RESEARCH ARTICLE

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Preliminary Investigation for A Potential Ameliorative Role of Methanolic Extract of Leaves of *Polyalthia longifolia* and *Trigonella foenum* and Seeds of *Murraya koenigii* on Lindane Treated Male Rats

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Abstracts: Lindane is reported have to detrimental effects on different tissues. In the present study the 90 male Albino Swiss rats were treated lindane at a dosage of 250ppm/kg body weight/day. Following the lindane treatment, shrinkage in the testis was observed but there was no physiological difference in complete body mass. Sperm production was also hampered and the generated sperms were also damaged. Profound histological changes were also observed in the testis and degeneration of the Leydig cells was noted along with reduction in serum testosterone levels. Oxidative stress was also observed to ascent during the lindane treatment course revealing significant generation of ROS. Following the plant extract treatment a significant recovery was observed. The fertility of the rats was restored whereas the functioning of the reproductive organs and accessory sex organs were also apt almost comparable to that of the control group. ROS generation was also observed to decline following the plant extract treatment

Key Words: Lindane, *Murraya koenigii*, Oxidative stress, *Polyalthia longifolia*, ROS, Sperm function, Testosterone, *Trigonella foenum*

INTRODUCTION

Reverse Pharmacology was proposed and initiated by Vaidya. [1] Reverse Pharmacology is possible only in those countries with pluralistic healthcare and where robust clinical and laboratory documentation of novel human, pharmacodynamic effects are possible by inter-system collaborative teamwork. [2] The scope of Reverse Pharmacology is to understand the mechanisms of action at multiple levels of biological organization and to optimise safety, efficacy and acceptability of the leads in natural products, based on relevant science. [3]

Lindane, the γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane, is a widely used organochlorine pesticide. The application of lindane in soil, foliar, and seed treatment for a large variety of fruit and vegetable crops, its application on livestock, pets, and on agricultural premises, in pharmaceutical preparations, as well as in public health pest control, could lead to the exposure of humans to low concentrations of this compound. Various experiments were carried out in order to assess the toxicity and carcinogenicity of lindane. [4, 5] Lindane HCH intoxication has been associated with male reproductive toxicity in experimental animals and lindane may have the potential to produce adverse effects on fertility in men [6] (Kamirin, 1997) as the lindane has the potential of passing through the testis. [7] Lindane had also reported to be inducing detrimental effects on various other tissues. [8, 9]

Reversal of the reproductive toxicology has already been using vitamin E, D [10] and carotene [11] respectively. Treatment with plant extract *Hypericum perforatum* had shown to have the reproductive protective role in metal ions damaged reproductive organs in male mice. [12] The antioxidant activities of reproductive parts were higher than those of the vegetative organs, with the pods having highest total phenolic, proanthocyanidin, and flavonoid contents and antioxidant potentials of *Cassia fistula* L. [13] However any of the extract had not reported to have proper sperm function and normal function of organs and maturation of the sperm.

The leaves of *Murraya koenigii* are as an herb in Ayurvedic medicine. Their properties include much value as an antidiabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and anti-hypercholesterolemic. [14] *Polyalthia longifolia* had also reported to have cytotoxic property, [15-18] Anti-inflammatory properties, [19] Anti-microbial properties, [20], Hypoglycemic properties. [21] *Trigonella foenum-graecum* Fenugreek is a plant in the family Fabaceae has shown many biological prospectuses predominantly includes hypoglycaemic [22-25] and cytotoxic properties. [26-28] None of the above three plants individually or in combination has been reported to have protective reproductive mechanism. Hence the present study was conducted to understand the toxicological effect of lindane and effect of methanolic extract on the leaves of *Murraya koenigii* and *Polyalthia longifolia* and seeds of *Trigonella foenum-graceum* on the reproductive organs of lindane treated organs.

MATERIAL AND METHODS

Experimental Animals

150 healthy adult male rats of Swiss albino strain, weighing between 250g to 300g, were obtained from Dept of Biochemistry, MS University, Vadodara for the experimental purpose. They were housed in an air conditioned animal house at temperature 25±1°C and exposed to 10 to 12 hours of daylight with free excess to food pellets and water. The Guidance for Care and Use of Animals for Scientific Research [*Indian National Science Academy 2000*] was strictly followed. The animals were acclimatized for five days prior to the experiments.

Experiment Schedule

The animals were divided into four groups and caged separately. Group I consisting of 5 animals served as Negative Control Group and was maintained with normal diet and water *ad libitum* through out the study duration. Group II consisted of 10 animals which were given olive oil @ 2 ml/day/rat orally for 90 days study duration feeded. Group III was further divided into 3 sub groups with 45 animals each. These sub groups were fed with lindane dissolved in olive oil at a dosage of 100, 150 and 250 ppm/kg body weight/day respectively for a period of thirty days through a oral gavage attached to a syringe. Group IV comprising of 30 animals from each of Group III sub groups, was fed with 2mL of plant extract per day. Five animals from Group III and IV were sacrificed every ten days in the present investigation, while Group I was sacrificed at the end of 90 day study and Group II was sacrificed at the and of 30 day and 90 days study period.

Plant Extract

Dried Dried seeds of *Trigonella foenum* were purchased from local dealer. Fresh leaves of *Murraya koenigii* and *Polyalthia longifolia* were collected from Nirma University Botanical garden. The seeds and leaves washed with distilled water. For preparation of extract, air dried powdered seeds of *Trigonella foenum*, leaves of *Murraya koenigii* and *Polyalthia longifolia* was mixed with 60% ethanol in the ratio of 1:10 individually and was soxhlated at 70°C for 72 h. The individual extracts were then mixed in the ratio 2:2:1 to obtain the crude slurry.

Biochemical Studies

Sperm Count and Sperm Viability

The cauda epididymal sperm suspension was prepared in normal saline. The percent motility and count of cauda epididymal spermatozoa of normal and all treated groups of mice were determined by the method of Jeyendra et al (1984) and expressed as percentage motility and millions/ml respectively. The ratio of live: dead

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Table 1: Experimental Protocol

Group	Treatment and Dose	Duration (days)	No of animal treated	Day of Autopsy
Group I	Untreated	-------	05	Scarified at the end of 90 days
Group II	Olive oil @ 2 ml/day/rat orally	-------	10	Scarified at the end of 30 days & 90 days
Group III a	Lindane @100 ppm/kg body weight/day	30	45	11th, 21st & 31st
Group III b	Lindane @ 150 ppm/kg body weight/day	30	45	11th, 21st& 31st
Group III c	Lindane @ 250ppm/kg body weight/day	30	45	11th, 21st & 31st
Group IV a	Plant extract 2 ml/day/rat orally	60	30	41st, 51st, 61st, 71st, 81st & 91st
Group IV b	Plant extract 2 ml/day/rat orally	60	30	41st, 51st, 61st, 71st, 81st & 91st
Group IV c	Plant extract 2 ml/day/rat orally	60	30	41st, 51st, 61st, 71st, 81st & 91st

Table 2: Complete Body weight (g) and testis weight (g%/ body weight) of control and treated group animals.

Group	Complete Body Weight (g)	Testis (g%/ body weight)
Group I	280±4.1	0.46±0.03
Group II	275±3.8	0.45 ±0.02
Group III a	279±0.52	0.43±0.05
Group III b	278±0.39	0.48±0.03
Group III c	277±5.9*	0.37±0.05[a]
Group IV a	280±0.81	0.44±0.09
Group IV b	279±0.65	0.45±0.04
Group IV c	282±4.9*	0.40±0.07[a]

Values are mean ± SD. * no significant [a] P<0.001 Group I = untreated animals, group II= vehicle (olive oil) treated animals; Group III a, b, c = Lindane treated animals for 30 days; group IV a, b, c= Plant extract treated animals for a period of 60 days.

Table 3: Sperm Analysis and Sperm Functional Analysis of control and treated group animals.

Parameters		Group I	Group II	Group III a	Group III b	Group III c	Group IV a	Group IV b	Group IV c
Sperm Analysis	Sperm Count$^{\alpha}$	57.2±4.02	56.4±3.04	55.9±2.76	51.1±4.52	23.32±2.78[a]	54.8±2.15	56.1±2.05	53.0±2.54[a]
	Sperm Viability$^{\beta}$	98.3±2.18	97.9±2.35	94.8±3.87	90.8±2.84	15.4±3.84[a]	91.2±1.29	90.7±1.41	79.0±1.58[a]
Sperm Functional Analysis	AIT$^{\gamma}$	95.4±2.36	97.0±1.58	92.4±5.43	90.7±2.15	18.4±2.07[a]	91.8±3.16	89.7±2.36	70.6±2.4[a]
	HOS$^{\gamma}$	69.2±1.83	68.3±2.12	66.8±3.25	63.0±1.79	22.4±3.50[a]	70.1±1.95	68.4±1.79	55.0±2.54[a]
	NCD$^{\gamma}$	96.3±2.19	96.0±1.87	92.2±3.45	89.4±1.28	28.2±2.38[a]	91.4±1.48	92.5±3.05	70.8±1.92[a]

Values are mean ± SD. [a] P<0.001 Group 0 = untreated animals, group I= vehicle (olive oil) treated animals; Group III a, b & c= Lindane treated animals for 30 days; group IV a, b and c = Plant extract treated animals for a period of 60 days. α: million/mL, β: live sperm %, γ: normal %

Table 4: Seminal Plasma Biochemistry analysis of control and treated animals.

Parameters	Group I	Group II	Group III a	Group III b	Group III c	Group IV a	Group IV b	Group IV c
Protein$^{\alpha}$	10.23±0.34	11.03±0.28	10.17±0.17	11.2±0.81	7.28±0.56[a]	9.7±0.41	10.2±0.62	10.34±0.19[a]
LDH$^{\beta}$	2.70±0.98	2.77±0.83	2.73±0.20	2.79±0.54	3.57±0.43[a]	2.75±0.57	2.76±0.31	2.69±0.33[a]
Acid phosphatase$^{\gamma}$	20.67±1.68	21.91±1.79	22.01±1.86	21.86±1.46	16.57±1.53[a]	21.44±1.26	20.97±1.37	18.67±1.87[a]
Fructose $^{\delta}$	22.82±3.16	23.02±3.01	22.17±1.86	22.47±2.15	22.34±3.78[a]	22.01±1.86	23.04±2.45	21.46±5.90[a]

Values are mean ± SD. [a] P<0.001, Group I = untreated animals, group II= vehicle (olive oil) treated animals; Group III a, b, c = Lindane treated animals for 30 days; group IV a, b, c = Plant extract treated animals for a period of 60 days. α: mg/100 mg of tissue, β: IU/mg of protein, γ: KA/mg of protein, δ: mM

Table 5: Tissue biochemical analysis of testis in control and treated animals

Parameters	Group I	Group II	Group III a	Group III b	Group III c	Group IV a	Group IV b	Group IV c
Total Protein*	1.45±0.33	1.40±0.29	1.42±0.19	1.38±0.81	0.82±0.16[a]	1.32±0.74	1.34±0.66	1.28±0.01[a]
GSH$^{\alpha}$	224.53±5.08	220.89±4.92	218.7±3.62	215.2±4.76	126.4±6.8[a]	210.8±4.51	212.5±3.78	210.02±1.05[a]
MDA$^{\beta}$	NT	NT	0.03±0.01	0.04±0.02	2.92±0.02[a]	0.06±0.01	0.05±0.02	0.07±0.02[a]
Catalase$^{\gamma}$	35.89±2.05	38.91±3.19	37.3±2.08	36.4±3.54	9.25±0.83[a]	36.7±3.14	38.2±3.16	37.7±1.37[a]
SOD$^{\delta}$	33.85±1.21	32.73±1.93	32.18±2.05	33.05±0.91	8.73±0.6[a]	31.7±3.15	32.8±2.57	33.92±0.41[a]

Values are mean ± SD. [a] P<0.001, Group I = untreated animals, Group II= vehicle (olive oil) treated animals; Group III a, b, c = Lindane treated animals for 30 days; Group IV a, b, c = Plant extract treated animals for a period of 60 days. *: g%, α: mg/L, β: [10^{-5}] (mg/L), γ: H_2O_2 degraded/min/mg of protein, δ: units/mg of protein

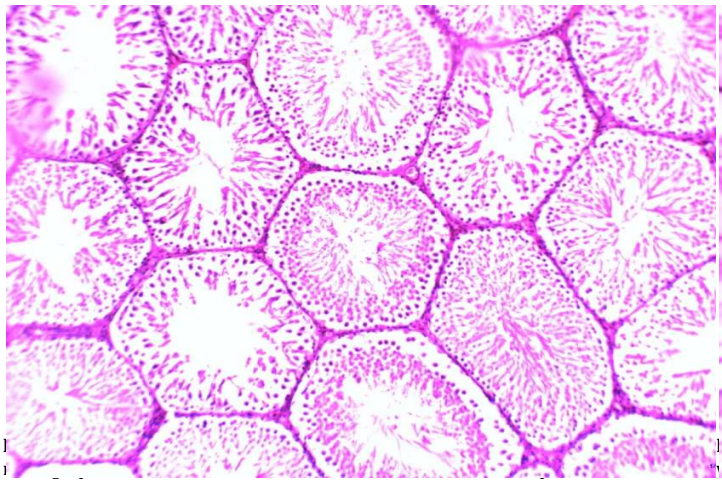
spermatozoa of control and all treated groups of mice were determined using eosin nigrosine staining given by WHO manual. [29] The number of stained and unstained spermatozoa was scored in 10-20 separate fields and percentages (%) were calculated.

Sperm Functional Analysis

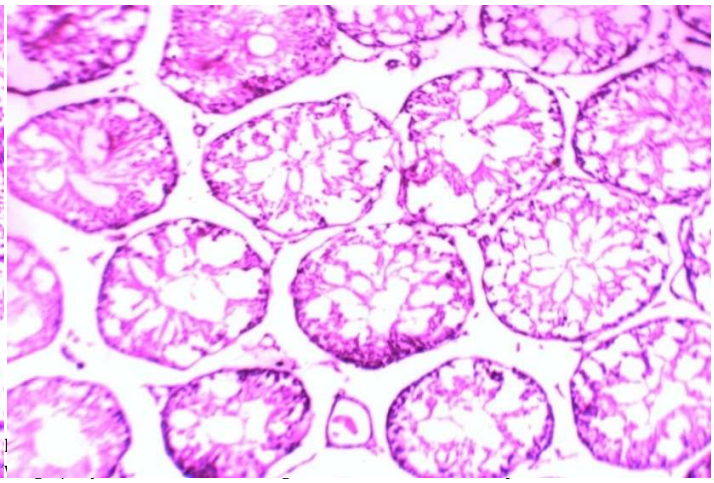
Spermatic fluid collected after the tearing of cauda epidydimis was used for the assessment of acrosome intactness test (AIT), [30] nuclear chromatin decondensation test (NCD) [31] and Hypo osmotic swelling test (HOS). [32]. The score above 50% in all functional test were considered normal and below the level were considered sub-fertile or infertile.

Seminal Plasma Biochemistry

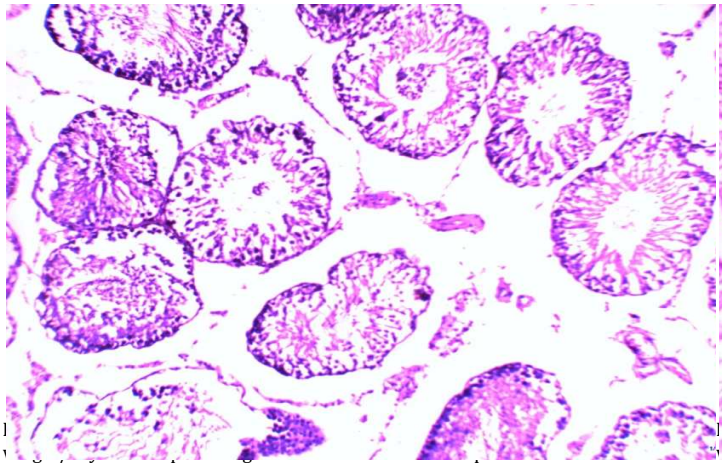
Sperm free seminal plasma was used for the quantitative determination of Fructose, [32] Protein, Acid Phosphatase, Lactate



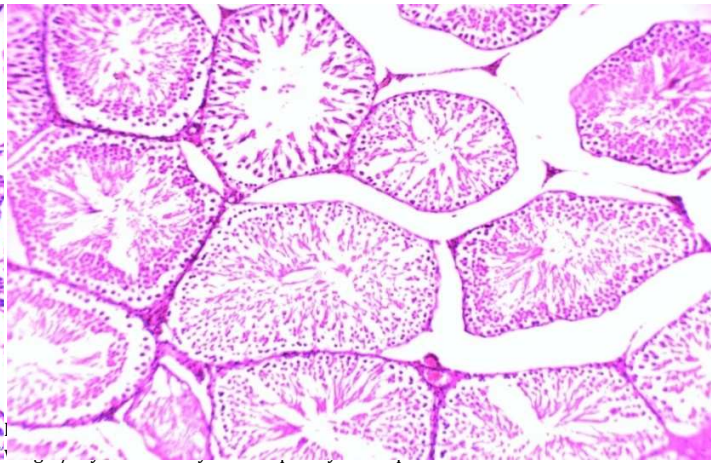
Internal arrangement was observed to be regular, while sperm accumulation (SA) was observed. ST was observed to be connected with interstitial space (IS) where endocrine cells called Leydig cells are located.



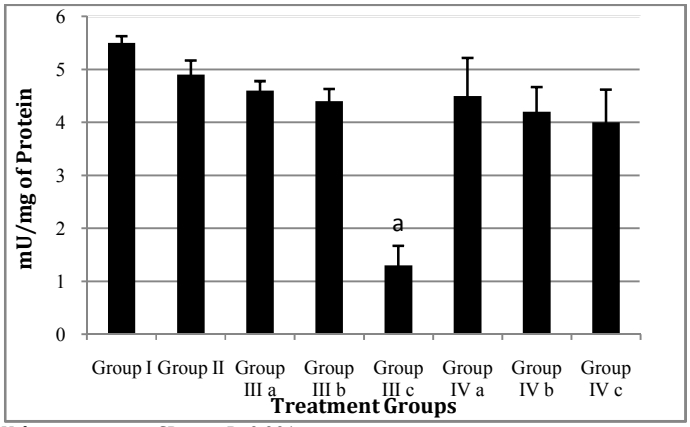
Treated Control.



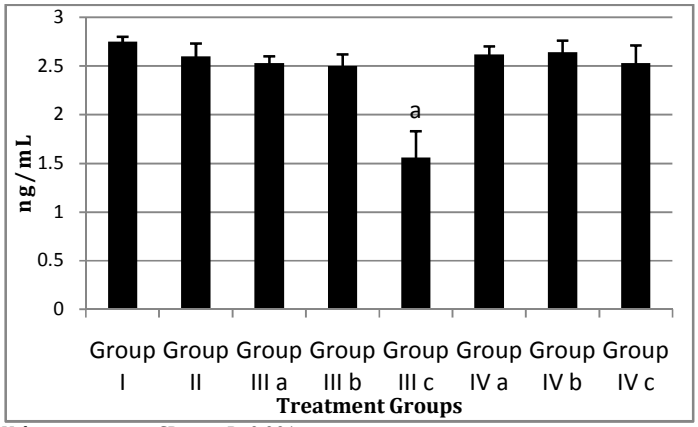
internal arrangement was also completely distorted. Leydig cells were observed to degenerate.



observed to be complete restored while the sperm accumulation was observed in the seminiferous tubules, almost comparable to that of the vehicle treated control animals.



Values are mean ± SD. a: P<0.001
Group I = untreated animals, Group II= vehicle (olive oil) treated animals; Group III a, b, c = Lindane treated animals for 30 days; Group IV a, b, c = Plant extract treated animals for a period of 60 days.
Figure5: GraphI:α-Glucosidase activity in control and treated animals



Values are mean ± SD. a: P<0.001
Group I = untreated animals, Group II= vehicle (olive oil) treated animals; Group III a, b, c = Lindane treated animals for 30 days; Group IV a, b, c = Plant extract treated animals for a period of 60 days
Figure 6: GraphII:Testosterone Levels in control and treated animals

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Dehydrogenase using (*Qualigen diagnostic kit, Mumbai, India*), and α-glucosidase (*Roche Applied Science, Germany*).

Histopathological Studies
Complete testis was removed, cleared of visible fat. The organ weight was taken and a small portion of testis was used for histological examination of control, following the treatment with Lindane and Plant extract. The tissues were cleared of the visible fats and were fixed immediately in Bouin's fluid for 24 hours, dehydrated in ethanol, cleared in benzene, infiltrated and embedded in paraffin wax. Five µm thick sections were cut and stained with Harris Hematoxylin and eosin.

Tissue Biochemistry
The remaining tissue was homogenized in phosphate buffer saline, centrifuged at 3500 rpm for the 15 min, the supernatant obtained was used for assaying tissue protein Qualigens Ltd. Mumbai, India, Malondialdehyde (MDA), [33], Reduced Glutathione (GSH), [34] Super oxide Dismutase (SOD) [35] and Catalase [36] activities of the vehicle treated control animals, lindane treated and Plant extract treated animals were estimated.

Testosterone Estimation
Testosterone was estimated using Chemiluminescent Immunoassay Abbott Architect, USA.

Statistics
For all biochemical estimations, a minimum of 8 to 10 replicates were done for each parameter. The data were statistically analysed using student's t test and Analysis of Variance ANOVA) followed by Scheffe's test for least significance. The P value determined was in comparison to control group II.

RESULT
Body Weight and Organ Weight
No physiological change in the complete body weight of any of the treated group was observed. Treatment with the lindane, a decline in the testis weight was observed in group III c ($p<0.001$) after the treatment 30 days in comparison to that of the group I, II, III a and III b. Following treatment with plant extract a gradual recovery was observed in group IV c ($p<0.001$) almost comparable to that of the control group II. Surprisingly, Group III a and b did not show any significant changes in their body and organ weight (Table 2).

Sperm Count and Viability
The sperm count in the cauda epididymis of group III c declined significantly ($P<0.001$) compared other groups. Treated group III a and b showed marginal drop in the sperm count. All the altered values recovered in group IV animals. The sperm viability data also showed similar trend (Table 3).

Sperm Functional Analysis
There were no changes observed in any of the sperm functional analysis in any of the study groups expect the Group III c. Group III c showed significant decline in the acrosome intactness test, hypo osmotic swelling test and nuclear chromatin decondensation test suggesting severe functional damage to the sperm. Significant recovery was observed in both HOS ($P<0.001$) and NCD ($P<0.001$) within a period of 60 days while AIT also showed gradual recovery comparable to that of the Group II (Table 3).

Semen Biochemistry
Lindane treatment @ 250 ppm/kg body weight resulted in very significant decrease in seminal plasma biochemical parameters viz., protein, lactate dehydrogenase C_4, Prostate specific Acid phosphatases activity, Fructose and seminal vesicle specific α-Glucosidase ($P<0.001$). while other treated groups ie., Group II, III a and b did not elicit any changes whatsoever. The altered parameters was again restored to almost comparable to that of the control of group I following 60 days of plant extract treatment (Table 4).

Histopathological Studies
The histology of testis was performed for Group II, Group III b, c and Group IV. The micrograph of testis in control group II demarcates the normal arrangement of the internal structure of the Sertoli cells in the seminiferous tubules. The tubules were observed to be connected through interstitial space containing endocrine cells, Leydig cells. Sperm accumulation was observed in the seminiferous tubules (Figure 1).

Following the lindane treatment @ 150 ppm/kg body weight/day (group III b) for a period of 30 days, did not result in complete degeneration of the interstitial space but there was evidence of initiation of degeneration of Leydig cell. The arrangement of Sertoli cells was also slightly distorted and there was focal degeneration observed. As compared to that Group III c (250 ppm/kg body weight/day) showed significantly distorted seminiferous tubules and in addition, the Leydig cells were completely absent and there was no visible sperm accumulation within the seminiferous tubules. (Figure 2 & 3).

A gradual restoration of the testicular arrangement was observed in animals following a plant extract treatment. During the 30 days of the plant extract treatment (group IV), re-appearance of the connecting tissue was observed in interstitial space indicating regeneration of the Leydig cells. The arrangement Sertoli cells in the seminiferous tubules were also observed to partially re-arranged compared to that of group II. Sperm accumulation was observed to initiate after 30 days of PE treatment indicating a speedy recovery (Figure not shown).

After the completion of the 60 days of the plant extract treatment the micrograph was almost comparable to that of the vehicle treated control group. The micrograph depicts almost normal arrangement of the interstitial space consisting of connective tissue, Sertoli and Leydig cells were observed to be almost comparable to that of the control group (Figure 4).

Tissue Biochemistry
The histological observations were confirmed by the tissue biochemical parameters. Following lindane treatment (group III c), a significant decline in the protein content of the tissue was noted. The activity of the oxygen free radical scavenging enzymes (OFR) viz. Super oxide dismutase and catalase were also observed to decline. The concentration of the reduced glutathione was also noted to vary significantly in group III c. In contrast, the levels of the malondialdehyde was noted to escalated while the concentration of malondialdehyde was below the detection limit in Group I, II, III a and b.

Following the plant extract treatment (group IV), gradual recovery was observed in all the altered parameters (Table 5).

Testosterone
There was a slight non significant drop in the testosterone levels in Group III a and b but Group III c showed a significant decline as compared to the Vehicle treated group. The levels recovered in the plant extract treated animals, almost comparable to that of the control groups (Graph II).

DISCUSSION
The present study was performed to explore the effects of the lindane and possible ameliorative role of methanolic extract of leaves of *Polyalthia longifolia* and *Trigonella foenum* and seeds of *Murraya koenigii* on the reproductive organs and functions.

Lindane is one of the environmental chemicals known to have an endocrine effect and has been associated with male reproductive alterations. [37-42] The mechanism of action of lindane on male reproductive system remains unclear. [43] During the lindane treatment, decline in the testicular mass was observed. Similar resulted were also reported by Beard and Rawling. [44] No notable difference in the body mass of the rat was observed during the lindane exposure also reported by Saradha and Mathur. [43] However other articles had reported a decline in the body mass following the lindane treatment. [9] Thus in the present experiment, shrinkage of

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testis was noted but no physiological difference was observed in the complete body mass.

A dropping off in the epididymal sperm count was pragmatic following the lindane treatment which too was also reported by Prasad et al. [39] Sperm viability was also observed to diminish along with damaged sperm. This reveals induction of infertility in male rats due to lindane treatment. An analogous result was also observed by Fausto et al in male rabbits. [45]

In acrosome intactness test (AIT), there was considerable decapitation of the sperm head. Nuclear decondensation (NCD) assay divulged a significant decline in the stability of the chromatin material in the sperm during lindane treatment. Hence the sperm observed to be normal in AIT, but has nuclear instability indicating disturbance in the sperm maturation process. Hypo osmotic (HOS) analysis in lindane treated animal noted to have a significant decline in motility of the sperm. Thus semen analysis and sperm functional analysis reveals that lindane treatment induces infertility and sperm produced are not properly matured viz. sperm produced are damaged, which goes in agreement with Samanta and Chainy. [46]

In the present study, the levels of protein in cauda epididymides showed a significant decrease after 30 days of lindane treatment. This decrease might be due to mutilation of protein metabolism/synthesis.

LDH-C_4, an isoenzyme associated with testis, routinely used as marker for determining proper functioning of the reproductive organs. [47] An increase in the LDH-C_4 value indicates that the sperm mitochondrial activity has declined; indicating improper functioning of the sperm and maturation. Semen biochemistry analysis showed an increase in activity of LDH-C_4 during lindane treatment, revealing obstruction in the functioning of mitochondria. This may be due to improper maturation or high oxidative stress in the cauda epididymides as reported by Saradha and Mathur. [43]

Acid phosphatase (ACP) and fructose both are associated sperm motility and are secreted by the accessory sex glands. [48]. Activity analysis of ACP revealed a decline during lindane treatment, revealing inappropriate environment for motility of the sperm which was also observed during HOS analysis.

The estimation of α-glucosidase activity in semen is widely studied as marker for epididymal function. [49]. Epididymal α-glucosidase does not play a crucial role in the development of sperm fertilizing capacity, but is involved in the providing optimum condition for sperm storage. [50] In the present study, a dwindle in the activity was noted in the lindane treated animals, indicating less number of sperm produced as mentioned earlier. The decrease in the enzyme activity has been in observed non-azoospermic men. [51]

Thus treatment of lindane in rats, the functioning of male reproductive organ is complete hampered and the male rats become oligo-necro-zoospermic. Treatment with lindane not only affects the sex organ but also hampered the functions of the accessory sex organs.

During treatment with the lindane, profound testicular changes were examined. The arrangement of Sertoli cells in the seminiferous tubules was completely damaged. This indicates that the Sertoli cells located in tubules are damaged, as effect of which sperm generation and maturation is also hampered, which was noted during studying semenolgy. No sperm accumulation was observed indicating dysfunction of the Sertoli cells. The interstitial space was observed to be damaged. Hence Leydig cells located in the interstitial space were degenerated, which can also be confirmed by reduction in the testosterone levels in the serum. Results obtained in this study were also reported by other articles. [52-54] A complete contradictory result was reported by Traina et al [55] indicating increase in the size of the Leydig cells due to lindane treatment. The exact mechanism of Leydig cells degeneration due to lindane treatment is yet unknown but increase in the oxidative stress is believed to be one of the major factors.

The antioxidant system plays an effective role in protecting testes and other biological tissues below a critical threshold of reactive oxygen species thus preventing testicular dysfunction. [56] Following the lindane treatment, the protein content of the tissue was observed to decline along with shrinkage of testis. The activity of the antioxidant enzymes viz. catalase and super oxide dismutase were noted to decline following the lindane treatment. The concentration of the reduced glutathione was also noted to decline in lindane treated animals. Thus the decline in the anti-oxidant parameters reveals inclination in the ROS generation in the tissue, which is also justified by inclination in the MDA concentration in the tissue. A similar result was also reported by Sujatha et al. [57] GSH, MDA and SOD levels following Quinolphos treatment and its subsequent reversal by *Withania somnifera* and *Aleo barbedensis* has also been reported in similar studies carried out on liver and kidneys. [58]

The plant extract treatment in lindane treated animals showed significant recovery. A significant recovery or restoration of the sperm and sperm function was noted at the completion of the plant extract treatment, comparable to that of the vehicle treated control animals. The viability of the sperm and sperm density were observed to be restored, almost comparable to that of the vehicle treated control group. Sperm functional analysis parameters were also noted to be almost comparable to vehicle treated control animals.

Seminal biochemistry had revealed a significant recuperation during the plant extract treatment. The activity of LDH-C_4 was noted to decline compared to that of lindane treated animals, almost comparable to that of the control; revealing the improvement in the mitochondrial function as well as reduction in the oxidative stress in cauda epidydymides.

ACP activity was also observed increase during the course of plant extract treatment almost comparable to that of the vehicle treated control animals group; indicating healing effect on the accessory sex organs.

The activity of the α-glucosidase was observed to almost equate to that of the vehicle treated control animals group at the completion of 60 days of plant extract treatment period. The result generated revealed the proper functioning of brush borders of cauda epidydymides, bestowing the optimum condition for sperm storage.

Thus treatment with the plant extract reinstated the functioning of reproductive organs and accessory sex organs; almost comparable to that of control. Due to the treatment the oligo-necro-zoosermic rats were observed to produce normal sperm and fertility was observed to be restored. Thus plant extract treatment for a period of 60 days has shown ameliorative effect for damaged sperm maturation and reproductive organs functioning.

A gradual and slow re-development of testis was noticed following the plant extract treatment. On completion of the treatment, the arrangement of the Sertoli cells in the seminiferous tubules was almost comparable to that of the vehicle treated control animals. Accumulation of the sperm was also observed in the seminiferous tubules, whereas semenolgy studies revealed that sperm produced were duly matured indicating the proper functioning of the Sertoli cells, which were damaged during the lindane treatment. The interstitial space was also observed to re-develop following the plants extract treatment with regeneration of the Leydig cells. The levels of the testosterone in the serum were also observed to increase following the plant extract revealing a apt functioning of the Leydig cells.

During the plant extract treatment the protein content in the tissue was noted to incline along with the tissue mass, almost comparable to that of the vehicle treated animals. The activities of the antioxidant enzymes viz. catalase and superoxide dismutase were observed to decline. The

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concentration of the reduced glutathione was also noted to increase during the treatment course. The concentration of the MDA was also observed to reduce. Thus plant extract treatment had diminishing effect on the oxidative stress enhanced due to lindane treatment. The antioxidant activity of the extract may be due euchrestine B, bismurrayafoline E, mahanine, mahanimbicine, and mahanimbine from *Murraya koenigii* [59] and trigonelline from *Trigonella foenum.* [24]

CONCLUSION

Thus, in nutshell, lindane treatment has an explicit effect on reproduction. Lindane treatment induces oligo-necro-zoospermic male rats. Functioning of the reproductive organs and accessory sex organs was also hampered. Oxidative stress was also observed to increase in the tissue. Following the plant extract treatment, a significant recovery was observed. The reproductive organs functions aptly producing functionally matured sperms. The functioning of the accessory sex organs was also restored. Oxidative stress was noted to decline thus preventing the tissue from ROS damage.

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Acknowledgement: The authors are grateful for the financial assistance from Nirma Education & Research Foundation (NERF), Ahmedabad for the present investigation. The present investigation is a part the Dissertation Thesis of Mr. Ankit Kumar Jain and Mr. Bandish Kapadia submitted to Nirma University, Ahmedabad.