Biomedical & Pharmacology Journal Vol. **1**(1), 65-78 (2008)

Effects of ethanol extract of leaves of *Helianthus annuus* on the reproductive system of male wistar rats: Testicular histology, epididymal sperm properties and blood levels of reproductive hormones

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(Received: May 18, 2008; Accepted: August 07, 2008)

ABSTRACT

The time and dose dependent effects of ingestion of the ethanol extract of the leaves of Helianthus annuus *L.*(the common sunflower),0.5g/kg and 2g/kg body weight for 28days,on the histology of the testes, Epididymal sperm properties, blood levels of Testosterone, Follicle stimulating hormone and Leutinizing hormone of male Wistar rats were investigated in two phases. The results in phase one revealed the presence of mild to severe histo-degenerative testicular changes in treated rats sacrificed by the end of day 14, 28 and 56 compared with their matched controls. This was accompanied with statistically insignificant ($p>0.05$) decrease in mean body weights and testicular weights in treated rats in the period of treatment. Between 28th and 56th day when extract treatment was withheld, previously treated rats showed gain in mean general body and mean testicular weight increased toward that of the control. Results in phase two revealed progressive lowering of the epididymal sperm count percentage sperm viability as well as the blood levels of gonadotropins and testosterone while the percentage abnormal sperm morphology progressively increased with time of treatment. Statistically significant ($p<0.05$) effects of extract treatment on these parameters were observed for both doses in animals sacrificed earliest and persisted in experimental animals spared extract treatment between day 28 and 56 before they were sacrifice. This study suggest that the 2 experimental doses of extract used were probably above threshold and a time dependent effect of extract treatment on epididymal sperm properties as well as blood levels of gonadotropins and testosterone. It also suggests that the anti-spermatogenic effect of the extract could be as a result of toxicity on the testes and/or the anterior pituitary gland. We conclude from this study that the ethanol extract of the leaves of helianthus annuus possesses anti-spermatogenic /antifertility potentials that may be harnessed for contraception but further investigation in this regard is required.

Key words: Ethanol Extract, Leaves, Helianthus annuus, Rats, Testicular Histology, Epididymal Sperm Number, Reproductive Hormones.

INTRODUCTION

In Nigeria and other parts of the world, the practice of traditional or folklore medicine involves the use of herbs, animal and mineral and/or the use of rituals and incantations: The use of herbs has however survived best(Ebomoyi *et al.,* 2004).As a result of the inability of modern medicines to meet all the health needs of man, everywhere attention is still given to the possibility

of finding cures in alternative medical systems(Okpako,1991).Hence the continued coexistence of traditional(herbal) medicines and their orthodox counterparts, especially in poor developing regions of the world.In recent times there have been a significant increase in the use of herbal medicines as a result of the World Health Organization's promotion of traditional medicines (WHO, 1991). The plant Helianthus annuus L is an annual perennial characterized by its rough hairy stem,3-12 feet high, broad coarsely toothed rough leaves 3-12 inches in wild specimens and often a foot or more in cultivation. Its composite flower consist of inner disc florets and outer ray florets .The inner disc florets mature into what is commonly referred to as the sunflower seed but are actually the fruits of the plant.(Wikipedia,2000;Putnam *et al.,*2002).The genus Helianthus has about fifty species all natives of North America .Like the other species in the family of Asteracea, they have well evolved having spread worldwide to now have a global distribution(Turner,1977) .Such plants that are most vigorous and have successfully adapted to different habitats are known to contain an effective arsenal of interesting biologically active compounds and it has been suggested that they should constitute the focus of the search for new sources of potentially useful natural products with potentials of becoming important as key structures for designing new drugs and/or agrochemicals (Bajaj,1991).

The leaves of Helianthus annuus are harvested as the plant come into flower and are dried for later use (Bown, 1995).A tea made from the leaves has been reported to be used as astringent, diuretic and expectorant as well as in the treatment of high fevers (Moerman, 1998). The crushed leaves are used as a poultice on sores, swellings, snake bites and spider bites (Foster and Duke, 1990). This may not be unrelated to the common practice in the southern parts of Nigeria of using the crushed leaves to topically treat recent skin lacerations.

The dried leaves have been used to treat asthma in Thailand (Zagari, 1992).Also tea made from the flowers has been reportedly useful in the treatment of malaria and lung ailments (Foster and Duke 1990; Moerman 1998).The flowering head and seeds have been used as febrifuge, nutritive and stomachic (Chiej, 1984).The seed is also considered to be a diuretic and expectorant (Grieve, 1984) It has been used with success in the treatment of many pulmonary complaints. The dried seed has been used for the treatment of diabetes mellitus in Taiwan (Lin,1992).A decoction of the roots have been used as a warm wash on rheumatic aches and pains(Moerman 1998).The anti-microbial (McChesney and Adams,1985) and anti-neoplastic effects(Eugester,*et al.,*1991) have also been reported. The oil of the seed is rich in polyunsaturated fatty acids especially Linolenic and Linoleic acids and has been used as dietary replacements to lower serum cholesterol in the treatment and prevention of atherosclerotic vascular related disorders (Rogers, 1999).

Other uses of Helianthus annuus could be summarily described as ornamental, nutritional and economical. Nutrition wise the seed can be eaten raw or cooked. Rich in fats, the seed can be ground into powder, made into sunflower butter and used to make seed yoghurt (Saunders, 1976; Sweet, 1962).Mixed with cereal flours it is used to produce nutritious bread (Philips and Foy, 1990).Young flower buds are steamed and served like globe artichokes. A high quality edible semi drying oil obtained from the seed characterized by a low cholesterol levels is used in cooking, salads and margarines(Duke,1983).Cultivars with up to 50% oil yield have been developed in Russia(Duke and Ayensu, 1985).

The roasted seed is a coffee and drinking chocolate substitute (Polunin, 1969).The leaf petioles are boiled and mixed in with other foodstuffs (Cheij, 1984).

The chemical constituents (Grieve, 2002) and the nutritional analysis (Duke and Ayensu , 1985.) of the flower and seed were available in the literature. Initial phytochemical screening of the ethanol extract of the leaves reported the presence of tannins,Saponins glycosides, steroids, carbohydrates and reducing sugar while alkaloids were absent(Ejebe *et al.,* 2008).

As regard the toxicity of the plant Helianthus annuus, the growing plant can

accumulate nitrates especially when fed on artificial fertilizers (Cooper and Johnson, 1984).The pollen or plant extracts may cause allergic reactions (Foster and Duke, 1990).The LD_{50} of the ethanol extract of the leaves in male Wistar rats was reported as 14g/kg of animal weight (Ejebe et al 2008). The aqueous extract of Helianthus annuus has been prescribed for the treatment of menstrual irregularities and of uterine leiomyomas for several years by traditional medical practitioners in southern Nigeria. The anti-spermatogenic effects of the seed have also been reported (Starikova 1970). An unpublished investigation of the effect of the aqueous extract of the leaves of Helianthus annuus on the testicular histology suggested atrophy of the Sertoli cells with extensive necrosis of the interstitial cells of Leydig. This study is aimed at investigating the effects of the crude ethanol extract of the leaves of Helianthus annuus *L* (Sunflower) on the histology of the testis, blood levels of the reproductive hormones as well as epididymal sperm characteristics of mature male Wister rats.

MATERIAL AND METHODS

Identification and preparation of the plant material

The wild variety of Helianthus annuus L was identified with the assistance of a plant physiologist, Mr H.B. Onyechasim. Young stems of the plant with healthy leaves were harvested from the bush growing behind the postgraduate hostels at the Abuja campus of the University of Port-Harcourt in March 2007.The plucked leaves were sun dried for seven days and then pulverized with NIPL blender into smooth powder.

Extraction process

Extraction was with absolute ethanol by the Maceration technique (Evans and Trease, 2004).

Preparation of stock solution

From 3.2kg of the pulverized leaves 200g of the caked paste of the extract was obtained. This was dissolved in 1250mls of distilled water and stirred to obtain a uniform stock solution of concentration 0.16g/ml, from where measured volumes that conveyed the selected experimental doses to the different animal groups were obtained. The stock solution when not in use was stored in freezer at 4°C.

Animal experiment

Seventy-two (72) adult male Wistar rats weighing between 200-300g were procured from the breeding colony of the Department of pharmacology of the College of Health Sciences, University of Port Harcourt and were housed in wooden cages in the animal house of the Faculty of Basic Medical Sciences Delta state university, Abraka.They were allowed 2 weeks to acclimatize to their new environment before the commencement of the experiment. Throughout the study period they were fed with standard rat feed and water ad libitum and maintained in standard environmental conditions of temperature; twelve hours dark/light cycle.

The study was organized in two phases

In phase one, we determined the effect of treatment with the extract on the Mean body and testicular weights as well as the histology of the testes of male Wistar rats using 36 animals. While in phase II another 36 animals were used to determine the effect of extract treatment on the Blood levels of some male reproductive hormones and Epididymal sperm properties. In each phase the animals were randomly allotted into two experimental dose groups that received 0.5g/kg and 2g/kg of extract daily for 28 days and control groups that had 5mls of distilled water for the same period. Each group consisted of 12 rats from which 4rats were randomly selected and sacrificed under chloroform general anesthesia by the end of day 14, 28 and 56. Between day 28 and 56, surviving rats in the experimental groups had only their routine feed and water without any extract. The solution of the extract was orally administered to the rats using a medicut intravenous cannula as an improvised oral cannula. The animals had their pre and post treatment weights determined in both phases.

Phase I animals at the time of sacrifice had their testes harvested via a laparotomy. Each testis was weighed using an electronic weighing balance before immediate fixation in 10% formosaline for at least 24hours.Cut 5µm Paraffin sections were stained with hematoxylin and eosin using Lillie's method (Lillie,1976). The slides were

analysed with the assistance of an histopathologist. The slides were later photographed at magnification of x 40 and photomicrographs developed.

Phase 2 animals at sacrifice, had 5 mls of blood collected, each into heparinized bottles using the technique of open cardiac puncture. Each specimen was immediately centrifuged at 5000g for 10minutes and the sera were separated and stored in a freezer until the blood levels of the reproductive hormones were assayed. Blood levels of Leuteinizing Hormone,Follicle Stimulating Hormone and Testosterone of each specimen were assayed using the technique of Enzyme Immuno Assay(2004).Each animal in this phase also had their testes harvested for immediate sperm analysis. Epididymal sperm count was determined using the technique described by Taylor et al 1985.Epididymal sperm morphology was determined by a modification of the method of MacCain et al (1989) and sperm viability according to the method described by Gyan *et al.,2*007.

Statistical analysis

Results were expressed as Mean± Standard Error of Measurement (SEM) and as percentage sperm viability and abnormal sperm morphology. The effects of the extract on the mean body weight and testicular weight of the experimental and control rats were analysed for statistical significance using the Paired t-Test of Microsoft Excel 2003 statistical software (Ogbeibu, 2005). The effects of treatment with the extract on the blood levels of the assessed reproductive hormones, Epididymal sperm number, Percentage abnormal sperm morphology and sperm viability were compared with controls within specified length of extract treatment before sacrifice as well as effect of duration of treatment before sacrifice within a specified dosage group. Here comparisms of these effects were carried out with the Single Factor ANOVA test using the EPI Info6 version 6.0 computerized statistical software. Level of statistical significance for all analyses was 5% ($P<0.05$).

Table1:**Pre-treatment and post- treatment mean weights of experimental and control rats**

Dose group	Day 14		Day 28		Day 56	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Low(0.5g/Kg) of Extract	251.5±0.65	258.75±3.48	228.5±3.12	217±7.88	228.5±5.75	277.25±8.14
High (2g/Kg) of Extract	274.5±5.42	252.85±7.44	242± 5.52	237± 4.20	256.5±4.37	278.50±9.35
Control (5mls of Distilled water)	239.30±5.90	216± 7.76	248.50±6.51	260±4..08	228.55±5.64	260± 1.87

Table 2: Changes in pre-treatment mean weights of experimental and control rats

Group/Treatment	Sacrifice Day		
	Day 14	Day28	Day 56
0.5g/kg (Extract)	-2.75g (1.2%)	-11.5g (5.0%)	+48.75g (17.56%)
2g/kg (Extract)	-21.65g (7.9%)	-5g (2.1%)	+22g (7.9%)
Control	+6.7g (2.8%)	+11.5g (4.6%)	+31.5g (13.8%)

Table 3: Pretreatment and posttreatment weights of experimeental and control rats compared using the student paired t-test

Sacrifice day	P-values
14	0.4196
28	0.2284
56	0.0507

Table 4: Comparing the effects of the 2 experimental doses on the mean animal weights with each other and with their control using the paired t-test

Sacrifice day	P-Value		
	0.5g/kg vs 2g/kg	0.5g/kg vs control	2g/kg vs control
14	0.3877	0.3589	0.3776
28	0.1120	0.2168	0.3247
56	*0.0349	0.5	0.1283

Table 5: Blood testosterone level in rats treated with helianthus leaves extract (nm)

Group/Treatment	Sacrifice Day		
	Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	0.75±0.03	0.66 ± 0.02	0.55 ± 0.02
Extract treated (2g/kg)	0.69±0.05	0.56± 0.08	0.34± 0.12
Control (0.5mls distilled H_2O)	0.90±0.15	0.89± 0.10	0.92± 0.12

Table 6(a): Blood gonadotropin level in rats treated with helianthus annuus leaves extract leuteinizing hormone (miu/ml)

Group/Treatment	Sacrifice Day		
	Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	2.82±0.3	1.98±0.25	1.66±0.15
Extract treated (2g/kg)	2.62±0.26	1.85±0.19	1.65±0.20
Control (0.5mls distilled water)	3.40±0.35	3.25±0.20	3.00±0.40

Table 6(b): Follicle stimulating hormone (mIU/ml)

Group/Treatment	Sacrifice Day		
	Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	2.04±0.04	1.75±0.05	1.66±0.03
Extract treated (2g/kg)	1.76±0.03	1.53±0.02	1.65±0.04
Control	3.39±0.02	3.20±0.04	2.95±0.03

Table 7: Epididymal sprem viability (Percentage)

Group/Treatment	Sacrifice Day Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	84%	62.5%	50%
Extract treated (2g/kg)	67%	58%	49.5%
Control (0.5mls distilled H_2O)	99.6%	95%	96.5%

Table 8: Epididymal sprem number (mean±sem) or rats treated with helianthus annuus leave extract

Group/Treatment	Sacrifice Day Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	8.6×10^6/ml (±0.03)	7.4×10^6/ml (±0.01)	5.6×10^6/ml (±0.02)
Extract treated (2g/kg)	5.4×10^6/ml (±0.01)	3.7×10^6/ml (±0.04)	2.5×10^6/ml (±0.02)
Control (0.5mls distilled H_2O)	11.3×10^6/ml (±0.5)	10.9×10^6/ml (±0.02)	10.7×10^6/ml (±0.03

Table 9: Abnorla sperm morphology (Percentages) for rats treated withhelianthus annuus leaves extract

Group/Treatment	Sacrifice Day Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	4.5	8.6	1.5
Extract treated (2g/kg)	6.4	10.5	20
Control 0.5mls distilled (H_2O)	2.5	3.0	5.0

Table 10: Epididyml sperm number (mean± sem) of rats treated with helianthus annuus leave extract

Group/Treatment	Sacrifice Day Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	8.6×10^6/ml (±0.03)	7.4×10^6/ml (±0.01)	5.6×10^6/ml (±0.02)
Extract treated (2g/kg)	5.4×10^6/ml (±0.01)	3.7×10^6/ml (±0.04)	2.5×10^6/ml (±0.02)
Control (0.5mls distilled H_2O)	11.3×10^6/ml (±0.5)	10.9×10^6/ml (±0.02)	10.7×10^6/ml (±0.03

Table 11: Abnormal sperm morpholgy (percentages) for rats treated with helianthus annuus leaves extract

Group/Treatment	Sacrifice Day Day 14	Day 28	Day 56
Extract treated (0.5g/kg)	4.5	8.6	1.5
Extract treated (2g/kg)	6.4	10.5	20
Control 0.5mls distilled (H_2O)	2.5	3.0	5.0

Table 12: P-values obtained when means of day 14,28 and 56 within a specified dose group were compared using single factor ANOVA test

	Gp1	Gp2	Gp3
Epididymal Sperm Number	*0.0000	* 0.0000	*0.5807
% Abnormal Sperm Morphology	*0.0013	* 0.0000	* 0.3548
FSH	*0.0022	* 0.0000	* 0.5931
LH	*0.0321	* 0.0188	* 0.6913
Testosterone	*0.0002	* 0.0095	* 0.9927

Table 13: P-values obtained when the Means of extract treated animals and controls were compared using single factor ANOVA test (0.5g/kg vs 2g/kg vs control)

	Day 14	Day 28	Day 56
Epididymal Sperm Number	*0.0000	*0.0000	*0.0000
% Abnormal Sperm Morphology	0.1554	*0.0188	*0.0000
FSH	*0.0000	*0.0030	*0.0001
LH	0.2109	*0.0036	*0.0051
Testosterone	*0.0037	*0.0006	0.2920

RESULTS AND DISCUSSION

Many herbs used in traditional medical systems are yet to be investigated for their potential toxicity on the male reproductive system. A possible therapeutic usefulness of herbal remedies discovered to possess a reversible toxic effect on male fertility would be as a male oral contraceptive agent. Current estimation suggest that the population of the world will rise to 9.2billion at best and 14.2 billion at worst by the year 2050 (Leo *et al.,* 1996).The uncontrollable rate of growth of global population and its adverse socio-economical consequences creates a considerable need for contraception. A survey of sexual attitudes and lifestyles in the United Kingdom showed that the combined oral contraceptive pills is the most commonly used method of contraception with condoms and vasectomy coming a close second and third respectively (Johnson *et al.,* 1994).While the use of oral contraceptive pills by female is similarly popular in third world countries like Nigeria, the same can not be said of methods of contraception like vasectomy directed at the male. In many parts of these developing countries with very low literacy level in the general population especially the rural areas, male not only dominates sexually but also socio-culturally. Hence he is more often than not unwilling to submit himself to contraception leaving the woman to bear this all important responsibility of family planning alone. Beside access to contraceptive advice and technology is largely limited to the urban areas.

The basic male condom consists of a thin, stretchable latex film which is molded into a sheath, lubricated and packed in a foil wrapper. The sheath has a teat end to collect the ejaculate. Many males do not prefer to use the condom with their wife even though they will not mind using it in an illicit affair because of the protection against sexually transmitted infections it offers them (Van Landingham *et al.,* 1995). Similarly even among commercial sex workers who routinely use condoms with clients, a lower rate of condom use with their boyfriends have been reported (Dorfman, Derish and Cohen,1992) The disadvantages of the sheath are that they need to be applied before intercourse

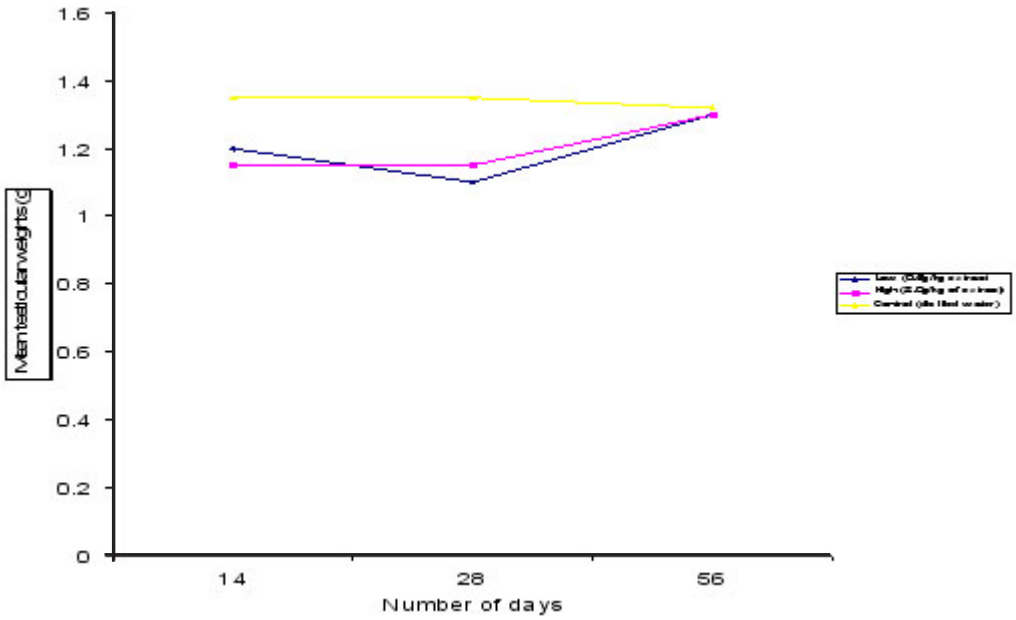

Fig. 1: Effects of extract treatment on mean testicular weights

and that they reduce the level of sensation (Symonds and Symonds,1998).Perhaps the discovery of an orally ingested contraceptive agent for males in the developing world especially for rural dwellers who are mostly disadvantaged as highlighted above will be more acceptable as long as erection is not affected adversely and its effect is reversible in the short term post discontinuation of its use.

The recognition by the W.H.O of population explosion as one of our planetary crisis has prompted this body to put great attention on the search for a safe, cheap and socially acceptable form of contraception. One aspect of this vital effort has focused upon folk use of anti-fertility herbs.

The anti-spermatogenic effect of the seed of Helianthus annuus has been reported (Starikova ,1970) and an unpublished investigation of the effects of the crude aqueous extract of the leaves of this plant on the testis of male rats seem to collaborate this effect.

This work is an attempt to investigate the time and dose dependent effects of the crude ethanol extract of the leaves of Helianthus annuus on the testes as well as the blood levels of reproductive hormone and Epididymal sperm characteristics of male Wistar rats

The LD_{50} of the ethanol extract of the leaves of Helianthus annuus had been reported as 14g/kg in a study where no mortality was recorded at a dose level as high as 8g/kg (Ejebe *et al.,* 2008).This was the basis of selecting 0.5g/kg and 2g/kg as the experimental doses in this study.

All the extract treated animals were observed to have lost between 1.2 -5.0 % of their pre-treatment weight during the period of treatment while the control animals gained between 2.8-13.8% of their pretreatment weights in the study period. Also the experimental animals that were sacrificed by the end of the 56th day having stopped ingestion of the extract after the 28th day were also noticed to have gained weight (7.9-17.56% of their pre-treatment weights) by the time they were sacrificed. However when the pretreatment and post-treatment mean weights of the extract treated animals were compared using the paired student t-Test there was no significant differences between the pre and

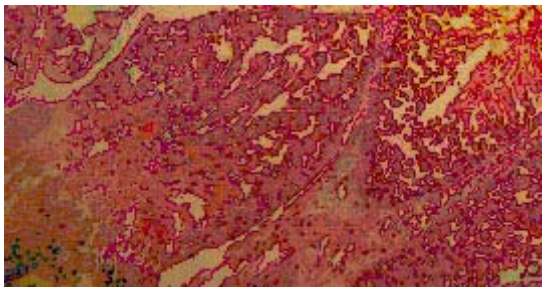

Fig. 2: Control (Day 14): Histology of the testis appears normal

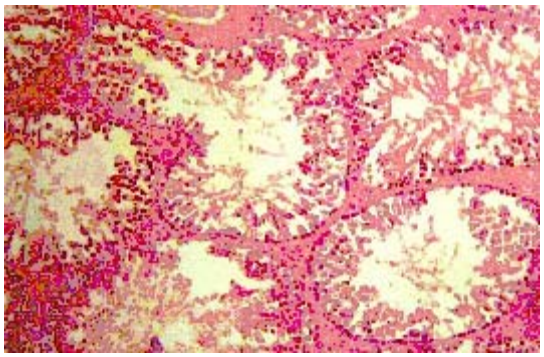

Fig. 3: 0.5g/kg of Extract (Day 14). Histology of testis show atrophic changes, hemorrhagic and necrotic areas with presence of many inflammatory cells

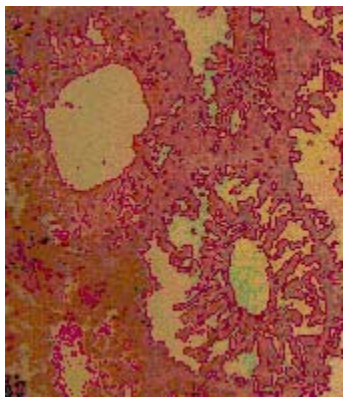

Fig. 4: 2g/kg of Extract treated (Day 14): Histology show germ cells arrest with more severe degenerative changes. There are empty lumens of seminiferous tubules with thickened basement membrane

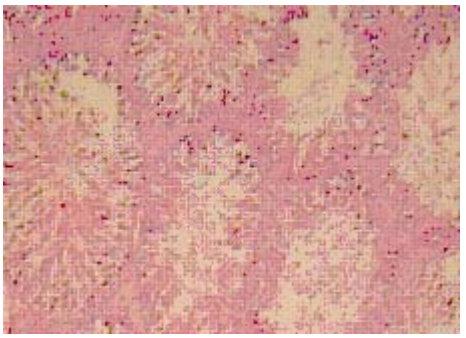

Fig. 5: Control (Day 28): Histology of Testis appears normal

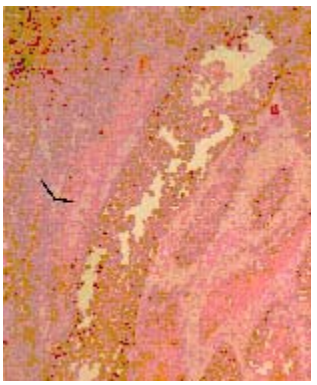

Fig. 6: 0.5g/kg of Extract (Day28): Histology of testis show moderate atrophic changes with several empty lumens of seminiferous tubule, germ cell arrest and thickening of the basement membrane

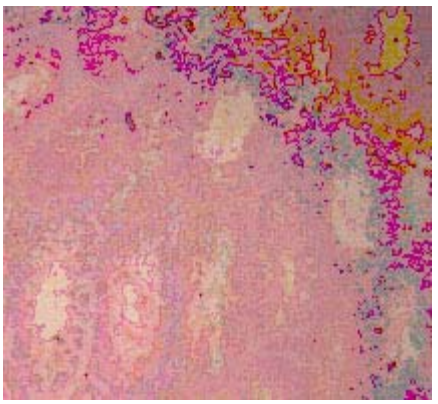

Fig. 7: 2g/kg of extract (Day28): Histology of Testis show extensive necrosis, haemorrhage and severe atrophic changes

post-treatment mean weight of animals sacrificed on day 14,28,and 56.While the observation that the plant extract produced weight loss during the period it was fed to the experimental rats may be suggestive of some form of general toxicity(Gyan *et al.,*2007),type II diabetes have been known to be due to insulin resistance , associated with obesity and responsive to weight loss. And considering the fact that the use of the dried seeds of Helianthus annuus as an anti-diabetic agent in Taiwan has been reported by Lin, 1992, it will not be so much out of place to investigate this extract for any anti-diabetic action. The mean testicular weights of the extract treated rats sacrificed by days 14 and 28 were both lower than those of their respective controls. This effect is expected in an atrophying organ and was anticipated as a possible outcome of this study. However on comparing the mean testicular weights of the extract treated rats with those of their respective controls using the paired student t-Test no dose or time dependent significant effects were observed (All p values >0.05).Importantly however is the observation that extract treated animals sacrificed by the 56th day had mean testicular weights that more closely approached those of the control rats. This is suggestive of a ready reversibility of any atrophic effect adducible to treatment with the extract on discontinuation of therapy.

Report on the histological slides of the testes showed the presence of variable degree of atrophic changes in those of extract treated rats compared with those of their respective controls which appeared normal. Reported histological changes in testes of extract treated rats included emptier lumen of the seminiferous tubules, thickening of the basement membrane, interstitial and intraluminal hemorrhages, arrested germ cell development, widespread necrotic changes and presence of inflammatory cells

Treatment of the experimental rats resulted in a progressive lowering of the blood levels of FSH, LH and Testosterone as well as in the sperm count and percentage viability. There was also a progressive increase in the percentage abnormal sperm morphology in the extract treated groups compared with their respective controls. When the effects of the plant extract on sperm properties and the reproductive blood hormone levels on the different sacrificial days were compared with each other within a given dose level and using the single factor ANOVA test the changes were all significant at both dose levels of the extract while for the control group they were all statistically insignificant (P>0.05).This may suggest that both experimental doses of the extract were above the threshold dose required to produce significant anti-spermatogenic effects which were significantly present as early as the 14th day and remained significant even after discontinuation of therapy between the 28th and 56th day. It was also observed that histo-degenerative changes were marked in those experimental rats sacrificed at the end of the 56th day even though they stopped receiving the extract by the 28th day. Testicular toxicants that arrest germ cell development have previously been reported to produce worse effects by the end of the duration of the cycle of the germinal epithelium, which last for 56 days in rats (Heller and Clermont,1963; Osinubi *et al.,* 2004). The effects of extract treatment on sperm properties are in line with the degenerative changes observed in histology. This further collaborate the presence of anti-spermatogenic activity in the extract.

The changes in the blood levels of testosterone, Folicle Stimulating Hormone and Leutinizing hormone, which were all lowered in the extract treated rats compared with the controls, provide some clues to the possible mechanisms of anti-spermatogenic action of the extract. The cooperative actions of testosterone and FSH are required for the initiation of spermatogenesis (Kent *et al.,* 1999) and testosterone production by the Interstitial cells of Leydig is stimulated by LH .The direct toxicity of the extract on interstitial cells of Leydig suggested in an unpublished work was not assessed in this work because of the poor outlining of these cells using H&E staining technique. While the possible existence of this effect explains the drop in LH, the pattern of changes in the levels of the blood Gonadotropins also suggests that the extract possibly act via effects on the Anterior Pituitary gland. This was not however assessed in this study. Another possible mechanism of the anti-spermatogenic actions of this extract could be the presence of steroids in its phytochemical constituents. Such steroids that are found in plants have been referred to as phytosteroids or

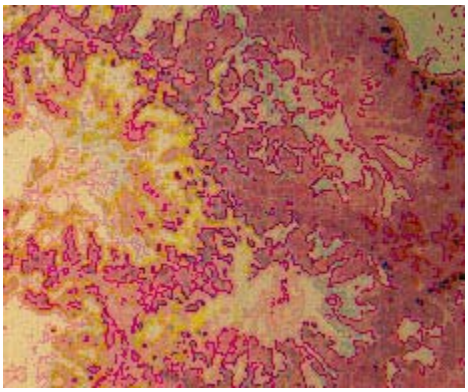

Fig 8: Control (Day57): Histology appears normal

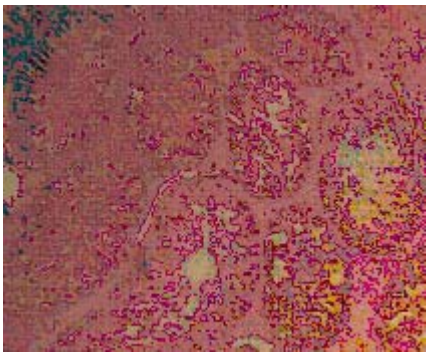

Fig. 9: 0.5g/kg of extract treated (Day57): Histology of testis show Atrophic changes, Necrotic tissues and areas of intra luminal haemorrhage

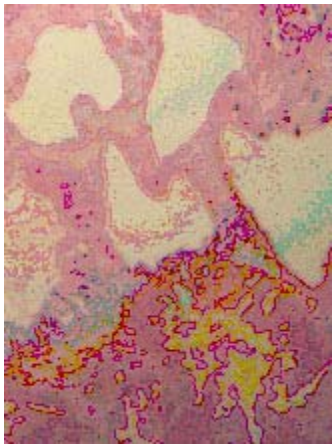

Fig. 10: 2g/kg of Extract treated (Day 57) Histology of testis show severe atrophic changes, empty lumen of tubules as well thickened basement membrane

phytoestrogens (Stansbury, 2007; Ward Dean, 2008). They have been reported to adversely affect the fertility in both male and female animals by acting as estrogen disruptors. Plants like soya beans which were initially widely promoted by nutritionist as rich sources of proteins (essential amino acids) have been found to be rich in these phytoestrogens and the antifertility effects of these agents have been confirmed in several disparate studies in animals and humans (Harrison *et al.,*1997.). As a result of this possibility it is now recommended that phytoestrogens in plants be avoided unless contraception is a desired goal. There is however a need for further identification of the steroids in the ethanol extract of the leaves of Helianthus annuus to be undertaken in order to ascertain if they are the sort incriminated to adversely affect spermatogenesis and male fertility.

CONCLUSION

This study strongly suggest the presence of anti-spermatogenic activity in the ethanol extract of the leaves of Helianthus annuus as indicated by the histo-degenerative changes of the testes and negative changes in the epididymal sperm characteristics seen in those rats treated with the extract compared with their respective controls. The site of action of the extract could be direct toxicity on the testes and/or the anterior pituitary gland. The identification of steroids as one of the several phytochemical constituent of the extract could provide a possible clue to the active anti-fertility constituent as phytoestrogens have been recognized to adversely affect fertility in male and female animals. There is need for further investigation into the effects of this extract on the anterior pituitary as well for actual fecundity test to be conducted in treated rats and matched controls to further collaborate some of the findings in this study before any strong claim can be made about the existence of a potential male oral contraceptive agent in this extract.

ACKNOWLEDGEMENTS

The authors acknowledge and are grateful to Morka Lucky, Ogbebor Martins ,Ahatty G.C, Madam Grace Eke, Aghogho, Ejiro for their immense contributions to the successful conduction of this study.

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