com/1472-6882/11/117 [
- Ouedraogo A, Kakai RG and Thiombiano A. (2013). Population structure of the widespread species, *Anogeissusleiocarpa* (DC.) Guilt. & Perr. across the climatic gradient in West Africa semi-arid area. *South African Journal of Botany.*;88:286-295.
- Pakia, M and Cooke, J.A. (2003). The ethanobotany of midzichenda tribes of the costal forest areas in Kenya. *South African Journal of Botany*, 74:76-84.
- Rahman MF, Siddiqui MK, Jamil, K. Effects of Vepacide (*Azadirachta indica*) on Aspartate and Alanine Aminotransferase Profiles in Sub-chronic Study with Rats. *Journal of Human Experimental Toxicology.* 2001;20:243-9.
- Radox Laboratories, 1997. Determination of Alkaline Phosphatase (Ec 3.13. 1). Radox Laboratories, Antlim, UK.
- Raskin T. (1992). The Dynamics and Changing Structure of Traditional Healing System in Nigeria, *International Journal of Health Research.*; 4(2): 99-106
- Read, B. E. 1982. Chinese materiamedica: insect drugs, dragon & snake drugs, fish drugs. Southern Materials Center, Inc., Taipei, The Republic of China.
- Reitman D. and S. Frankel, 1957. A colometric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-59.

- Rotimi VO. Activities of Nigerian chewing stick extracts against *Bacteroidesgingivalis* and *Bacteroidesmelaninogenicus*. *Antimicrob Agents Chemother.* 1988;32(4):598-600.
- Muhammad SA (2009). Reproductive toxicologic evaluations of *Bulbinenatalensis* Baker stem extract in albino rats. *Theriogenology.* 72(3): 322–332
- Sherbahn, R. (2015). Side effects and adverse effects of clomid, clomiphene citrate. Advanced Fertility Center of Chicago, USA.
- Singh NS, Vats P, Suri S, Shyam R, Kumria MML, Ranganathan S, et al. Effect of an Antidiabetic Extract of *Catharanthusroseus* on Enzymic Activities in Streptozotocin Induced Diabetic Rats. *Journal of Ethnopharmacology.* 2001;76:269-77.
- Tietz, N.W. (1995). *Clinical guide to laboratory tests*, 3rd edition W. B. Saunders, Philadelphia 1-997.
- Varely, H., Gowenlock, A.H. and Bell, M. (1987): *Practical Clinical Biochemistry. Hormones, Vitamins, Drugs and Poison*, 6th Edn., Heinemann Medical Books, London, pp: 477 549.
- Victor YA. (2013) In-Vitro Assessment of Antioxidant and Antimicrobial Activities of Methanol Extracts of Six Wound Healing Medicinal Plants. *Journal of Natural Sciences Research.* ;3(1):74-82.
- Wakil AM, Sandabe UK, Mbaya AW, Ngulde SI, Sodipo OA, Shettima MS, et al. Evaluation of Trypanocidal efficacy of Aqueous extract of *Ficussycomorus* Linn. (Moraceae) stem bark in Albino rats. *Vom J Vet Sci* 2016;11:58–72. www.scopemed.org/?mno=249174
- WHO, (2005), Resolution – promotion and development of training and research in traditional medicine, WHO Document No.WHA, 30- 49 [35].
- WHO, (2008), Resolution – Drug policies and management: Journal of medicinal plants. WHO Document NO.WHA, 31- 33.
- WHO: Traditional Medicine Strategy 2002–2005, [whqlibdoc.who.int/hq/2002/who, 22 February, 2014: 12:05](http://whqlibdoc.who.int/hq/2002/who_22_Feb_2014_12_05.pdf)
- Wolf PL, Williams D, Tsudaka T, Acosta L. *Methods and Techniques In: Clinical Chemistry.* John Wiley and Sons USA. 1972;23-9.
- World Health Organization (2008): *Traditional Medicine Strategy 2002-2005.* WHO/EDM/TRM/2002.1, [<http://whqlibdoc.who.int/hq/2002/>, 02 march, 2014
- Yasar, H.Y. and Ertugrul, O. (2009). Clomiphene citrate-induced severe hypertriglyceridemia. *Fertil. Steril.*, 92(1):396.e7-8.

Yakubu MT, Afolayan AJ. (2009) Reproductive toxicologic evaluations of *Bulbinenatalensis* Baker stem extract in albino rats. *Theriogenology*. 72(3): 322–332

Comment [u20]: 1. Journal should be italicized.
2. Use recent reference rather than old reference
3. Generally, the reference writing style should be based on Inter. Journal of Biochemistry guideline, so follow the guideline.

UNDER PEER REVIEW