Herbal Medicines Journal **2018; Vol. 3, No. 1:1-7**
DOI: 10.22087/hmj.v3i1.675 **ISSN:** 2538-2144

Original Article

The Reproductive Toxicity of the Ethanolic Extract of *Tetrapleura tetraptera* Pods on Male and Female Swiss Albino Mice (*Mus musculus*)

Adeola Michael Oloyede[1*]

[1] Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba, Lagos State, Nigeria

Received: 01.3.2018; Accepted: 28.03.2018

Abstract

Background and Aim: *Tetrapleura tetraptera* is used to treat various maladies in southwestern Nigeria, particularly among the Yoruba tribe. This research was designed to examine the reproductive toxicity of the ethanolic extract of *T. tetraptera* in male and female mice respectively.

Materials and Methods: To conduct a spermatogenic study, 20 male mice weighing averagely 19 – 25g were divided into four groups of 5 mice. Groups 1 – 3 were administered *T. tetraptera* extract of 200, 100, and 50m/kg^{-1} bwt. orally respectively. The control group was administered distilled water. All the groups were treated for 40 days after which they were sacrificed via jugular puncture. The testes were excised and sperms collected from epididymis for the preparation of slides. Various histologic studies have been carried out on the testes. For teratogenic studies, 20 female mice weighing averagely between 15– 20g were divided into four groups based on body weight, and then a male mouse was placed in each cage to ensure mating. Animals in group 1 – 3 received 200, 100, and 50m/kg^{-1} bwt of the extract orally, while the control group received distilled water. The animals were treated for 40 days after which their uteri were excised to observe embryos or foetuses.

Results: In the spermatogenic studies, some abberrant sperm cells such as sperms without hook, armophous head, two tails, pin head and clusterd tails were observed. Histology revealed slight modification of histoachitechure of the testes like necrotic shreded cells with degenerating basal membrane, non-differentiated germinal cells, artrophy of sertoli cells and vacuolated sertoli cells.

No embryo or foetus was found in the female reproductive organs after excission in all the mice treated with extract, but each mouse in the control group conceived and delivered an average of 6 mice.

Conclusion: *T.tetraptera* may contain some cytotoxic agents inhibiting implantation and distorting sperm morphology. The extract may be unsafe for consumption at the doses investigated.

Keywords: Spermatogenic, Teratogenic, Toxicity, *Tetrapleura tetraptera*, Testes, Embryo

***Corresponding Author:** Oloyede Adeola Michael, Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba, Lagos State, Nigeria. Email: oloism@yahoo.com.

Please cite this article as: Oloyede M A. The Reproductive Toxicity of the Ethanolic Extract of *Tetrapleura tetraptera* Pods on Male and Female Swiss Albino Mice (*Mus musculus*). Herb. Med. J. 2018;3(1):1-7.

Introduction

Tetrapleura tetraptera widely referred to as Aidan tree, belongs to the fabaceae family. It is highly valued in Nigeria and other regions for its nutritional and medicinal properties (1). The fruits and seeds of this plant have been reported to produce good aroma and flavour in foods (2). Since it has distinct medicinal

properties, *T. tetraptera* is used in traditional medicine for several ailments (3-7). Although the plant has been examined in various studies, the teratogenic and spermatogenic activities of the ethanolic extract of the pods have not been previously investigated. This study, hence, was carried out to evaluate the spermatogenic and teratogenic activities on male and female reproductive organs of male and female mice respectively.

Materials and Methods

Plant Material

T. tetraptera pods were purchased in Bariga market, and then they were eamined for authentication in the Forestry Research Institute (FRIN) Ibadan Oyo state with voucher No. FHI 1O7984. It was dried and pulverized to powder form. The pulverized plant weighing 317.0g was soaked in 1000ml absolute ethanol for 72hrs. The crude extract was then sieved using cotton wool and then it was placed on the hot plate at 40 ± 1°C to evaporate the ethanol present in the crude extract until it was solidified. 18.1g extract was realised after heating, and it was subsequently kept in air tight amber bottles.

Animals

Twenty male *Mus musculus* of 13-15 weeks old, weighing 19-25g, considered as the bioassay organisms, were purchased from the Federal Vaccination Production Laboratory (FVLP), YABA. The mice were fed ad-libitum with mice pellets purchased from Nigerian Institute for Medical Research (NIMR) every day. The animals were kept in cages for at least 14 days in order to be acclimatized to laboratory conditions (29 ± 2˚C and Relative Humidity 70 ± 2%) before the beginning of bioassay. For teratogenic studies, 20 female mice of 13-15 weeks' old weighing averagely between 15–20g were divided into four groups based on body weight, and then a male mouse was placed in each cage to ensure mating. Animals in group 1 – 3 received 200, 100, and 50m/kg^{-1} bwt of the extract orally. The choice of doses was based on folkloric measurement as used traditionally and having observed that the LD 50 value is 3240.37 mg kg-1 (8), while the control group received distilled water. The extract was administered orally to the mice for 40 days in the morning. On the 40th day, the male mice were sacrificed by jugular puncture and excised to remove the epididymis and testes while female mice were anaesthesized and then sacrificed to observe uteri.

Sperm Head Abnormality Assay

Following anesthetization, the male mice were sacrificed by cervical dislocation. The epididymides were removed and cut up with sharp scissors in physiological saline in a petri dish. Subsequently, smears were prepared on some slides which were clean following staining the cells with a mixture of normal saline (9:1) for 45 min. The slides were air-dried and coded for subsequent investigation that was supposed to be carried out under microscope, and then various abnormalities were recorded. Cytological investigation for sperm-head abnormalities was conducted by the use of a binocular microscope at X1000 magnification. Only one slide was prepared for each mouse, which was used for scoring using the tally counter.

Histology

Testes were kept in formalin 10%, and following the processing of tissue were stained with H&E (Hematoxilin and Eosine) for histological investigation to be carried out under a light microscope. Three slides were prepared per animal, and 3 sections were observerd per slide. The histopath of the testes was carried out using microtome model of Leita Rm 2235, and histomorphometric parameters such as seminiferous epithelium, seminiferous tubules, accumulation of lipids in sertoli cells, spermatogonia cells and intestitial cells of leydig were evaluated.

Statistical Analysis

All data were analyzed with IBM® PASW/SPSS® Statistics 18.0-2009, the mean± standard error were calculated for the different aberrations in the mice.

Ethics statement

Ethical approval was received from ethical committee of the University of Lagos, College of Medicine, University of Lagos in November 2016.

Results and Discussion

Table 2 shows a few abberrant sperms such as absence of hook (most prominent), two tails, knobbed hooks, Amorphous head, bent hook, hook at wrong angle, tail folder overhead, pin head, banana shaped head, and clustered head observed in treated mice though insignificant. More sperms were observed in the group administerd 200mg/kg^{-1} than in the control group.

Histology of Testes Administered T. tetraptera

Histology of testes of mice exposed to T. tetraptera (HE X10). A – Showing Non-differentiated germ cells (ndGC) and arrow indicating degenerating basal membrane at 200mg/kg-1. B- Intact basal membrane (BM) with gradual atrophy of cytoplasmic content at 100mg/kg-1. C-Necrotic cells (NC), disorganized seminiferous tubules (DST) and hyalinized seminiferous tubules at 50 mg/kg-1. D-Atrophy and vacuolated cytoplasm of sertoli cells. Control- showed conserved histoarchitecture of the testes with normal seminiferous tubules, which are linked by thick germinal epithelium, nomal leydig cells. Within the seminiferous tubules are seen spermatogonia at the base of the tubule, Primary spermatocyte and spermatid at different stages of maturation and embedded in the germinal epithelial closer to the lumen. Spermatids tails protruded into the seminiferous tubules. Sertoli cells were also seen.

Spermatogenic studies have indicated a series of abnormal sperm morphology which was dose-dependent. These damages to sperm cells can be by

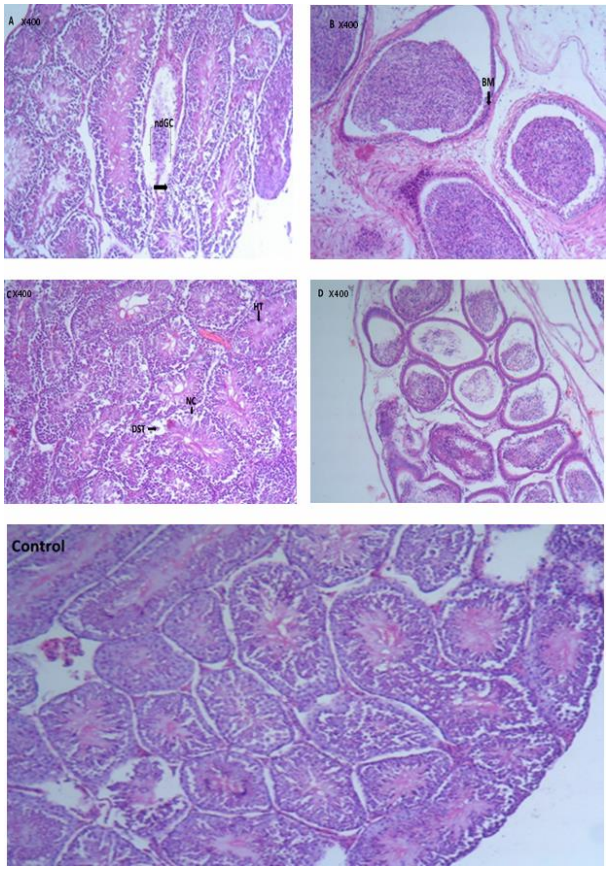

Figure 1. Histology of Testes Administered *T. tetraptera*

Histology of testes of mice exposed to *T. tetraptera* (HE X10). A – Showing Non-differentiated germ cells (ndGC) and arrow indicating degenerating basal membrane at 200mg/kg^{-1}. B- Intact basal membrane (BM) with gradual atrophy of cytoplasmic content at 100mg/kg^{-1}. C-Necrotic cells (NC), disorganized seminiferous tubules (DST) and hyalinized seminiferous tubules at 50 mg/kg^{-1}. D-Atrophy and vacuolated cytoplasm of sertoli cells. Control- showed conserved histoarchitecture of the testes with normal seminiferous tubules, which are linked by thick germinal epithelium, nomal leydig cells. Within the seminiferous tubules are seen spermatogonia at the base of the tubule, Primary spermatocyte and spermatid at different stages of maturation and embedded in the germinal epithelial closer to the lumen. Spermatids tails protruded into the seminiferous tubules. Sertoli cells were also seen.

physiological, cytotoxic or genetic mechanism (10). Implying that variation in DNA content of spermatozoa and gross morphological deficiencies might be genetically controlled (10). In genetic mechanism, such abnormality may be adduced to damages that occurred during pre-meiotic stages of spermatogenesis (11). Physiologically, the series of complicated and synchronized mophorlogical and biochemical steps involved in the formation of normal sperm heads during spermatogenesis may be responsible for the abnormality (12). *T. tetraptera* has been described to elicit numerous advantageous biological responses due to the presence of various compounds such as flavonoids, phenols, tannins, saponins, terpenoids and phlebotannin (13; 5). GC–MS analysis indicated the existence of D-fructose, piperazine, octodrine, glycidol, glyceraldehydes, 6-octadecenoic acid and 9, 12-octadecenoic acid. Meanwhile, D–fructose was the principal compound (14). In addition to these important phytochemicals, Essien *et al*., (15) also confirmed the presence of toxic constituents such as hydrogen cyanide, phytates and oxalates in *T. tetraptera*. Hydrogen cyanide is rated as a systemic poison which inhibits cytochrome oxidase, and prevents the cellular use of oxygen. It also inhibits the final stage of electron transport in the cells of the brain which leads to the loss of consciousness, respiratory arrest, and death (16). Odeigah (14)

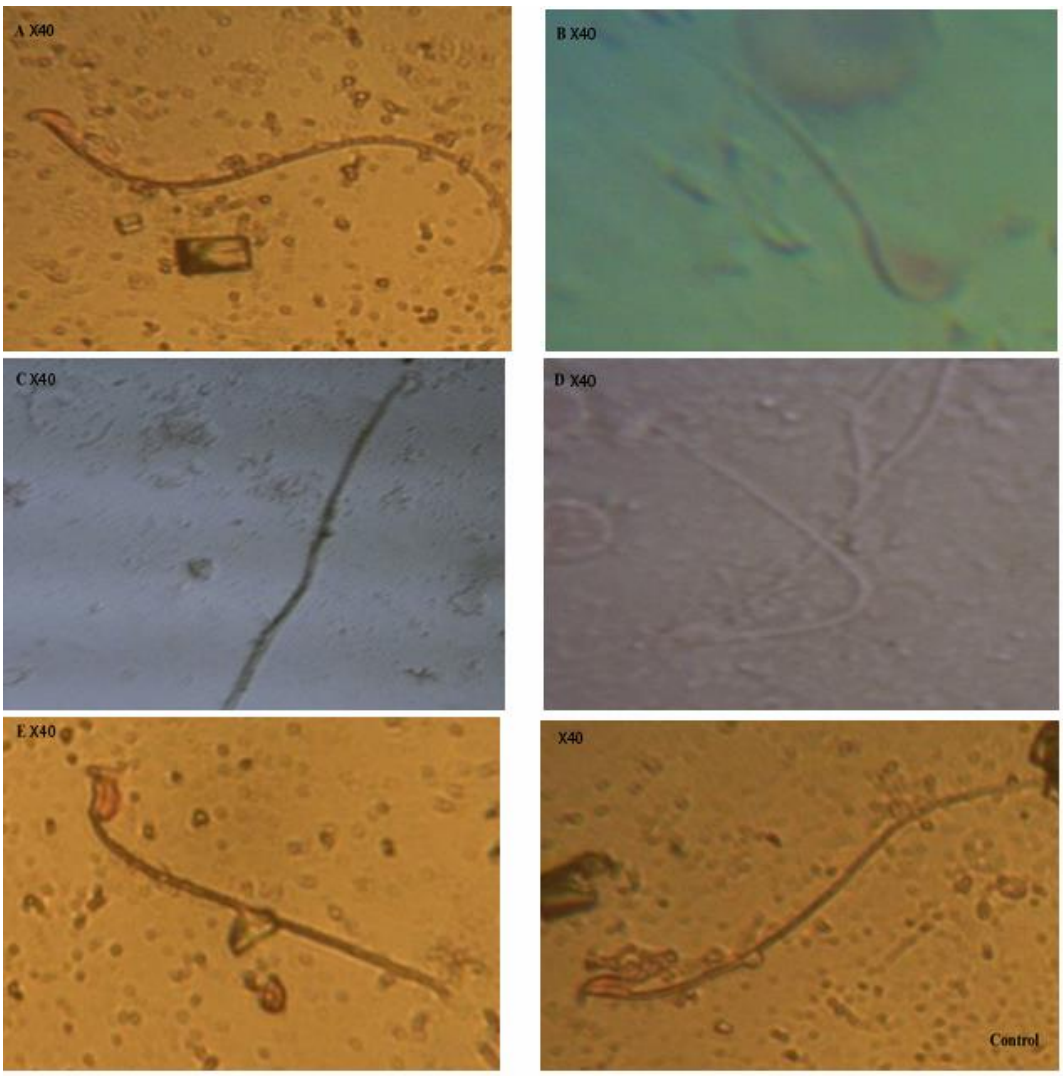

Figure 2. A-E: A- sperm without hook, B- amorphous head, C- pin head, D-banana shaped sperm, E- hook at wrong angle, Control-normal sperm morphology.

indicated that exposure of mice to the chemicals might result in pituitary hypothalamic or sex hormonal effects, which in turn could affect spermatogenesis, or exposure could bring about abnormalities in seminal fluid leading to functional or structural impairment of sperm.

Hydrogen cyanide, which is also a chemical found in T. tetraptera, may have affected spermatogenesis via this mechanism. Oxalates found in this plant has been reported as a component part of the most common form of kidney stones which induces toxic reactions in renal epithelial cells, such as altered membrane surface properties and cellular lipids. Moreover, it brings about changes in gene expression, disrupts mitochondrial functions, forms reactive oxygen species, and reduces cell viability (17). The toxicity of this plant is also supported by the histologic observation on the testes. The histoarchitecture of the testes undergo degenerative changes which may induce antiandrogenic and reversible infertility in male albino mice. These chemicals may also cause mutation in the primary spermatocytes and spermatogonia at the pre meiotic phases of spermatogenesis. Taylor (18) indicated that mutation in germ cells before or during the reproductive period could be passed to later generations resulting in reproductive deficiencies. Hence, the abnormalities found in sperm heads presumably happened during spermatogenesis, becasuse as the sperm head develops its shape, it is highly stable (19). A similar study on the toxicity of the ethanolic extract of *T. tetraptera* revealed that it could cause 59.17% mortality on the second instar larvae of Anopheles gambiae (20)

No embryo or foetus was found in teratogenic studies in female mice. Phytochemical screening has also indicated the presence of some bioactive and toxic substances of plant extracts that can influence the regulation of oestrus cycle, conception and reproduction (21). Alkaloids have been indicated to decrease plasma concentrations of LH, FSH and estradiol (22). Elizzi *et al*. (23) exhibited that the stem

Figure 3. A – C: A-Yellow plug (YP) showing sign of mating, B-Embryo (E) absent, C- Foetuses (F) present. Treated animals did not conceive or implantation was inhibited.

Table 1: The Effect of *T. tetraptera* on Sperms Mophology.

Treatment	No. of sperms scored	No. of normal sperms	Abnormal % of sperms
200mg/kg^{-1}	3000	237.00 ± 46.40	8
100 mg/kg^{-1}	3000	206.00 ± 48.05	6
50 mg/kg^{-1}	3000	173.6 ± 17.34	6
Control (distilled)	3000	221.33 ± 39.49	2

Values are Mean±SEM (N=5).

Table 2: Statistical Analysis of the Sperm.

Phenotype	Normal head	No hook	Two tails	Knobbed head	Amorphous head	Bent head	Hook at wrong angle	Tail folded over head	Pin head	Banana shaped head	Clustered head
Mean ±SEM	58±2.00	4±1.80	4±0.37	2±0.90	2±0.39	2±092	3±1.00	4±0.39	3±1.00	2±0.5	2±0.37

bark of *T. tetraptera* had an inhibitory impact on luteinizing hormone released by pituitary cells. This inhibition obviously prevents the occurrence of ovulation in mice. Quantitative analysis indicated that phytochemical contents such as saponins, alkaloids, tannin, flavonoid, and phenol were present in the pod of *T. tetraptera* (24). It has been reported that the plants contraceptive properties have saponins, alkaloids, and glycosides compounds that can account for the antigonadotropic properties of plants which are used as natural contraceptives (23). Furthermore, glycoalkaloids of some plants, such as potato, have toxic impacts capable of causing the death of embryo as well as absorbed and dead fetuses (25). Therefore, it is probable that induction of infertility by the components of *T. tetraptera* can occur by affecting the hypothalamus or direct toxic effect on embryo. Hence, the presence of alkaloids, phenolics, steroids, cyanogenic glycosides, oxalates and saponins in the extract of *T. tetraptera*, which might act alone or even as a synergy, might be somehow responsible for the observed pregnancy-terminating impacts, sperm abnormalities and alteration of the histoarchitecture of the reproductive organs in the present study.

Conclusion

Apart from cyanogenic glycosides, phytates and oxalates, found in this plant, food poisoning generated from plants' secondary metabolites has not been adequately investigated in the majority of developing countries. People have died out of ignorance, poverty and inadequate phytomedical information and education, especially within the African societies. Further investigations are required to evaluate lesser doses and explore in vitro studies to ascertain some other physiological implications in some biological systems.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre Kenya. 2009.
2. Adesina SK, Iwalewa EO, Johnny II. Tetrapleura tetraptera Taub- Ethnopharmacology, Chemistry, Medicinal and Nutritional Values-A Review British Journal of Pharmaceutical Research. 2015;12(3):1-22.
3. Tsala DE, Habtemariam S, Simplice FH, Ndzana MTB, Jital AA, Dimo T. Topically Applied Tetrapleura tetraptera Stem-Bark Extract Promotes Healing of Excision and Incision Wounds in Rats. Journal of Intercultural Ethnopharmacology. 2014;3(2):63-7.
4. Ishola IO, Afolayan GO, Olugbade IO, Olasunmbo Owolabi-Afolayan O. Protective effect of Tetrapleura tetraptera (Schum. & Thonn.) fruit extract against haloperidol-induced catalepsy and

scopolamine-induced memory impairment: Involvement of antioxidant system. West African Journal of Pharmacology and Drug Research. 2015;30.
5. Salahdeen HM, Omoaghe AO, Isehunwa GO, Murtala BA, Alada ARA. Gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extracts of kolanut (Cola nitida) (vent) and its toxicity studies in rats. Journal of Medicinal Plants Research. 2015;9(3):56-70.
6. Adeleye SA, Oguoma OI, Braide W, Osogbase EE. Phytochemistry and Antimicrobial Properties of 2:1 and 1:2 Ethanol-methanol Extracts of Tetrapleura Tetraptera. American Journal of Current Microbiology. 2016;4(1).
7. Onuka AE, Okechukwu NC, Maxine KM, Bond AU. Evaluation of the Effects of Tetrapleura tetraptera Extract and Clomiphene Citrate to Determine Influence of Reproductive Hormones and Estrous Cycle on Leukocyte Counts: Novel Evidence for a Potential Developmental Link Between Luteinizing Hormone and MID Cells. Hematology & Transfusion International Journal. 2017;5(6).
8. Okokon JE, Udokpoh AE, Antia BS. Antimalaria activity of ethanolic extract of Tetrapleura tetraptera fruit. Journal of Ethnopharmacology. 2007;111:537-40.
9. Odeigah PGC. Sperm-head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutation Respodents. 1997;389:141-8.
10. Bakare AA, Mosuro AA, Osibanjo O. An in vivo evaluation of induction of abnormal sperm morphology in mice by landfill leachates. Mutation Res. 2005;582:28–34.
11. Monesi V. Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine, Journal of Cell Biology. 1962;14:1–18.
12. Otubanjo OA, Mosuro AA. An in vivo evaluation of induction of abnormal sperm morphology by some antihelmintic drugs in mice. Mutation Research. 2001;497:131-8.
13. Eloho Akin-Idowu P, Olubunmi Ibitoye D, Temitope O. Chemical Composition of the Dry Fruit of Tetrapleura tetraptera and its Potential Impact on Human Health. Journal of Herbs, Spices & Medicinal Plants 2011;17:52-61.
14. Ochuko L, Olayinka F, Odjobo B, Tosin A, Olaseinde Adesewa O, Tugbogbo A, et al. Ethanol extract of Tetrapleura tetraptera fruit peels: Chemical characterization, and antioxidant potentials against free radicals and lipid peroxidation in hepatic tissues. Journal of Taibah University for Science. 2017;11:861-7.
15. Essien EU, Izunwanne BC, Aremu CY, Eka OU. Investigation into the toxic constituents of the dry fruit of Tetrapleura tetraptera, International Journal of Food Sciences and Nutrition. 2009;44:55-8.
16. National Research Council U.S. (NRC) Subcommittee on Acute Exposure Guideline Levels. Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 2. Washington (DC): National Academies Press (US); Hydrogen Cyanide: Acute Exposure Guideline Levels. 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK207601/.
17. Jonassen JA, Kohjimoto Y, Scheid CR, Schmidt M. Oxalate toxicity in renal cells. Urol Res. 2005;3(5):329-39.
18. Taylor RJ. Food Additives. John Wiley and Sons, New York. 1980.
19. Wyrobek AJ, Bruce WR. Chemical induction of sperm-head abnormalities in mice, Production of National Academic of Science. USA. 1975;72:4425-9.
20. Aina SA, Banjo AD, Lawal OA, Okoh HI, Aina OO, Dedeke GA. The Toxicity of Extracts of Tetrapleura tetraptera (Aridan), Delonix regia (Flame of the Forest) and Raphia vinifera (Raffia Palm) on the Larvae of Anopheles gambiae. Academic Journal of Entomology. 2009;2(2):67-70.
21. Benie T, Duval T, Thieulant ML. Effects of some traditional plant extracts on rat oestrous cycle compared with clomid. Phytotherapia Resources. 2003.;17:748-55.
22. Bianco F, Basini G, Grasselli F. The plant alkaloid Sanguinarine affects swine granulosa cell activity. Reproductive Toxicolology. 2006;21:335-40.
23. El-izzi A, Bennie T, Thieulant M, Dural L. Inhibitory effect of saponins from T. tetraptera on the leutei-nizing hormone release by pituitary cells. Planta Medica. 1990.;6:357-9.
24. Ekwenye UN, Okorie CF. Antibacterial activity of Tetrapleura Tetraptera Taub. Pod extracts. International Journal of Pharmacy and Biological Sciences. 2010.1:71–8.
25. Wang L, waltenberger B, Pferschy-Wenzig EM, Blunder M, Liu X, Malainer C, et al. Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ). Biochemistry Pharmacology. 2014;4:2-9.