ROLE OF AQUEOUS LEAF EXTRACT OF PSIDIUM GUAJAVA L. ON SPERMATOGENESIS AND SEX HORMONES IN MALE ALBINO RAT

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Abstract: The effect of aqueous leaf extract of Psidium guajava on spermatogenesis and some sex hormones on male albino rats. The male rats were divided randomly in to 4 groups of six rats each. The rats were oral administered with guava leaf extract at 0,250,500,750 mg/kg BW respectively for the duration of 30 and 60 days. The result showed dose dependent significant in reduction of body weight, increase in sperm count and decrease in sperm abnormalities in experimental rats. The level of sex hormones like testosterone, Oestrogen and FSH increases in all experimental rats during 60 days of oral administration. FSH level increases in experimental rats during 60 days, LH and prolactin almost remains the same in control and experimental rats. The level of cholesterol, glycogen increases and protein level decreases in testis and accessory male sex organs. Based on the results obtained the effect of aqueous leaf extract of Psidium guajava has positive effect on spermatogenesis and enhance fertility.

Introduction: Infertility has been a recurring problem among male and female individuals. Today, orthodox medicine has almost exceeded its limits in resolving problems of infertility. This is why the use of phyto-medicine is becoming a main stay in the treatment of infertility. It has been reported that alternative medicines have proven efficacious in the treatment of male and female infertility (Rabia et al., 2008). Locally grown plants have been used worldwide as supplements and therapies for several ailments. In recent times, many plant extracts, have been reported to enhance fertility (Chaturapanichetal.,).More than 90% of male infertility cases are due to low sperm counts, poor semen quality or both (Lindheim et al., 1996).

The fertility enhancing capacity of plant extract has been reported in numerous studies (Mehrotra etal.,1978). In recent time, paramount attention is been shifted from synthetic drugs to natural plant product. Various plants that were once considered of little or no importance are now studied and subsequently developed into drugs,with outside effects (Francis et al., 1969). Some wild herbs and species have efficiency to improve fertility factor. The genus *Psidium*belongs to the family*Myrtaceae*, It comprises approximately 150 species of small trees and shrubs in which 20 species produce edible fruits. The most commonly cultivated species of genusis *Psidium guajava* L.is known as the commonguava, widely grown in tropical and subtropical areas of the world like Asia, Egypt, Hawaii, Florida (Goncalves.etal.,2005).The guava leaves are 2 to 6 inches long, 1 to 2 inches wide and appear stiff dull-green but coriaceous with pronounced veins. Toxicity studies of guava plant in animal models has shown that its fruit, leaf and root are safe and without side effects on humans (Khan et al.,1985).There are bioactive components in the guava leaf that can fight against pathogens, regulate blood glucose levels, and can aid in weight loss. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts and a number of other fixed substances (Zakaria et al., 1994).

Flavonoids are phenolic compounds widely distributed in plants, which display avariety of biological activities, such as antioxidant, anti-inflammatory, blood lipid-lowing, and anti-carcinogenic activities (Kipniset al.,2001). Androgenic activity (Yousef *et al*. 2005).The active principles such as phenols, alkaloids, saponins and most specially flavonoids are known to have estrogenic (Das et al., 2004) and androgenic (Yousef et al., 2005) activity. Guava fruits are also a good source of pectin, a dietary fiber(Ekereet al., 2013).

Crude extracts obtained from plants are usually composed of crude oils, essential oils and miscellaneous compounds. These plant extracts as well as mineral oils have several benefits (Cariacetal.,2003).The leaves contain essential oil with the main components being α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide,β-copaneneand guayavolic acids (Iwu et al., 1993).

The leaves contain fixed oil 6%, volatile oil 0.365% (Burkill et al., 1997) 3.15% resin, 8.5% tannin and a number of other fixed substances. The essential oil contains eugenol confirmed (Nadkarni&Nadkarni et al., 1991), mallic acid and tannin from 8-15%. The fruit contains 'glykosen' 4.14% - 4.3%, quercetin, its 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside with strong antibacterial action (Oliver-Bever et al., 1986).

Based on supportive aids of WHO in the field of public health, family planning and reproductive health, consumption of herbal drugs has been considered as an alternative to synthetic drugs for fertility enhancement. Lack of research on the effects of *P.guajava* aqueous leaf extract on fertility in albino rat, Hence the present study was carried out.

Material And Methods:

Plant material: Fresh leaves of *Psidiumguajava* were collected from Malleshwaram, Bangalore. The leaves were washed with distilled water, cut into small pieces and added into hot water to get brown colour leaf decoction (5 gm in 50 ml DW). The concentrated decoction was cooled and preserved in a refrigerator at 4^0c (stock solution 1ml=0.1 gm of extract).

Phytochemical study: The presence of various phytochemical constituents in aqueous extract of *P. guajava*was carried out by the method of Harborne (1973) for glycoside, flavonoid, saponin, tannins and terpenoids.

Animal: 30 days old male Wistar albino rats weighing about 100-110/gm body weight were taken for the study. The animals were maintained in plastic cages, under controlled temperature (24±27^{o}c) and photoperiod (12L,12D).The rats were acclimatized for 10 days to the best laboratory conditions prior to experiment maintained the balanced diet, water was provided daily.

Experimental Procedure: The rats were divided randomly into four groups of six animals each. Group I treated as control, Group II –IV treated as experimental. The control groups were fed with normal diet.Group II: treated 250 mg/kg body weight of aqueous extract for 30 and 60 days. Group III: treated 500 mg/kg extract for 30 & 60 days. Group IV: treated 750mg/kg extract for the same duration by oral administration. The experimental animals were allowed free access to water and feed throughout the period of the experiment. Body weights of the animals were recorded every week. Generally, the study was conducted in accordance with animal ethical committee. After 30 and 60 days the animals were anesthetized in chloroform vapour and blood samples were collected through cardiac puncture for hormonal assay.

Sperm count: Sperm viability and morphology were evaluated by obtaining spermatozoa from the cauda epididymis. After exposing the epididymis, transect at the point of origin of vas-deference at the distal end and at the boundary between the corpus and cauda epididymis at the proximal end. Place the tissue in a watch glass containing 0.5 ml of normal saline. Epididymal spermatozoa were obtained quickly by a puncture of the cauda epididymis. Minced the tissue carefully with the help of fine forceps and scissors to ensure the extrusion of spermatozoa from the cauda epididymis. The tissue fraction is then removed by using forceps and a needle. And suspension is used for sperm analysis according to WHO laboratory manual (1992).

The dilution is made using a white blood cell pipette; the sperm suspension is drawn to the 0.5 mark half way up the stem and the spermicidal solution subsequently to the 1 ml mark the top of the bubble chamber. The precipitation is then thoroughly mixed and one drop of it is added to both sides of the haemocytometer. The number of spermatozoa is counted in the four corner squares of the haemocytometer under a microscope at 400X. When a spermatozoon crosses the line of the grid, only those at the top and right hand sides of the squares are counted. Spermatozoa on both sides of the haemocytometer are counted and the average number is recorded.

Concentration of spermatozoa = Average number of spermatozoa counted *multiplication factor (10000)*dilution factor (20)

Sperm morphology: Sperm morphology is assessed by using supra vital staining technique. A drop of sperm suspension on a clean glass slide on which a drop of eosin stain is placed and then thoroughly mixed with the help of glass rod. Transfer a portion of mixture to a second slide and prepare a thin film. Examine the slide under the microscope at 400X and score about one hundred spermatozoa from different fields of the slide. Evaluation of sperm abnormality is based on the criteria as mentioned by Wyrobek and Bruce (1975. Spermatozoa are considered abnormal, if they show any of the following types of abnormalities; amorphous head, hook head etc.

Biochemical estimation: The tissues liketestis,seminal vesicle, prostate gland, and Cowper's gland were removed carefully along with the fat bodies, weighed and processed for biochemical analysis like protein by Lowry's et al.,(1951), glycogen by Dubois et al., cholesterol by Zlakis et al.,(1953) and fructose by Somagi (1965)and Roa et al., (1989)

Hormone Assay: Serum was collected and processed for testosterone, oestrogen; follicular stimulating hormone and luteinizing hormone were by RADIO IMMUNO ASSAY (RIA).

Objectives: The proposed work is designed to understand and to evaluate the possible fertility effect of aqueous leaf extract of *P.guajava* of male albino rat.

RESULTS: In the present experiment, one month old rats were daily fed with *Psidiumguajava* leaf extract for 30 and 60 days, the following results were observed.

Body weight: The mean body weight of 30 days control rat showed 229.18±35.03 gm. whereas the animal administered orally with 250 mg leaf extract showed lesser body weight (223.33±19.27 gm). During 30 days treatment 500mg showed decrease in mean body weight(208.33±12.67gm). Further significant reduction in body weight (206.66±13.02gm) was observed in 750 mg leaf extract when compare to that of control rats for 30 days.

The mean body weight of 60 days control rat showed 285±5.34 gm. whereas the animal administered

orally with 250 mg leaf extract showed lesser body weight (271.83±13.10gm). During 60 days treatment 500mg extractshowed decrease in mean body weight(247.83±6.88gm). Further significant reduction in body weight 240±6.03gm was observed in 750 mg leaf extract when compare to that of control rats for 60 days.

Organ Weight: The organ weight of testis, seminalvesicle,prostategland and Cowper's gland weight were summarized in Figure II and Table 2:

Testis: The mean testis weight of 30 days control rat showed 2.74±0.023gm. Whereas the animal administered orally with 250 mg leaf extract showed increase in testis weight (3.32±0.051gm). During 30 days treatment with 500mg showed decrease in mean body weight of (3.20±0.52gm). Further significant reduction in testis weight 3.03±0.017gm was observed in 750mg leaf extract when compare to that of control rats for 30 days.

The rats administered orally for 60 days in 250 mg body weight showed slight increase of testis weight of 3.44±0.051gm when compared with that of control (2.69± 0.017gm). Further, in 500 and 750mg body weight showed increase in testis weight of 2.67± 0.05gm and 2.81±0.01gm respectively.

Seminal vesicle: Seminal vesicles of control rat showed 0.65±0.023gm and 0.46± 0.392gm/organ weight for 30 and 60 days. Whereas the animal administered orally for 250, 500 and 750 mg/body showed fluctuation in organ weight of (0.59± 0.0115,0.97± 0.0112 and 0.41±0.0112) for 30 days. During 60 days the experimental animal showed gradual increase (0.81±0.0113) in 250 mg of leaf extract. Whereas, in 500 and 750 mg showed decrease in mean organ weight of 0.46±0.0115 and 0.19±0.057 respectively.

Prostate gland: Control Prostate gland showed 0.29±0.0115 and 0.09±0.0115 gm/organ weight during 30 and 60 days. In experimental group II and IIIshowed increase of organ weight of 0.41±0.011 and 0.53± 0.17in 250 and 500 mg of leaf extract. In group IV showed decrease of organ weight (0.19±0.011) in 750 mg of leaf extract for 30 days. During 60 days the experimental animal showed increase organ weight in group II and III (0.15±0.0115 and 0.18±0.0057). Group IV showed decrease in organ weight of 0.085±0.0557gm/organ weight when compare with control group for 60 days.

Cowper's gland: During 30 days the control Cowper's gland showed 0.12±0.023gm/organ weight. Whereas fluctuations of organ weight were noticed in group II, III and IV (0.08±0.0115, 0.1±0.023 and 0.065±0.0057). 60 days of control rat showed increase in gm/body weight. In group II, III and IV showed decreased in organ weight of 0075.11±0.115, 0.07±0.0057 and 0.075±0.0288 when compare with control for 60 days respectively.

Hormone Analysis-Serum testosterone Level: Administration of aqueous extract of *Psidiumguajava* leaves for 30 days brought about significant increase in serum testosterone level in group II (392.34ng/dl) when compared with that of control rat (333.78ng/dl), Whereas rat fed with 500mg, 750mg of extract for 30 days, the testosterone level found to be decreaseof group III and group IV 306.07ng/dl and 135.9ng/dl respectively.

Similarly after 60 days the testosterone level in control rat showed339.43ng/dl. When animal administered with leaf extract of group II,III and IV showed significant increase of testosterone level 340.23, 382.31 and 423.6ng/dl (Fig III and table 3) when compared with control.

Oestrogen: The oestrogen level of control rat showed the value of 15.77pg/ml during 30 days, where as in group II showed increase of 17.55pg/ml. Group III and IV showed decrease of oestrogen level(11.8 and 14.19 pg/ml) when compare with control rats. During 60 days of leaf extractadministration, the control rat showed 21.93pg/ml. In experimental group of II, III, and IV showed increase of oestrogen level (28.3, 39.1 and 38.9 pg/ml) compare with control.(Fig III and table 3)

FSH and LH: The FSH showed 0.3mIU/ml in control rats. Whereas the same trend were noticed in all the experimental groups for 30 days. During 60 days of oral administration of leaf extract, the control animal showed the value of 0.5mIU/ml. whereas, in group II,III and IV showed variation in FSH level (0.6,0.5 and 0.8 mIU/ml) were noticed in all groups.

The LH level remains constant in control and experimental groups during 30 and 60 days (0.07ng/ml) of oral administration of aqueous leaf extract of *P. guajava*(Fig III and table 3).

Prolactin: Prolactin levels were constant in all the groups of (control and experimental) 30 days (0.3 ng.ml). Further, a slight increase of prolactin level (0.5 ng.ml) was noticed in control and experimental groups during 60 days of treatment, when compare to that of control(Fig III and table 3).

Sperm morphology: Control sperm morphology showed normal histo-architecture. Whereas oral administration of 250mg, 500mg and 750 mg/kg body weight aqueous extract of *Psidiumguajava*leaves for 30days showed variations of sperm morphology. During 60 days the experimental animals showed increased in sperm count, increase in normal sperm with significant decrease of sperm abnormalities of treated groups when compared to control groups.

Cauda epididymis Sperm count 30 and 60 days: Group I animal (control) rats fed with normal feed for 30 days showed 10,000,000 mm^3/ml Sperm count. Experimental animal (Group I) fed with Oral administration of 250mg per kg body weight of leaf extracts of *P.guajava* showed increase of sperm count

(1,40,00,000 mm^3/ml)when compared with normal control. Group III and IV rats were fed with 500mg and 750 mg per kg body weight of aqueous extract of *Psidiumguajava* leaves result in significant decrease of caudaepididymal sperm count of rats when compared to the control group of male albino rats (9,000,000 and 5,000,000 mm^3/ml).Similarly after 60 days of treatment, the sperm count of control rats were 1,60,00,000 mm^3/ml whereas rats fed with 250mg, 500mg and 750mg of extract showed gradual increase of sperm count ranging from 6,40,00000 mm^3/ml, 7,50,00000 mm^3/ml, 9,00,00,000 mm^3/ml when compare with 30 days respectively.

Biochemical changes: Biochemical changes of fructose, protein, cholesterol and glycogen levels were summarised in table 5 and Vfigures:

Fructose level 30 and 60 days: Fructose level was highest in Testis of group II (2.5±0.7) when comparison with control rat (2±0.5mg). When experimental rats showed decrease of fructose content in (500 mg and 750 mg (1.6±0.7 and 1.4±0.6mg/gm). Fructose level was highest in Seminal vesicle (6±3.2) where the rat was fed with 500 mg of *Psidiumguajava* extraction comparison with control rat(4±2.2mg/gm) and other groups showed constant of fructose level were noticed in 250 mg and 750 mg (3.3±1.2 and 3.3±1.2). Fructose level was lowest in prostate gland (0.3±0.1) when the rat was administered with 750 mg of *Psidiumguajava* extract and it was constant at 1.6±0.2 in the control and experimental rats(250 mg and 500 mg). Fructose level remained the same in Cowper's gland at 1.6±0.7 in control rats and experimental rats is 2.3±0.45 and3±1.0 (500 mg and 750 mg), but there was a reduction of fructose level in the experimental rats where 250 mg of extract was given (1.3±0.5) (Table 5)

Fructose level was highest in the Testis (6±1.9) of 60 days administration of leaf extract the rats that were fed with 750 mg of *Psidiumguajava* extract in comparison with control rat (3±1.0 mg) and experimental rats(4.3±1.2 and 5±1.5 in 250mg and 500 mg). Fructose level was highest in the Seminal vesicle (13.3±2.9) in the rats that were fed with 750 mg of *Psidiumguajava* extract in comparison with control rat (7.6±2.1).In experimental rats showed 10±2.5mg and 11±2.7mg in 250mg and 500 mg oral administered with leaf extract. Fructose levels were constant in prostate glands, All the experimental rats were at (2.5±0.5) in comparison to the control rats (1.6±0.2). In the Cowper's gland rats with 250 mg and 750 mg showed the same level of fructose in them (3±1.0). Control rats and 500 mg rats showed the same level of fructose as well (2.3±0.4).

Protein level 30 and 60 days: Protein levels in Testis were highest in the control rats (3.76±1.9) when compared to the experimental rats. In seminal vesicle the control and experimental rats treated with 750 mg of *Psidiumguajava* extract showed same level of protein (1.6±1.1) and this was more when compared to the experimental rats (250 mg and 500 mg). In prostate gland the levels of protein found were more (2.5±1.0) in control rats when compared to the experimental rats. Similarly in Cowper's gland the protein level was high (1.6±0.06) in control rats when compared with other experimental rats shows the protein content as (1.3±.3,0.98±0.06 and 1.5±0.5) in group II,III and IV.

During 60 days the Protein levels in Testis were reduced in the experimental rats (0.37±0.02) when compared to the control rats.In seminal vesicle the control and experimental rats treated with 750 mg of *Psidiumguajava* extract showed same level of protein (0.04±0.002) and this was more when compared to the experimental rats (250mg and 500mg). In prostate gland the levels of protein found were more (0.07±0.003)in rats treated with 750 mg of *Psidiumguajava* extract when compared to the control rats and experimental rats (250mg and 500mg). In Cowper's gland the protein level was high (0.07±0.0035) in the rats treated with 750mg extract of *Psidiumguajava*in comparison with other experimental rats 0.05±0.0025 and 0.06±0.003 (250mg and 500mg) and control rat. The protein level is indirectly proportional to the spermatogenesis, if protein level increases which intern reduces spermatogenesis (Table 6).

Cholesterol level 30 and 60 days: Cholesterol levels were highest in Testis in the rats treated with 500 mg of *Psidiumguajava* extract(1020±53) when compared to control rats 875±25and experimental rats 1000±52 and 875±25 (250mg and 750mg). In seminal vesicle the cholesterol levels were same in the control rat and in the rats treated with 250 mg of extract (1125.0±63). This was high when compared to the other experimental rats 1000±51 and750±23 (500mg and 750mg). The cholesterol levels in the prostate gland were the same in all 3 experimental rats (50±8) and least in the control rat (30±6). In Cowper's gland, rats treated with 250 mg and 500 mg(50±8)extract showed same levels of cholesterol. This was high when compared to the control rat and other experimental rats is 40±7 and 30±6 (750 mg)

During 60 days of treatment the cholesterol levels were highest in Testis in the rats treated with 500mg of *Psidiumguajava* extract (1025±28) when compared to control rats 875±20 and experimental rats 250mg and with 750mg of extract (875±20 and 1000±25). This was high when compared to the other experimental rat (250 mg). The cholesterol levels in the prostate gland were the same in rats treated with 500mg and 750mg of extract (100±6). This was high when compared to the control rat and other experimental rat is 50±3 and 75±5 (250mg) In Cowper's gland, rats treated with 500mg extract showed highest levels of

cholesterol (112.5±7). The level of cholesterols remained the same in the rats treated with 250 mg and 750mg of extract (100±6). The least level of cholesterol was found in the control rat (75±5) (Table 7).

Glycogen level 30and 60 days: High glycogen levels were found in testis in the rats treated with and 750 mg (8±1.2) whereas 250,500 (6±0.9) is constant when compared (5±0.8). In seminal vesicles the highest glycogen levels were found in rats treated with 500 mg of extract (0.3±0.07). In the control rat and other experimental rats it remained constant at 0.2±0.06. The levels of glycogen in prostate gland were the same in rats treated with 500mg of extract and 750mg of extract(0.2±0.06). It was the same in controlrat and in other experimental rat (250 mg) 0.1±0.03. There was no significant variation in the levels of glycogen found in Cowper's gland. As the control and rats treated with 250 mg, 750 mg of extract were the same at 0.2±0.06. The rat treated with 500 mg was least (0.1±0.03).

High glycogen levels were found in testis in the rats treated with 750mg of extract for 60 days (1.5±0.9). In seminal vesicles the highest glycogen levels were found in rats treated with 750mg of extract (1±0.6). In other experimental rats it remained constant at 0.5±0.025. The levels of glycogen in prostate gland were highest in the rats treated with 750 mg of extract (0.9±0.05). This was more in comparison with the control rat (0.1±0.03)and other experimental rats,0.1±0.03 and 0.6±0.04 (250 mg and 500 mg) (Table 8).

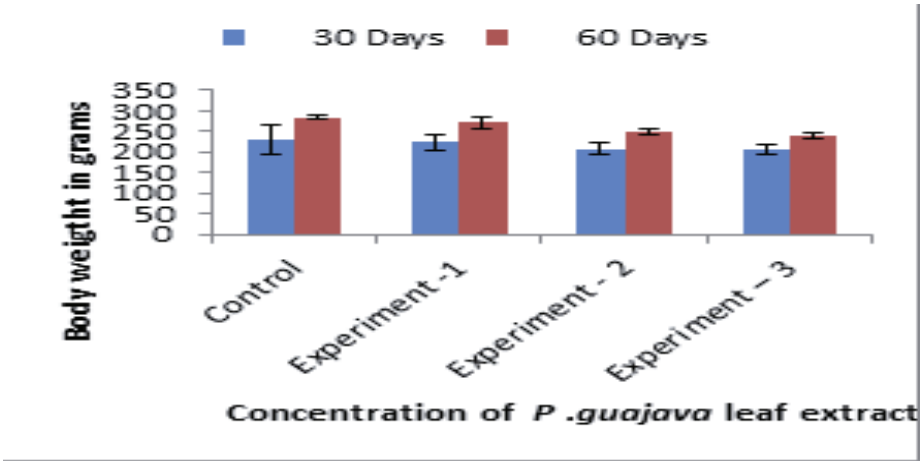


Figure I and Table 1: Showing Mean Body Weight of*Rattusnorvegicus*oraladministration of *P.gaujava*leaf extract duration of 30 and 60 days.

	Control	Experiment -1	Experiment - 2	Experiment – 3
30 Days	229.18±35.03	223.33±19.27	208.33±12.67	206.66±13.02
60 Days	285±5.34	271.83±13.1	247.83±6.88	240±6.03

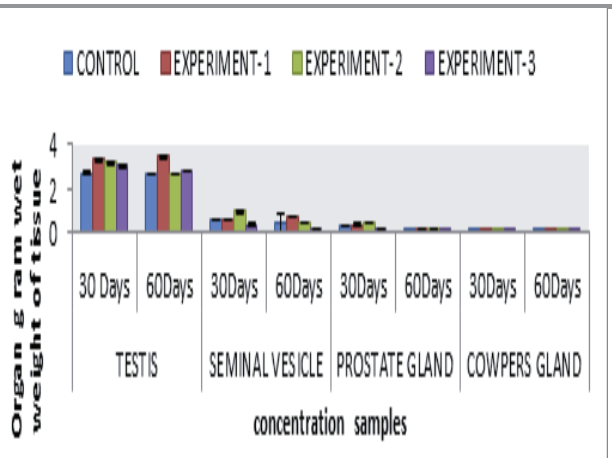
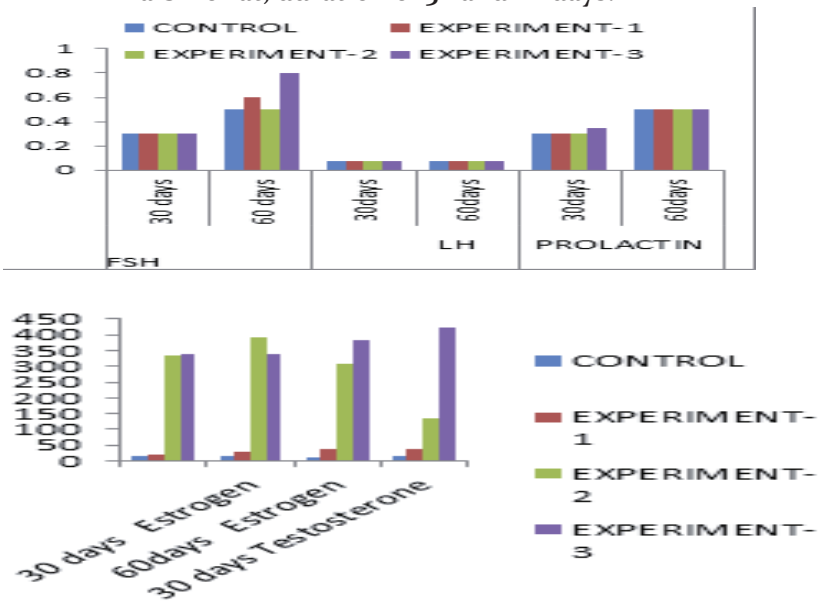


Figure II and Table 2: Showing Mean reproductive organ weight of *Rattusnorvegicus*oral administration of *P.gaujava*leaf extracts duration of 30 and 60 days:

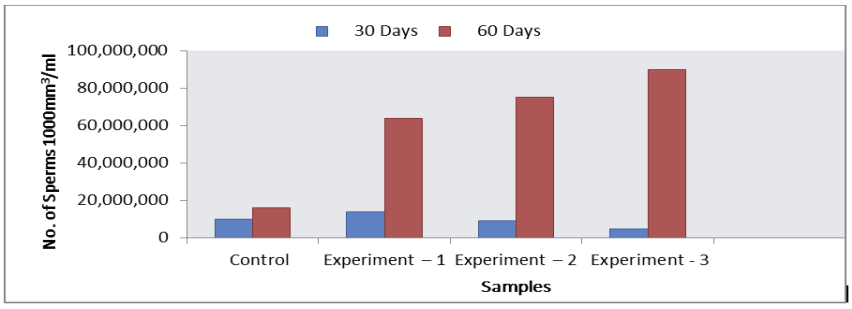
Organs	Duration	CONTROL	EXPERIMENT-1	EXPERIMENT-2	EXPERIMENT-3
TESTIS	30 Days	2.74±0.023	3.32±0.051	3.2±0.052	3.03±0.017
	60Days	2.69±0.017	3.44±0.0115	2.67±0.0057	2.81±0.0115
SEMINAL VESICLE	30Days	0.65±0.023	0.59±0.0115	0.97±0.0112	0.41±0.0112
	60Days	0.46±0.392	0.81±0.0113	0.46±0.0115	0.19±0.0057
PROSTATE GLAND	30Days	0.29±0.0115	0.41±0.011	0.53±0.017	0.19±0.011
	60Days	0.09±0.0115	0.15±0.0115	0.18±0.0057	0.085±.0057
COWPERS GLAND	30Days	0.12±.023	0.08±0.0115	0.1±0.023	0.065±.0057
	60Days	0.075±.0.0057	0.11±.0.115	0.07±.0057	0.075±0.0288

Figure III and Table 3: Showing Hormones level of *P.gaujava*leaf extract in albino rat, duration of 30 and 60 days:



HORMONES	DURATION	CONTROL	EXPERIMENT-1	EXPERIMENT-2	EXPERIMENT-3
ESTROGEN (pg/ml)	30 days	15.77	17.55	11.8	14.19
	60days	21.93	28.33	39.1	38.9
TESTOSTERONE ng/dl	30 days	333.78	392.34	306.07	135.9
	60 days	339.43	340.23	382.31	423.6
FSH mIU/ml	30 days	0.3	0.3	0.3	0.3
	60 days	0.5	0.6	0.5	0.8
LH mIU/ml	30days	0.07	0.07	0.07	0.07
	60days	0.07	0.07	0.07	0.07
PROLACTIN ng/ml	30days	0.3	0.3	0.3	0.35
	60days	0.5	0.5	0.5	0.5

Figure IV and Table 5: Showing Caudaepididymal sperm Count of *P.gaujava*leaf extract duration of 30 and 60 days



No. of sperms 1000mm3/ml	Control	Experiment – 1	Experiment – 2	Experiment - 3
30 Days	10,000,000	14,000,000	9,000,000	5,000,000
60 Days	16,000,000	64,000,000	75,000,000	90,000,000

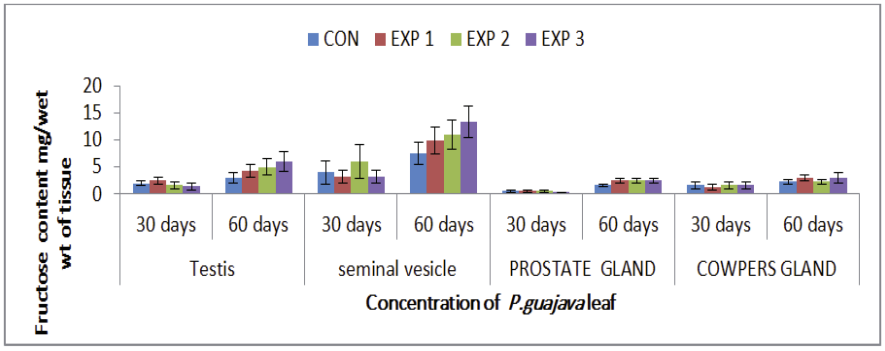


Figure V and Table 6 showing Fructose Content oral administration of *P.gaujava*leaf extract duration of 30 and 60 days

ORGANS	Duration	Control	Experiment- 1	Experiment-2	Experiment- 3
TESTIS	30 DAYS	2±0.5	2.5±0.7	1.6±0.7	1.4±0.6
	60 DAYS	3±1.0	4.3±1.2	5±1.5	6±1.9
SEMINAL VESICLE	30 DAYS	4±2.2	3.3±1.2	6±3.2	3.3±1.2
	60 DAYS	7.6±2.1	10±2.5	11±2.7	13.3±2.9
PROSTATE GLAND	30 DAYS	0.6±0.2	0.6±0.2	0.6±0.2	0.3±0.1
	60 DAYS	1.6±0.2	2.5±0.5	2.5±0.5	2.5±0.5
COWPERS GLAND	30 DAYS	1.6±0.7	1.3±0.5	1.6±0.7	1.6±0.7
	60 DAYS	2.3±0.4	3±0.6	2.3±0.4	3±1.0

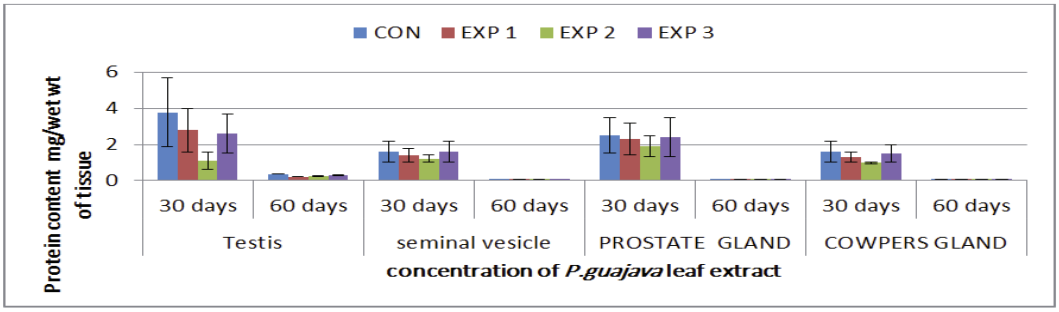
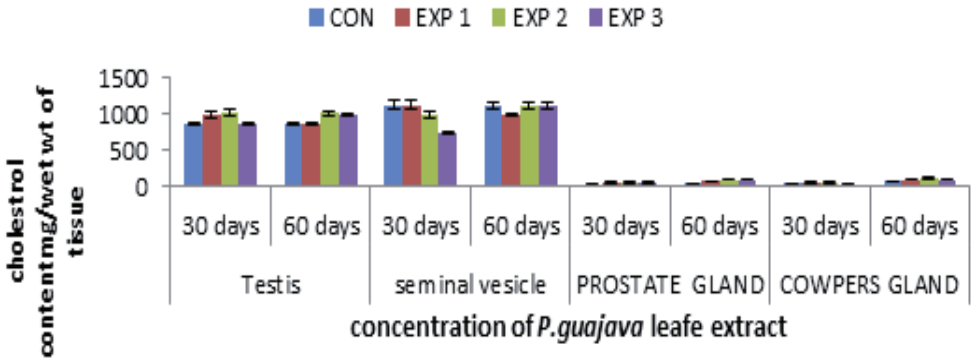


Figure VI and Table 7 showing Protein Content oral administration of *P.gaujava*leaf extract duration of 30 and 60 days:

ORGANS	DURATION	CON	EXP 1	EXP 2	EXP 3
TESTIS	30 DAYS	3.76±1.9	2.8±1.2	1.1±0.5	2.6±1.1
	60 DAYS	0.37±0.02	0.22±0.01	0.25±0.01	0.30±0.03
SEMINAL VESICLE	30 DAYS	1.6±0.6	1.4±0.4	1.2±0.2	1.6±0.6
	60 DAYS	0.04±0.002	0.06±0.003	0.05±0.0025	0.04±0.0020
PROSTATE GLAND	30 DAYS	2.5±1.0	2.3±0.9	1.9±0.6	2.4±1.1
	60 DAYS	0.02±0.001	0.06±0.003	0.05±0.0025	0.07±0.003
COWPERS GLAND	30 DAYS	1.6±0.06	1.3±0.3	0.98±0.06	1.5±0.5
	60 DAYS	0.05±0.0025	0.05±0.0025	0.06±0.003	0.07±0.0035

Figure VII and Table 8 showing Cholesterol Content oral administration of *P.gaujava* leaf extract duration of 30 and 60 days:



ORGANS	DURATION	CONTROL	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
TESTIS	30 DAYS	875±25	1000±52	1020±53	875±25
	60 DAYS	875±20	875±20	1025±32	1000±25
SEMINAL VESICLE	30 DAYS	1125±63	1125.0±63	1000±51	750±23
	60 DAYS	1125±38	1000±25	1125±38	1125±38
PROSTATE GLAND	30 DAYS	30±6	50±8	50±8	50±8
	60 DAYS	50±3	75±5	100±6	100±6
COWPERS GLAND	30 DAYS	40±7	50±8	50±8	30±6
	60 DAYS	75±5	100±6	112.5±7	100±6

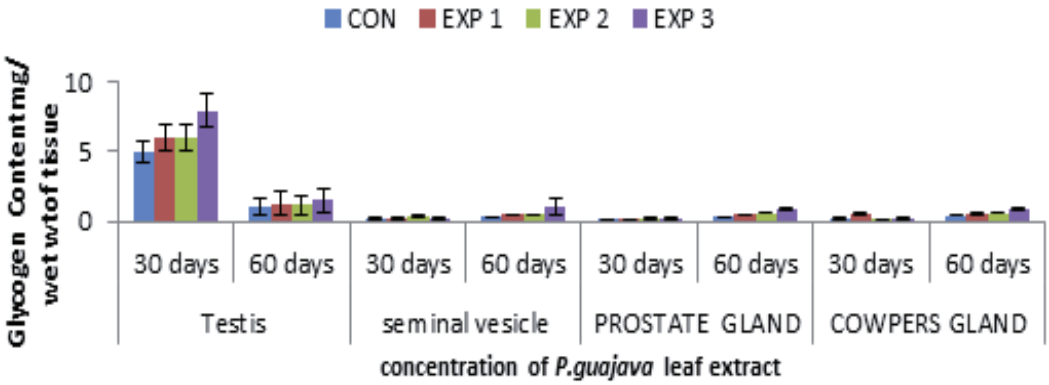


Figure VIII and Table 9 showing Glycogen Content oral administration of *P.gaujava*leaf extract duration of 30 and 60 days

ORGANS	DURATION	CONTROL	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
TESTIS	30 DAYS	5±0.8	6±0.9	6±0.9	8±1.2
	60 DAYS	1±0.6	1.3±0.8	1.2±0.7	1.5±0.9
SEMINAL VESICLE	30 DAYS	0.2±0.06	0.2±0.06	0.3±0.07	0.2±0.06
	60 DAYS	0.3±0.015	0.5±0.025	0.5±0.025	1±0.6
PROSTATE GLAND	30 DAYS	0.1±0.03	0.1±0.03	0.2±0.06	0.2±0.06
	60 DAYS	0.3±0.015	0.5±0.025	0.6±0.04	0.9±0.05
COWPERS GLAND	30 DAYS	0.2±0.06	0.2±0.06	0.1±0.03	0.2±0.06
	60 DAYS	0.4±0.03	0.6±0.04	0.6±0.04	0.9±0.05

Discussion: In the present study, there was a significant reductionin body weight and testicular weight in *P.guajava* treated animals. Administrationof *P.guajava* extract orally for 250,500 and 750 mg/body weight showed reduced the net gain in body weight of treated animal. This result may be due to weight of the testis as it is dependent on mass of the differentiated sperm cells, hence the reduction in weight. Increases in testicular weight, which is known to be mostly related to the number of spermatozoa present in the tissue (Shams Lahijani et al., 2008).

Organ weights can be used as indices for toxicity tests and their loss in weight indicates androgen deficit state (Rao etal.,1996).It has been reported that testosterone plays a major role in the maintenance of structural integrity and functional activity of accessory reproductive organs (Moor, Price and Gallagher et al., 1930).Weight of the Accessory sex organs was also decreased in dose dependent manner of *P.guajava*treatment in the present study. Weight loss of accessory reproductive organ for 30 days showed decrease in the serum testosterone concentration.

In the present study administration of *P.guajava*extract for 60 days showed increased the sperm qualities which are in accordance with the report of Oyeyemi et al.,(2008) and Longe et al.,(1983) in which there was increase in sperm parameters of rat treated with *P.guajava* leaves extract.This could increase glucose metabolism leading to the production of pyruvate which is known to be the preferred substrate essential for the activity and survival of sperm cells (Egbunike et al.,1986;Dua and Vaidya 1996).

In the present study the improved sperm characteristics in the treatment groups for 60 days suggested that *P.guajava* extract could produce the stimulatory effect on hypothalamus.Although our result showed the variation in FSH hormone,the increase in the level of serum testosterone in all experimental groups indicates the modulating potentials of *P.guajava* leaf extract in rats. These findings suggest that administration of extract from leaf successfully increased the sperm qualities which are in accordance with the report ofOyeyemi et al., (2008) and Longe et al., (1983) in which there was a significant improvement observed in all the sperm parameter of rats treated with extract.

Epididymis is the main organ for active maturation of sperms in rats (Blaquer et al., 1970). The structure and function of epididymis are androgen dependant, it secretes components essential for sperm maturation.

Testosterone level significantly decreased during 30 days treatment. This reduction in serum testosterone may be due to decrease synthesis or increased metabolic clearance. It has been stipulated thatas testosterone levels decrease, levels of FSH and LH are expected to increase to stimulate the production

ofmore testosterone (Emanuele and Emanuele, 2001).In this study, low serum testosterone levels in animals treated with *P.guajava* extract was accompanied by low levels of LH and FSH. This suggests that thehypothalamic cells which produce LH may not function correctly to the feedback when testosterone level decreased. The inability of the anterior pituitary to respond to a decline in testosterone may imply that high glucose has a central effect on the interaction between the nervous system and endocrine system as suggested by Maneesh et al., (2006). The decrease in serum LH and FSH may result from impairment in their production and secretion.

The suppression of gonadotropin that consequently produced the concentration of testosterone accompanied by the androgendeprivation effects on the testicular and spermatogenic activities by the alkaloids from the *P.guajava* leaves will adversely affect the process of reproduction in animals.Although the findings on the gonadotropic hormones in the present study is in accordance with Yakubu et al., (2007). In males, androgen play a pivotal role in the development of the reproductive system, spermatogenesis and for the expression of male sex behaviour (Akingbemi, 2005; Wang et al., 2009; Schulz et al, 2010). In this study, pituitary gonadotrophs (FSH and LH) following treatment with *P.guajava* were regressed during 30days. This may imply that the plant extract showed constant serum hormone level wasnoticed through the experimental period for 30 and 60 days.

This implies that the plant acted directly on the anterior pituitary to inhibit synthesis of gonadotropins. The low TSH obtained in the treated groups may be due to destruction of the Leydig cells but a reflection of the complex hormonal interplay at the level of the hypothalamic-pituitary-testicular axis. The extract could disrupt the functioning of the LHRH receptor or its interaction with LHRH resulting in diminished LH release. Studies in animals and humans have shown that when TSH levels decrease, LH levels do not increase as would be expected (Van, 1983; Maneesh et al., 2006).

According RahmatBano et al., (1993) testicular soluble proteins are required for sperm viability, cell division, growth and differentiation of germ cells during spermatogenesis.

Protein content was reduced in all the treated groups during 30 days this resultmay be due to the protein content in any organ is directionally proportional to growth rate. Reduction in protein content in testis in treated rats may also be the reason for the reduction in organ weight. Similar result was also noticed by Gosh et al., 1992.

Glycogen, protein and cholesterol are major components which play an important role in the body construction and energy metabolism. Glycogen is stored as food materials and is considered a major source of energy in animals. It also plays an important role in various physiological activities through glucose and lactic acid.Glycogen is a reserve carbohydrate found in sertoli cells and spermatogonia in testis, it serves as asource of glucose which is an energy supplier to the tubular cells. In the present study the decrease in glycogen content for 30 and 60 days resulted in impaired glycolysis. Similar results were also noticed by Mohri et al., (1975) in alpha–chlorohydril in spermatozoa.

Cholesterol is a major precursor in the biosynthesis of steroid hormone such as testosterone, is required for normal testicular activity. In the present study decrease of cholesterol content in experimental groups for 30 days is due to the testicular cholesterol alkaloids might suggest an effect of steroidogenic pathway in the testis. Such reduction in cholesterol level may be inimical to the normal functioning of the testis of the animals. During 60 days of administration of plant extract found to be increased in the experimental group, this may be due to variation of the synthesis of hormone.

Fructose acts as a donor of energyto the spermatozoa, which breaks it down selectively andconverts it into energy.Glucose, as well asgalactose, is also present in seminal fluid, although to amuch lesser extent. The motility of spermatozoa isvery closely connected with fructose break down. It has been demonstrated that thereis a definite ratio between the fructose level and thenumber of spermatozoa in the ejaculate; therefore, the number of spermatozoa is usuallyaccompanied by significant fall of fructose in the semen.

Conclusion: A slight increase of body weight in group II was noticed. Group III and IV showed decrease in body weight. The weight of Testis increased in group II in comparison with group III and IV in 30 days. Group II for 60 days increased organ weight and slight decrease in body weight in comparison to control rats. The weight of accessory sex organs decreased in all the groups (30 and 60 days) in comparison to control.

Epididymal sperm count showed a decrease in group III and IV (30 Days), the sperm count increased in all the groups (60 days). The number of abnormal sperms was less in group II, III and IV during 60 days.

Testosterone level increased in group II (30 days) a reduction was observed in the other experimental groups. Testosterone level increased in all experimental groups when compared with control (60 days).Oestrogen levels fluctuated in the experimental groups (30 days). It increased in the experimental groups (60 Days) when comparison to the control. FSH, LH and prolactin remained constant in all the groups (both exp. and control)

Protein content in testis decreased in all the groups (30 days and 60 days). The protein content in accessory sex organs varied when compared to control.Cholesterol increased in testis and accessory sex organs when compared to control (30 days and 60 days). Glycogen level increased in testis and accessory sex organs when compared to control (30 days and 60 days). Fructose content in testis was more in group I and decreases in groups III and IV when compared to control (30 days). During 60 days fructose content increased in all the experimental groups in comparison to control. Fructose content in accessory sex organs showed fluctuation (30 Days). In 60 days there was increase of fructose when compared to the control.

In conclusion, administration of aqueous extract of *P. guajava*leaves increased sperm count, testosterone level and fructose content in dose dependant manner and has fertility enhancing ability. This increasemay act directly or indirectly on the pituitary gland secretary function causing to anincrease in the androgen. It has been demonstrated that the process of spermatogenesis and the accessory reproductive organs functions are androgen dependent. Therefore, any changes in the androgen production would reflect and explain the increase in the number of sperms.

The exact cause for this increase is unknown. Further histological studies, if conducted might reveal the exact cause.Function of seminal vesicles and their role on male fertility of rats. Dixit et al., (1982) reported the effect of chronic administration of garlic (*Allium sativum*) of testicular function in albino rat. Effects of *Terminaliacatappa*seeds on sexual behaviour and fertility of male rats were reported by Ratnasooriya et al., (2000).

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