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ALTERATIONS IN LIPID METABOLISM INDUCED BY THE BETEL LEAF STALK EXTRACT IN MALE ALBINO RATS

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ABSTRACT

The aim of this study was to investigate the antifertility efficacy of betel leaf stalk extract on lipid metabolites. The betel leaf stalk extract was administered at the dose of 50 mg/Kg/day through oral gavages method for 15 days. The impaired lipid metabolisms were observed in testis. The higher lipids in seminal vesicle and prostate gland indicate the elevation in lipid metabolism and lipogenesis, suggesting some alterations in the chemical composition of the seminal plasma and prostatic fluid. The accumulation of lipids in prostate causes the inhibition in lipase activity. The elevated testicular Phospholipids due to administration of extract results the disruption in integrity of the spermatozoal membrane and its adequate phospholipid composition leads to infertility. Significant reductions in triglycerides represent no risk of cancer. Increased concentration of cholesterol in testes suggests the impairment of spermatogenesis. The decreased cholesterol content of the epididymis and prostate gland indicated impairment in its synthesis and may hinder steroidogenesis, thus suggesting anti-steroidogenic potential of the plant extract.

KEYWORDS: Betel leaf stalk, lipogenesis, Phospho lipids, Infertility, Spermatogenisis, Steroidogenisis.

INTRODUCTION

Many plants have been identified and tested for their antifertility effect in male rats and mice (Thejashwini *et al*., 2012). Natural products are in great demand owing to their extensive biological properties and bioactive components which have proved to be useful against large number of diseases (Chakraborty & Shah, 2011). Although very few contraceptives have been developed from plant extracts, their potentiality has not been determined accurately, and their mode of action has been beyond our knowledge until now because there are many problems in assessing plant extract including batch to batch variation and a lack of definite active portion of the extract used for the development of herbal contraceptives. In mice Piper nigrum (Piperaceae) administered with 100mg dose for 90 days, degenerative changes were observed in all the tubules. Affected seminiferous tubules showed intraepithelial vacuolation, loosening of germinal epithelium, occurrence of giant cells, and mixing of spermatids of different stages, in severe cases, the tubules were lined by mainly a layer of sertoli cells (Mishra & Singh, 2009). *Piper betel* also known to have antifertility effect in rodents (Sarkar *et al*., 2000 and Kushwaha *et al*., 2007). Contraceptive like properties have also been reported in women by local tribes of Rajasthan and Bengal region of India as they use these for birth control also. (Arvind Singh *et al*., 2011). It mainly contains amide alkaloids, and piperine is the major active component (Suresh

C. Joshi *et al*., 2011). Hence, this study aimed to evaluate the *Piper Betel* leaf stalk extract as an antifertility through different metabolites and enzymatic processes involved in lipid and energy metabolism.

MATERIAL AND METHODS

In the present study healthy adult (3-4 months old, weight 215±10g) male wistar strain albino rats were used. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. The male albino rats were taken and divided in to two groups, each group contains 6 rats. First group rats were control rats administered with 1 ml of distilled water. Second group rats were experimental administered with betel leaf stalk extract, at the dose of 50 mg/Kg/day through oral gavages for 15 days. The ethanol extract was prepared according to WHO (1983) protocol CG-04. Stalks were shed-dried, powdered and extracted with 95% ethanol (v/v) at 55-60ºC for 3h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24ºC until use. Animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean air conditioned room, with photoperiod of 12 hours light and 12 hours dark cycle, at 25 ± 2ºC with a relative humidity of 50 ± 5%. The rats were fed with standard laboratory feed (Hindustan Lever Ltd, Mumbai) and water *ad libitum*. Twenty four hours after the last dose, the animals were autopsied. The tissues like testes, epididymis, seminal vesicle prostate gland and liver were isolated, chilled immediately and blood was collected, serum was separated and used for biochemical analysis. Total lipids (Folch *et al.,* 1957), Lipase activity (Colowick & Kaplan 1957), free fatty acids (Natelson 1971a), glycerol (Colowick and Kaplan 1957), Phospholipids (Zilversmidt and Davis 1950), Triglycerides (Natelson 1971b) and Total Cholesterol (Natelson 1971c) were estimated in both control and experimental rats.

RESULTS AND DISCUSSION

Lipids are key elements in the chemistry of life. Many plants and animals store chemical energy in the form of triglycerides, which are sparingly soluble in water. Defects in lipid synthesis or processing contribute to the development of many diseases, including obesity. Therefore, a lipid as substrates for spermatozoa, this study was focused on lipid metabolites.

The levels of total lipids (table-1) in reproductive tissues like testes and epididymis were significantly decreased while in the seminal vesicle and prostate gland they are significantly elevated (Hasim Basha & Changamma, 2013). Apart from carbohydrates, lipids are the second major fuel for mammalian organisms. The lipid content and distribution of the mammalian germ cell change in an ordered fashion from the first spermatogonial cell division, through spermatogenesis and epididymal maturation to capacitation and fusion with the oocyte in the female genital tract (Jones, 1998 and Flesch FM & Gadella BM. Dynamics, 2000). The lipid composition of the sperm membrane exerts a significant effect upon the functional quality of spermatozoa (Zalata *et al*., 1998a and Kusemiju *et al*., 2002). Spermatozoa are rich in polyunsaturated fatty acids, which are more liable for lipid peroxidation by reactive oxygen species (ROS) (El-Sweedy *et al.,* 2007).

The degradation of lipids indicating impaired lipid metabolism in the testis (Changamma *et al*., 2011). It is known that the lipid composition of the sperm membrane exert a significant effect upon the functional quality of spermatozoa (Zalata *et al*., 1998a). As spermatozoa are rich in lipids with phospholipids occupying approximately 70% of the total, lipids have an important role in maintaining sperm cell viability, maturity, fertility and function (Zalata *et al*., 1998b). Therefore, the *Betel* leaf stalk extract exert impaired lipid metabolism in the testis which leads to impaired spermatogenesis.

The epididymis plays a vital role in sperm maturation. It is a dynamic system that influences the spermatozoa directly or indirectly through the environment within which the spermatozoa exist. The sperm maturation process is therefore dependent on functional epididymal cells (Thomas Saether, 2003). Thus,

the extract shows its antifertility mechanism by lowering the lipid concentration, leading to alterations in epididymal sperm maturation process, sperm motility and fertilizing capacity.

In accessory secretary glands like seminal vesicle and prostate gland total lipids were elevated due to *Betel* leaf stalk administration. The higher lipid indicates the elevation in lipid metabolism and lipogenesis, suggesting some alterations in the chemical composition of the seminal plasma and prostatic fluid. The seminal fluid formed from the lipofusein granules from dead epithelial cells gave the secretion its yellowish color. Hence the stalk extract increases the lipofusein granules in seminal fluid (Lohiya *et al.*, 1999 and Hasim Basha & Changamma, 2013).

The reduced lipids in liver (table-2) indicate there were some alterations in hepatic lipid metabolism as the liver is the major metabolic tissue. The liver also performs several roles in lipid metabolism: Cholesterol synthesis, lipogenesis and the production of triglycerides (fats). Bulks of the lipoproteins are synthesized in the liver. Hence, due to the administration there was mild metaplasia of hepatocytes as well as proliferation of kupfer cell and hepatic cells cirrhosis (Udoh & Udoh, 2005).

Table1: Effect of *Betel* leaf stalk extract on Total Lipids, Lipase activity, Free fatty acids, Glycerol, Phospholipids, Triglycerides and Total Cholesterol in Testes, Epididymis, Seminal vesicle and Prostate gland over control rats.

S.No	Parameter	Control, Betel leaf stalk extract, % change and significance			
		Testis	Epididymis	Seminal vesicles	Prostate gland
1.	Total Lipids (mg/g wet wt.)	80.10±6.32 65.81±3.42 -17.84*	68.77±4.28 58.27±3.89 -15.26*	73.63±5.32 89.16±7.43 +21.09*	79.47±6.31 95.19±6.97 +19.79*
2.	Lipase activity (µmoles of PNPA cleaved/mg protein/hr)	0.574±0.02 0.750±0.03 +30.66*	0.634±0.01 0.769± 0.02 +21.20*	0.523±0.02 0.655±0.03 +25.25*	0.74±0.04 0.601±0.05 -19.65*
3.	Free Fatty acids (mg/g wet wt)	21.60±1.82 22.16±1.74 +2.59NS	20.76±0.76 21.60±0.85 +4.05***	19.91±0.32 20.76±0.84 +4.22***	35.34±2.87 32.54±2.34 -7.3***
4.	Glycerol (mg/g wet wt)	18.83±1.23 36.67±2.01 +94.74*	55.64±3.37 47.73±3.21 -14.21*	40.56±2.21 69.27±5.37 +70.79*	113.58±8.63 99.57±7.42 -12.33**
5.	Phospholipids (mg/g wet wt)	113.23±8.42 148.32±9.43 +30.99*	94.11±6.02 113.73±8.41 +20.84*	87.05±6.63 110.29±9.27 +26.69*	103.67±8.39 138.23±9.43 +33.33*
6.	Triglycerides (mg/g wet wt)	52.43±3.42 43.2±2.7 -17.60*	45.43±2.72 39.43±1.48 -13.20*	58.97±3.81 42.57±2.17 -27.81*	63.4±4.44 39.69±1.25 -37.39*
7.	Total Cholesterol (mg/g wet wt)	2.01±0.12 3.19±0.23 +58.70*	4.6±0.34 2.63±0.17 -42.82*	3.76±0.26 5.18±0.28 +37.76*	7.13±0.53 5.10±0.42 -28.46*

Mean ± SD of six individual observations.+ and – percent increase and decrease respectively over control rats. * P<0.001, ** P<0.01 indicates the levels of significance.***P<0.05 indicates the levels of significance. NS-no significant changes.

Table 2: Effect of *Betel* leaf stalk extract on Total Lipids, Lipase activity, free fatty acids, Glycerol, Phospholipids, Triglycerides and Total Cholesterol in Liver and Serum over control rats.

S.No	Parameter	Control, Betel leaf stalk extract, % change and significance	
		Liver	Serum(mg/100ml)
1.	Total Lipids (mg/g wet wt.)	52.83±2.37 35.15±2.35 -33.46*	35.23±2.37 27.18±1.58 -22.83*
2.	Lipase activity(µmoles of PNPA cleaved/mg protein/hr)	0.932±0.05 0.601±0.04 -35.51*	0.62±0.08 0.881±0.05 +29.17*
3.	Free Fatty acids (mg/g wet wt)	21.86±0.21 23.04±0.99 +5.39***	84.16±3.48 92.02±4.72 +9.33**
4.	Glycerol (mg/g wet wt)	70.56±5.03 75.51±5.32 +7.01 NS	109.57±7.26 93.84±8.17 -14.35**
5.	Phospholipids (mg/g wet wt)	108.61±8.63 94.11±7.32 -13.35**	69.11±3.31 95.73±7.97 +38.51*
6.	Triglycerides (mg/g wet wt)	55.69±3.40 35.43±1.82 -36.37*	82.72±3.24 63.42±5.01 -23.33*
7.	Total Cholesterol (mg/g wet wt)	3.20±0.21 1.19±0.03 -62.65*	77.01±6.37 126.02±11.82 +63.66*

Mean ± SD of six individual observations.+ and – percent increase and decrease respectively over control rats. * P<0.001, ** P<0.01,***P<0.05 indicates the levels of significance. NS-no significant changes.

The reduction in serum lipids speculate that this stalk extract may be helpful in reducing the complications of hyperlipidemia (Nabil A. Khouri & Haytham Daradka, 2012; Hasim basha & Changamma, 2013a).

The *Betel* leaf stalk extract showed significant elevation in lipase activity in testes, epididymis & seminal vesicle, but in prostate gland it was significantly reduced. The elevated lipase activity may be due to lowered lipids and inhibited lipogenesis. On the other hand the lipase activity in prostate gland was inhibited. The accumulation of lipids in prostate causes the inhibition in lipase activity.

The endogenous lipids may not mobilized and used as a source of energy in prostate gland due to administration.

The lipase activity in liver (table-2) is reduced and elevated in serum. It may be due to the efflux through circulation, in consonance with other findings (Hasim Basha & Changamma, 2013a). Hepatic lipase has a central role in the removal of phospholipids and triglycerides from sub fractions of high-density lipoprotein (HDL2) particles, but it may also function in the lipolysis of triglyceride-rich particles (Matti J Tikkanen & Esko A Nikkilä, 1987). These changes in liver and serum may be due to anti androgenic effect of *Betel* leaf stalk extract.

In the present study the testicular free fatty acids were not affected by extract, but in accessories like epididymis and seminal vesicle slightly significant ($P<0.05$) elevation was observed. The observed elevation in lipase activity causes elevation in free fatty acids. The reduced free fatty acids in prostate may be due to inhibited lipase activity. On the other hand the free fatty acids were slightly elevated in both liver and serum due to the administration of extract. This rise was attributed to the increased mobilization of free fatty acids in response to the extract. The increase in free fatty acid flux resulting from increased lipolysis. The level of non-esterified fatty acids (NEFA) and glycerol in serum or plasma is indicative of endogenous or induced adipocyte lipolysis. Fatty acids and glycerol are released by adipocytes in response to lipolytic hormones and enter the bloodstream for utilization by other tissues. However, an excess of circulating fatty acid over energy needs may contribute to hyperlipidemia, which is a risk factor for cardiovascular disease.

Glycerol is a precursor for synthesis of triacylglycerols and of phospholipids in the liver and adipose tissue. When the body uses stored fat as a source of energy, glycerol and fatty acids are released into the bloodstream. In some organisms, the glycerol component can be converted into glucose by the liver and, thus, provide energy for cellular metabolism. The elevated testicular glycerol is the index of the alterations in spermatogenesis with reference to extract administration.

In accessories, there was significant reduction in epididymis and prostate gland but elevation in seminal vesicle. The depletion in glycerol in epididymis results the lack of stimulation of 0_2 consumption of spermatozoa and also lack of energy substrates. Glycerol is a potential substrate for spermatozoa during their passage through the epididymis (Brooks, 1979). Hence, the sperm capacitation was disturbed in epididymis (Cooper & Brooks, 1981) due to the *Betel* leaf stalk extract. However, the elevation in glycerol content may be an indication that the secretary ability of the seminal vesicle was hindered by the extract and this will adversely affect its nutritive potentials for the semen which will in turn affect sperm motility.

Hepatic glycerol does not show any significant changes over control. But serum glycerol was reduced over control. These results were supported by other findings (Hasim Basha & Changamma, 2013a). Serum levels of total glycerides and free glycerol are important indices of lipid metabolism and cardiovascular disease risk. Serum levels of glycerol, FFAs, monounsaturated FAs, saturated FAs, and n-7 and -9 FAs are biomarkers for an increased risk of development of hyperglycemia and type 2 diabetes (Mahendran *et al*., 2013). Circulating glycerol concentration indicates the rate of hydrolysis of triglyceride in brown adipose tissue. Hence, the reduction in serum glycerol concentration predicts the lowered rate of hydrolysis of triglycerides. This was evidenced by reduced triglycerides in the present study due to extract administration.

The elevated phospholipids were observed in all reproductive tissues supported with other findings (Hasim Basha & Changamma, 2013). Successful fertilization depends on both the integrity of the spermatozoal membrane and its adequate phospholipid (PL) composition (Flesch and Gadella, 2000). It is well known that reduced integrity of the spermatozoal membrane is characteristic of spermatozoa from infertile men. Infertility can be caused, for instance, by reactive oxygen species (ROS) generated by neutrophilic granulocytes (Schiller *et al*., 2000). The elevated testicular phospholipids due to administration of extract results the disruption in integrity of the spermatozoal membrane and its adequate phospholipid composition leads to Infertility. Several investigators have reported that the phospholipid content of spermatozoa decreases during migration through the male reproductive tract. The loss of phospholipid during maturation may be associated with the utilization by the spermatozoa of the fatty acid side chains as a source of energy. However, the changes in phospholipids during maturation could also be due to adaptation of the sperm membranes to the new environment in the epididymis (Evans & Setchell, 1979). So the

accumulated phospholipids in testes and epididymis are the direct evidence of antispermatogenic effect of *Betel* leaf stalk extract.

Phospholipids regulate androgen biosynthesis at the level of the endoplasmic reticulum enzymes. Alterations in the constitution of the phospholipid microenvironment of rate limiting enzymes in the metabolic pathway may regulate not only the rate of pregnenolone metabolism but also determine the metabolic profile. Changes in the phospholipid microenvironment may regulate the biosynthesis of testosterone (Jacqueline Leßig *et al*., 2004). The accumulation of phospholipids in accessory organs may be due to depletion in testosterone levels (Govardhan Naik and Changamma, 2014) as these were androgen dependents. Thus extract showed its antiandrogenic effect.

There was slight reduction in hepatic phospholipids and elevation in serum phospholipids due to administration. Few aspects of lipid metabolism are unique to the liver, but many are carried out predominantly by the liver. The liver synthesizes large quantities of cholesterol and phospholipids. Some of this is packaged with lipoproteins and made available to the rest of the body. The remainder is excreted in bile as cholesterol or after conversion to bile acids (Bowen, 2007). Hence, the reduced liver phospholipids in the present study are due to more excretion into the bile. The enhanced serum phospholipids due to efflux from circulation.

The *Piper Betel* leaf stalk extract showed significant reduction in triglycerides in testes, accessory sex organs like epididymis, seminal vesicle, prostate gland and liver & serum over control rats. These results are supported with previous studies carried out in rats (Hasim basha and Changamma, 2013, 2013a). Triglycerides may serve as a vehicle for transport of PUFA from sertoli cells to germ cells so lowered triglycerides will affect the germ cell membrane in testes (Natalia E. Furland *et al*., 2003). Incubation of seminal plasma with spermatozoa shows that these cells use triglycerides for their metabolism (Montagnon *et al*., 1990). The seminal plasma provides a nutritive and protective medium for the spermatozoa during their journey through the female reproductive tract. The components in the seminal plasma attempt to compensate for this hostile environment. Hence, due to depletion in triglycerides the sperm metabolism disturbed, thus leads to infertility. Prostate Cancer linked to high triglyceride levels. Hypertriglyceridemia may increase the risk of prostate cancer (Wuermli *et al*., 2005). Thus lowered prostatic triglyceride due to extract administration indicates no risk of prostate cancer.

In liver reduced triglycerides were noticed because more triglycerides were oxidized to produce energy. A reduction in serum triglycerides (Kang *et al*., 1997) were noticed It has been reported that the elevated levels of plasma triglycerides associated with a higher incidence of coronary heart disease (Hasim Basha & Changamma, 2013a). But in present study there was reduction in serum triglycerides indicates no risk of cardiovascular disease.

The P. *Betel* leaf stalk extract showed significant elevation in testicular total cholesterol content (Meera agarwal *et al*., 2011). Cholesterol is the starting material for androgen biosynthesis (Mbongue *et al*., 2005). Cholesterol is involved in testicular steroidogenesis and is the most important precursor in the synthesis of steroid hormones and its level is related to fertility (Watcho *et al*., 2004; Thejashwini *et al*., 2012; Sharanabasappa *et al*., 2014). Testes synthesize the steroid hormone like, testosterone. The lowered testosterone levels by the administration of *Betel* leaf stalk extract (Govardhan Naik and Changamma, 2014) support the impaired utilization of cholesterol in steroidogenesis. Increases in the cholesterol level indicate the non utilization of these precursors for steroidogenesis which may be due to an inhibition in the availability of gonadotrophins (Satishgoud *et al*., 2009). Thus increased testicular cholesterol may result due to impaired utilization in steroidogenesis, (El-Sweedy *et al*., 2007), associated with impaired testicular activity (Azza M. El-Wakf *et al*., 2011) leads to

impaired spermatogenesis (Joshi *et al*., 2005) due to administration of extract.

However, in accessory sex organs like epididymis & prostate gland, cholesterol content were depleted while in Seminal vesicle it was increased. The physiological and biochemical integrity of accessory sex organs are dependent on androgens. The decreased cholesterol content of the epididymis and prostate gland after the administration of P. *Betel* leaf stalk extract indicated impairment in its synthesis and may hinder steroidogenesis, thus suggesting anti-steroidogenic potential of the plant extract (Yakubu *et al*., 2007a). All these organs play important roles in the maturation and mobility of the sperm and formation of semen. Hence, the administration of stalk extract caused reduction in the spermatogenesis, steroidogenesis and androgen production, it may alter the sexual behavior and may cause antifertility (Vijaykumar *et al*., 2003).

There was significant reduction in liver and elevation in serum cholesterol levels. This is in agreement with earlier reports (Changamma & Hasim Basha, 2013a). The liver is central to the regulation of cholesterol levels in the body. Not only does it synthesize cholesterol for export to other cells, but it also removes cholesterol from the body by converting it to bile salts and putting it into the bile where it can be eliminated in the feces. Furthermore, the liver synthesizes the various lipoproteins involved in transporting cholesterol and lipids throughout the body. Cholesterol synthesis in hepatocytes is under negative feedback regulation: increased cholesterol in the cell decreases the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis. So reduction of cholesterol levels could also be as a result of the inhibition of hydroxyl methyl glutaryl (HMG) CoA reductase in the liver. It indicates the inhibition of hepatic cholesterol biosynthesis and also efflux in to serum (Afolabi *et al*., 2011).

Serum cholesterol significantly increased after administration of *piper Betel* leaf stalk extract. The major lipids in the blood stream are cholesterol and triglycerides. Elevated serum levels of cholesterol are a major risk factor for coronary artery disease. Increase in serum cholesterol levels could possibly be due to either increase in the synthesis and/or decrease in the breakdown of fatty acids due to peroxisomes (Shashi Gupta, 2001),

A direct relationship exists between liver function and cholesterol levels. In fact, liver problems can lead to elevated cholesterol in blood and vice versa. High cholesterol levels can contribute to a variety of chronic health problems, such as cardiovascular disease. The increase in testicular and serum cholesterol may be co-related with its no utilization by the system, leading to a fall in circulating androgen and hence the altered histoarchitecture observed. Therefore the *Betel* leaf stalk extract exerts both antifertility and antiandrogenic activities.

Antifertility activity of *Betel* leaf stalk extract has been attributed due to the presence of certain phytochemical compound such as stigmasterol, psoralon, bergapten, orientin, vitedin, sapononis, tannins, Piperine and beta-sitosterol. Saponins are important mainly because of their steroid structure. They are precursors for the hemi synthesis of birth control pills (with progesterone and esotrogens) as well as similar hormones and corticosteroids (Shajeela *et al*., 2011; Muthulakshmi *et al*., 2013). Thus, saponins present in *Betel* leaf stalk extract inhibits the steroidogenesis, hence it possessed antifertility activity.

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REFERENCES

1. Thejashwini M.S, Krishna Ram H, Shivabasavaiah. 2012. Reversible antifertility effect of *cyamposis psoralioides* in male swiss albino mice. International Journal of advanced biological research. 2(4):657-665.
2. Chakraborty D, Shah B.2011. Antimicrobial antioxidative and antihemolytic activity of

Piper betel leaf extracts. Int J Pharm Pharm Sci. 3:192-199.
3. Mishra R.K, Singh S.K. 2009. Antispermatogenic and antifertility effects of fruits of *Piper nigrum* L. in mice. Indian journal of Experimental Biology. 47: 706-714.
4. Sarkar M, Gangopadhyay P, Basak B, Chakrabarty K, Banerji J, Adhikary P, Chatterjee A. 2000. Contraception. 62:271-274.
5. Kushwaha S, Aggarwal M, Mutreja A, Chauhan A, 2007. Egyptian Journal of Biology. 9:42-46.
6. Arvind Singh, Sushila Kala, Deepak N. Kapoor, Richa Gupta, Antar Virk, Samarjeet Singh, Jyoti Chaudhary. 2011. Effect on human sperm mitochondrial activity by *Piper betle* and *Calendula officinalis*. *Annals of Biological Research*, 2 (5):622-627.
7. Suresh C. Joshi, Aksha Sharma, Mridula Chaturvedi. 2011. Antifertility potential of some medicinal plants in males: An overview. International Journal of Pharmacy and Pharmaceutical Sciences. 3(5): 204-217.
8. WHO: Protocol CG-40, Preparation of Alcoholic Extract for Bioassay and Phytochemical Studies. 1983: (APJF/IP, 1001 A). Geneva, World Health Organization.
9. Folch J.M, Lees M.P and Stana-stanley G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226: 497-505.
10. Colowick S.P, Kaplan N.O. 1957. Methods in Enzymology. New York: Academic Press. 3:50-54.
11. Natelson S. Free fatty acids procedure in: techniques of clinical chemistry. Charless, C. Thomas Publishers, Springfield, Illinois, USA, 3rd edn 1971a.
12. Zilversmidth, D.B and Davis B.S. 1950. Micro determination of plasma phospholipids by trichloroacetic acid precipitation. J Lab Clin Med. 35: 155-160.
13. Natelson S. 1971b. Triglyceride's procedure in: techniques of clinical chemistry. Charless, C. Thomas Publishers, Springfield, Illinois, USA, 3rd edn 1971b; 273-280.
14. Natelson S. 1971c.Total cholesterol procedure Liebermann-Burchard reagent. In: techniques of clinical chemistry. Charless, C. Thomas Publishers, Springfield, Illinois, USA, 3rd edn. 263-270.
15. Hasim basha S and Changamma C. 2013. Effect of Carica Papaya (L.) seed extract on Lipid metabolites in male albino rats. Int. J. Pharm Pharm Sci, 5 (4), 527-529.
16. Jones R. 1998. Plasma membrane structure and remodeling during sperm maturation in the epididymis. Journal of Reproduction and Fertility Supplement. 53: 73–84.
17. Flesch F.M and Gadella B.M. 2000. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochimica Biophysica Acta.197–235.
18. Kusemiju O, Noronha C, Okanlawon A. 2002. The effect of crude extract of the bark of Carica papaya on the seminiferous tubules of male Sprague-Dawley rats. The Nigerian Postgraduate Medical Journal. 9(4): 205-209.
19. El-Sweedy M, Abdel-Hamid N, El-Moselhy M. 2007. The role of a mixture of green tea, turmeric and chitosanin the treatment of obesity related testicular disorders, J. Appl. Biomed. 5: 131–138.
20. Changamma C, Lakshman J, Hasim Basha S, Govardhan Naik A. 2011. Effect of 5Thio-D-Glucose on Testicular Metabolism of Albino Rat. Journal of Applied Sciences Research. 7(2): 98-101.
21. Zalata A.A, Christophe A.B, Depuydt C.E, Schoonjans F, Comhaire F.H.1998a. White blood cells cause oxidative damage to the fatty acid composition of phospholipids of human spermatozoa. Int. J. Androl. 21: 154-162.
22. Zalata A.A, Christophe A.B, Depuydt C.E, Schoonjans F. Comhaire F.H. 1998b. The fatty acid composition of phospholipids of spermatozoa from infertile patients. Molecular Human Reproduction. 4:111-118.
23. Thomas Saether. 2003. Metabolism of polyunsaturated fatty acid in rat testis. Department of Bio chemistry faculty of Mathematics and Natural Sciences, university of Oslo.

24. Lohiya N.K, Pathak N, Mishra P.K, Manivannan B. 1999. Reversible contraception with chloroform extract of *Carica papaya* Linn. Seed in male rabbit. Reprod Toxicol. 13:59-66.
25. Hasim basha S and Changamma C. 2013. Effect of Carica Papaya (L.) seed extract on Lipid metabolites in male albino rats. Int. J. Pharm Pharm Sci. 5 (4), 527-529.
26. Udoh F.V & Udoh P.B. 2005. Hepatotoxicity of the methanol extract of Carica papaya (paw-paw) seeds in wistar rats. Pharmaceutical Biology, 43(4): 349-52.
27. Nabil, A. Khouri and Haytham Daradka. 2012. Blood Biochemical changes associated with acute treatment of *Orchis Anatolica* plant roots ethanol extract in adult Albino rats, *International Journal of Applied Biology and Pharmaceutical Technology*, 3(1):73-81.
28. Hasim Basha S and Changamma C. 2013a. Effect of Carica Papaya Seed Extraction on Liver and Serum Profiles with Special Reference to Lipid Metabolism in Male Albino Rats. International Journal of Advanced Research in Pharmaceutical & Bio sciences. 3(3):26-31.
29. Matti J Tikkanen & Esko A Nikkilä. 1987. Regulation of hepatic lipase and serum lipoproteins by sex steroids. American Heart Journal. 113(2):562–567.
30. Cooper T.G and Brooks D.E. 1981. Entry of glycerol into the rat epididymis and its utilization by epididymal spermatozoa, *Journals of Reproduction & Fertility*, 61:163-169.
31. Mahendran Y, Cederberg H, Vangipurapu J, Kangas AJ, Soininen P, Kuusisto J, Uusitupa M, Ala-Korpela M, Laakso M. 2013. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. Diabetes Care. 36(11):3732-8.
32. Schiller J, Arnold K. 2000. Mass spectrometry in structural biology. In: Meyers, R.A. (Ed.), Encyclopedia of Analytical Chemistry. John Wiley and Sons. Chichester. 559–585.
33. Evans R.W and Setchell B.P. 1979. Lipid changes during epididymal maturation in ram extract of *Albizzia lebbeck* (L.) Benth in male rats. Asian J Androl. 6: 155-159.
34. Jacqueline Leßig, Claudia Gey, Rosemarie Su¨ß, Ju¨rgen Schiller, Hans-Ju¨rgen Glander, Ju¨rgen Arnhold. 2004. Analysis of the lipid composition of human and boar spermatozoa by MALDI-TOF mass spectrometry, thin layer chromatography and 31P NMR spectroscopy, Comparative Biochemistry and Physiology Part B 137: 265–277.
35. Govardhan Naik A and Changamma C. 2014. Hormonal changes in piper betel Linn. Leaf stalk extract administered male albino rats. World journal of pharmaceutical sciences. 3(4):1952-1959.
36. Bowen R. 2007. Secretion of Bile and the role of Bile acids in Digestion, Colorado State Hypertext book article on Bile, 2001, http://www.vivo.colostate.edu/hbooks/pathphys/digestion/liver/bile.html.
37. Natalia E. Furland, Eduardo N. Maldonado, Marta I. Aveldaño.2003. Very Long Chain PUFA in Murine Testicular Triglycerides and Cholesterol Esters, m Lipids, 38 (1): 73-80.
38. Montagnon D, Valtat B, Vignon F, Koll-Back M.H. 1990. Secretory proteins of human Seminal vesicles and their relationship to lipids and sugars. Andrologia. 22(1):193-205.
39. Wuermli L, Joerger M, Henz S, Schmid H.P, Riesen W.F, Thomas G, Krek W, Cerny T, Gillessen S. 2005. Hypertriglyceridemia as a possible risk factor for prostate cancer. Prostate Cancer Prostatic Dis.8 (4):316-20.
40. Kang J-J, Wang H-W, Liu l T-Y, Chen YC, Ueng T.H. 1997. Modulation of Cytochrome P-450-dependent oxygenases, Glutathione and Glutathione S-transferase in Rat Liver by Geniposide from Gardenia jasminoides. Food and Chemical Toxicology, 35:957-965.
41. Meera Agarwal, Priyanka Sharma, Sonalika Kushwaha. 2011. Antifertility efficacy of 50% ethanolic extract of *Calendula Officinalis* in male rats. International Journal of Pharmacy and Pharmaceutical Sciences.3 (5):192-196.
42. Mbongue F.G.Y, Kamtchouing P, Essame O.J.L, Yewah P.M., Dimo T, Lontsi D.

2005.Effect of the aqueous extract of dry fruits of Piper guineense on the reproductive function of adult male rats. Indian J Pharmacol. 37(1): 30-32.
43. Watcho P, Kamtchouing P, Sokeng S.D, Moundipa P.F, Tantchou J, Essame J.L, Koueta N. 2004. Androgenic effect of Mondia whitei roots in male rats. Asian Journal of Andrology. 6:269–272.
44. Sharanabasappa A Patil, Sujaya M, Saraswati B Patil. 2014. Aphrodisiac and phytochemical studies of Cocculus hirsutus extracts in albino rats. Asian Pacific Journal of Reproduction. 3(1): 23-29.
45. Satishgoud S, Sharangouda, Vishwanatha T. Saraswati B. Patil. 2009. Contraceptive effect of Terminalia Bellirica (bark) extract on male albino rats. Pharmacology online. 2: 1278-1289.
46. Joshi S.C. Gulati N. and Gajraj A. 2005. Evaluation of Toxic Impacts of Mancozeb on Testis in Rats. Asian J. Exp. Sci. 19(1): 73-83.
47. Azza M. El-Wakf, EL-Said M. Elhabiby, Waffa M. El-kholy, Eman Abd El-Ghany. 2011. Use of Tumeric and Curcumin to Alleviate Adverse Reproductive Outcomes of Water. Nitrate Pollution in Male Rats, *Nature and Science,* 9(7) 229-239.
48. Yakubu M.T, Akanji M.A, Oladiji A.T. 2007a. Evaluation of antiandrogenic potentials of aqueous extract of Chromolaena odoratum (L.) K. R. leaves in male rats. Andrologia. 39:235–243.
49. Vijaykumar B, Sangamma I, Sharanabasappa A, Saraswati B. Patil. 2003. Antifertility Activity of Various Extracts of *Crotalaria juncea* Linn. Seeds in Male Mice. Philippine Journal of Science. 132 (1): 39-46.
50. Afolabi I.S, Akuiyibo S.M, Rotimi S.O, Adeyemi A.O. 2011. In vivo evaluation of lipid and antioxidants qualities of Carica papaya Seed oil. Journal of Natural Products, (4):125-135.
51. Shashi Gupta, Meena Katana, P.K. Gupta, V.K. Vijjan. 2001. Effect of Petroleum ether extracts of different parts of Neem seed (Azadirachta Indica) on Hematological and biochemical parameters in rats. Indian J. Anim. Res. 35(1): 21-26.
52. Shajeela P.S, Mohan V.R, Louis Jesudas L, Tresina Soris P. 2011, antifertility activity of ethanol extract of Dioscorea esculenta (L.) Schott on male albino rats. International Journal of PharmTech Research, 3(2):946-954.
53. Muthulakshmi A, Jothibai Margret R, Mohan V.R. 2013. Antifertility Effect of Ethanol Extracts of *Feronia elephantum* Correa Leaf and Bark on Male Albino Rats. International Journal of Pharmaceutical Sciences and Drug Research. 2013. 5(1): 23-27.

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