

Research Article

## Triterpenoids of *Nerium oleander* shows Antifertility effect in Male Albino Rats

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**Abstract:** Antifertility activity of methanolic extract of the stem of *Nerium oleander* was carried out on male albino rats. After the careful experiments, the results revealed that oral administration of methanolic extract of the stem of *Nerium oleander* affected male fertility by two means, antispermatogenic and antiandrogenic action. It might be due to the presence of some triterpenoids compounds. So it could be a scope of developing an ideal male contraceptive, due to its antispermatogenic effects.

**Keywords:** *Nerium oleander*, Methanolic extract, Triterpenoids, Antifertility.

### 1. Introduction

Over a long period, several approaches for the induction of infertility have been investigated. However, no suitable method has emerged that is effective and free from side-effect [1,2]. Although contraceptives containing estrogen and progesterone are effective and popular. The risks associated with these drugs have paved the way to develop newer compounds from medicinal plants. Hence, there is a need for searching suitable products from indigenous medicinal plants that could be effectively used in the place of contraceptive pills. Compounds isolated from *Nerium oleander* have been reported for antimicrobial activity [3], anti-inflammatory activity [4], anticancer activity [5,6], CNS-depressant activity [7] and neuroprotective effects [8]. Effect of the sap of *Nerium oleander* on male fertility and spermatogenesis in tobacco budworm (*Helicoverpa assulta*) [9] motivate toward the antifertility activities. So, this study is preliminarily concerned with antifertility activity of dry methanol extract of the stem of *Nerium oleander* on male albino rats.

### 2. Material and Methods

#### 2.1 Animal model

For this study, colony bred, healthy, fertility proven adult Wistar rats (*Rattus norvegicus*) of 60 days aged were selected. Before using the animals for experiments, their initial body weights were recorded and blood samples were examined. Supervision of qualified veterinarian was available throughout the

study. The animals were kept in plastic cages and well-ventilated room under controlled conditions (12 hrs light: 12 hrs dark) [10]. The rats were maintained on pellet standard rat feed supplemented with soaked gram, wheat and water were provided. Two experiments were conducted during the course of study with dry methanol extract of the stem of *Nerium oleander*. A suspension was prepared daily (100 and 200mg/ml) before administration. The required drug was administered orally for a period of 60 days.

#### 2.2 Plant material

Plant material of *Nerium oleander* was collected from the roadside lining, Jaipur (India). It is abundantly available in this part of the country.

#### 2.3 Isolation of marker

*Nerium oleander* stem was collected from the roadside lining, Jaipur (India). Herbarium, Department of Botany, University of Rajasthan, Jaipur, India had authenticated and identified this Plant material. 1.0 kg Plant material was crushed then, shocked in methanol overnight, boiled exhaustively for 24 hours and finally filtered. The filtrate was concentrated under the reduced pressure and obtained (65gm) methanol extract of this plant for the experiment. A part of this extract (solvent-free) was fed to rats at 100 and 200mg/kg body weight per day [11].

#### 2.4 Tests for the presence of triterpenes

On treatment with Liebermann-Burchard and Noller's reagents fraction [petroleum ether: benzene (1:1)] gave a positive test showing its triterpenoid

nature. It also gave a positive test with TNM, which confirms that the given fraction is unsaturated triterpenoids.

## 2.5 Treatment protocol for antifertility activity study

Methanolic extract were orally fed to know about its hormonal nature and antifertility effect. Animals were distributed into two treatment groups, each consisting of 6 animals.

**Group-A:** Only sterile distilled water were given orally for 60 days to animals of this group. This group served as a control-treated vehicle.

**Group-B:** Animals of this group were orally fed with methanol extract at the dose of 100mg/kg body weight per day, for 60 days. Doses were freshly prepared and administered orally during the study.

**Group-C:** Animals of this group were orally fed with extract at the dose of 200mg/kg body weight per day for 60 days.

A suspension of the triterpenoids was made every day in DMSO and the required drug was administered orally with a glass syringe fitted with a feeding needle.

## 2.6 Study parameters

### 2.6.1 Sperm motility and density

For determining sperm motility and sperm density, 100mg of cauda epididymis was minced in 1ml of physiological saline within a scarification period of 5 minutes. The motility percentage was determined by counting motile and immotile spermatozoa per unit area [12]. Next cauda epididymis sperm density was determined by routine procedure and expressed as million/mm<sup>3</sup> of suspension [12].

### 2.6.2 Fertility test

Successful mating was carried out in all animals (male-female ratio 1: 2). The mated females were allowed to complete the gestation period. Five days prior to sacrifice period, numbers of pups were recorded. After this litter size and percent of fertility were calculated [11].

### 2.6.3 Body and organ weights

The initial and final body weight of the animals were recorded. Then the testes, epididymis, seminal vesicle and ventral prostate were dissected out and weighed accurately up to milligram level.

### 2.6.4 Serum biochemistry

Serum was isolated and stored for the detection of protein content [13], total cholesterol [14], phospholipids [15], alkaline phosphatase [16] and LDH [17] by respective calculations.

### 2.6.5 Hormone assay

Blood samples were collected for estimation of serum testosterone, FSH and LH by using radioimmunoassay. Serum samples were separated by standard procedures and stored at -20°C for subsequent analysis. Serum levels of testosterone, FSH and LH were assayed in duplicate by using radioimmunoassay kit [18].

### 2.6.6 Hematology

The blood samples from the heart were also collected and analyzed for blood urea [19], blood sugar [20] RBC, WBC and hematocrit levels [21].

### 2.6.7 Histological Study

Contralateral side of the testis, epididymis, seminal vesicle, kidney and liver were fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut at 6 $\mu$  was stained with Harris's hematoxylin and eosin to observe under a light microscope. The same sections were used for percentage of normal tubules. Similarly, seminiferous tubules and intertubular areas were observed, finally expressed of seminiferous tubules, intertubular area and sertoli cell nuclear area [22] was also determined.

### 2.6.8 Statistical Analysis

Data are expressed as mean  $\pm$  S.E. and analyze for statistical significance by using student's "t" test. The data are considered as significant at  $p \leq 0.01$  and non-significant at  $p \leq 0.001$  [23].

## 3. Results

### 3.1 Body and organ weight

The weight of testes, epididymis, vas deferens and seminal vesicle of rats treated with methanol extract of the stem of *Nerium oleander* at the dose level of 100mg/kg body weight (Group-B) ( $p \leq ns$ ) was non-significantly changed while treatment at the dose level of 200mg/kg body weight (Group-C) weight of testes significantly reduced ( $p \leq 0.001$ ) as compared to control intact rats (Group-A) (Table 1). The weight of ventral prostate, kidney, heart, liver and adrenal gland did not alter at any significant level in rats following methanol extract of the stem of *Nerium oleander* treatment (Group-B and C) as compared with control rats (Group-A) (Table 1).

### 3.2 Sperm motility and density

The sperm motility of cauda epididymis and sperm density in cauda as well as testes of rats after treatment of methanol extract of the stem of *Nerium oleander* for 60 days at the dose level of 100mg/kg body weight (Group-B) were changed non-significantly ( $p \leq ns$ ) while treatment at the dose of 200mg/kg body weight (Group-C) sperm motility and density were decreased

significantly as compared to control (Group-A) (Table 2).

### 3.3 Fertility

It was observed that the fertility was significantly ( $p \leq 0.001$ ) reduced in methanol extract of the stem of *Nerium oleander* treated rats at the dose level of 100mg/kg body weight and 200mg/kg body weight (Group-B and C) respectively by 77.77% & 46.66% (Table 2).

### 3.4 Hematological observations

The hematological parameters, i.e. hemoglobin, W.B.C., R.B.C, hematocrit, MCHC, blood sugar and blood urea were observed within normal range ( $p \leq ns$ ) in rats after 60 days treatment with methanol extract of the stem of *Nerium oleander* in all groups (Group-B and C) as compared to control rats (Group-A) (Table 3).

### 3.5 Serum biochemistry

Changes in protein, cholesterol, triglycerides and phospholipids level in serum of all treated rats at dose levels (100 and 200mg/kg body weight), were non-significant ( $p \leq ns$ ). Observation in all the hematological indices and serum biochemistry parameters are free from side effects (Table 4).

### 3.6 Hormone levels

The levels of luteinizing hormone, follicle-stimulating hormone and testosterone hormones were significantly decreased ( $p \leq 0.001$ ) at the dose of 200mg/kg body weight of methanol extract of the stem of *Nerium oleander* (Group-C) as compared to control rats (Group-A), while LH level in other groups (Group-B) were non-significantly changed after treatment ( $p \leq ns$ ) (Table 4).

### 3.7 Tissue biochemical analysis

The protein level of testis, cauda, seminal vesicle in rats at the dose level of 100mg/kg body weight (Group-B) was non-significantly ( $p \leq ns$ ) change while protein level in rats treated at the dose levels of 200mg/kg body weight (Group-C) was significantly decreased ( $p \leq 0.001$ ) as compared to protein level of control intact rats. The protein level of ventral prostate was non-significantly ( $p \leq ns$ ) change in all treated groups as compared to protein level of control intact rats (Table 5).

It was observed that cholesterol level in adrenal was significantly decreased ( $p \leq 0.001$ ) at the dose level of 100 and 200mg/kg body weight (Group-B and C) in dose-dependent manner as compared to cholesterol level in testis of control intact rats (Group-A). Cholesterol levels of testes, liver and heart were non-significantly ( $p \leq ns$ ) change in all treated groups as compared to cholesterol level of control intact rats (Table 5).

The glycogen content of rats in testis was decreased significantly at the dose level of 200mg/kg body weight (Group-C) while glycogen contents were non-significantly reduced ( $p \leq ns$ ) at the dose level of 100mg/kg body weight (Group-B) as compare to glycogen level of control intact rats. The glycogen levels of liver and heart were non-significantly ( $p \leq ns$ ) change in all treated groups as compared to cholesterol level of control intact rats (Table 5).

The fructose level of seminal vesicle in rats treated at the dose level of 100 and 200mg/kg body weight (Group-B and C) was decreased non-significantly ( $p \leq ns$ ) as compared to control intact rats (Group-A) (Table 5).

Table 1: Changes in the body and various organ weight of rat after 60 days of treatment of dry methanolic extract of Kaner (*Nerium oleander*).

Treatment	Initial b. wt. (gm.)	Final b. wt. (gm.)	Testes mg/100gm b. wt.	Epididymides mg/100gm b. wt.	Vas Deferens mg/100gm b. wt.	Seminal vesicle mg/100gm b. wt.	Ventral prostate mg/100gm b. wt.	Kidney mg/100gm b. wt.	Heart mg/100gm b. wt.	Liver mg/100gm b. wt.	Adrenal mg/100gm b. wt.
<b>Group-A</b>											
Control	103.33±2.10	165.00±1.82	1091.39±23.18	473.62±11.05	153.31±2.33	400.53±23.25	95.84±2.57	567.66±16.49	272.84±12.76	2355.29±38.72	19.39±0.56
<b>Group-B</b>											
100mg/kg b. wt.	103.33±2.11	166.67±2.11	1065.11±17.55 <sup>ns</sup>	455.57±10.13 <sup>ns</sup>	146.18±1.80 <sup>ns</sup>	359.80±9.09 <sup>ns</sup>	84.87±3.25 <sup>ns</sup>	559.90±17.66 <sup>ns</sup>	265.10±11.15 <sup>ns</sup>	2325.69±44.51 <sup>ns</sup>	17.71±0.35 <sup>ns</sup>
<b>Group-C</b>											
200mg/kg b. wt.	105±2.23	173.33±1.66	971.87±27.00*	421.43±11.87*	136.68±4.14*	315.38±13.50*	83.96±3.48 <sup>ns</sup>	536.97±18.75 <sup>ns</sup>	258.70±12.76 <sup>ns</sup>	2237.36±38.95 <sup>ns</sup>	17.50±0.32 <sup>ns</sup>

Data exposed as Mean ± S.E, ns = non-significant, \* Significant ( $p \leq 0.01$ ), \*\* Highly Significant ( $p \leq 0.001$ ).

**Table 2: Effect on sperm motility, density and fertility after treatment of dry methanolic extract of Kaner (*Nerium oleander*) in male rats.**

Treatment	Sperm motility %	Sperm density		No. of Pups delivered	Fertility %
		Cauda (million/mm <sup>3</sup> )	Testes (million/mm <sup>3</sup> )		
Group-A Control	72.32±0.62	14.72±0.18	2.97±0.06	45	100 %
Group-B 100mg/kg b. wt	69.96±0.78 <sup>ns</sup>	14.11±0.20 <sup>ns</sup>	2.68±0.11 <sup>ns</sup>	35	77.77 %
Group-C 200mg/kg b. wt	62.14±0.58*	13.97±0.07*	2.35±0.12*	21	46.66 %

Data exposed as Mean ± S.E, ns = non-significant, \* Significant ( $p \leq 0.01$ ), \*\* Highly Significant ( $p \leq 0.001$ ).

**Table 3: Blood biochemical changes in control and dry methanolic extract of Kaner (*Nerium oleander*) treated male rats.**

Treatment	HB %	WBC th/mm <sup>3</sup>	RBC mill/cu.mm	Hematocrit %	MCHC gm/dl	Blood Sugar mg/gl	Blood urea mg/dl
Group-A Control	12.96±0.57	11.07±0.28	5.54±0.10	38.59±2.58	34.50±3.09	96.66±2.70	26.67±1.67
Group-B 100mg/kg b. wt.	13.00±0.36 <sup>ns</sup>	11.36±0.29 <sup>ns</sup>	5.58±0.07 <sup>ns</sup>	39.68±1.71 <sup>ns</sup>	33.01±1.42 <sup>ns</sup>	98.56±2.52 <sup>ns</sup>	29.33±1.68 <sup>ns</sup>
Group-C 200mg/kg b. wt.	12.96±0.56 <sup>ns</sup>	11.86±0.38 <sup>ns</sup>	5.46±0.19 <sup>ns</sup>	38.58±2.57 <sup>ns</sup>	34.49±3.09 <sup>ns</sup>	96.66±2.70 <sup>ns</sup>	26.66±1.68 <sup>ns</sup>

Data Exposed as Mean ± S.E, ns = non-significant, \* Significant ( $p \leq 0.01$ ), \*\* Highly Significant ( $p \leq 0.001$ ).

**Table 4: Serum biochemical changes in control and dry methanolic extract of Kaner (*Nerium oleander*) treated male rats.**

Treatment	Protein gm %	Cholesterol mg/dl	Triglycerides mg/dl	phospholipids mg%	Testosterone ng/ml	LH mIU/ml	FSH mIU/ml
Group-A Control	10.86±0.58	125.00±7.21	133.28±0.48	123.81±5.35	2.09±0.08	3.38±1.90	4.80±0.16
Group-B 100mg/kg b. wt.	10.76±0.40 <sup>ns</sup>	131.25±5.35 <sup>ns</sup>	133.28±0.47 <sup>ns</sup>	124.99±2.60 <sup>ns</sup>	1.82±0.06 <sup>ns</sup>	2.83±0.10 <sup>ns</sup>	4.33±0.15 <sup>ns</sup>
Group-C 200mg/kg b. wt.	10.84±0.58 <sup>ns</sup>	125±7.21 <sup>ns</sup>	133.28±0.47 <sup>ns</sup>	123.80±5.35 <sup>ns</sup>	1.61±0.07*	2.35±0.14*	3.55±0.16*

Data exposed as Mean ± S.E, ns = non-significant, \* Significant ( $p \leq 0.01$ ), \*\* Highly Significant ( $p \leq 0.001$ ).

**Table 5: Tissue biochemical analysis after the treatment of dry methanolic extract of Kaner (*Nerium oleander*), in male albino Rats.**

Treatment	Protein mg/gm				Cholesterol mg/gm				Glycogen mg/gm			Fructose mg/gm
	Testes	Cauda	Seminal Vesicle	Ventral prostate	Testes	Liver	Heart	Adrenal	Testes	Liver	Heart	Seminal vesicle
Group-A Control	380.70±7.75	343.67±13.51	329.60±13.10	231.83±27.72	12.60±0.46	15.20±0.67	15.41±0.41	8.85±0.69	3.11±0.16	4.98±0.34	3.88±0.04	7.77±0.97
Group-B 100mg/kg	334.78±14.81 <sup>ns</sup>	292.56±17.68 <sup>ns</sup>	274.04±15.25 <sup>ns</sup>	199.98±22.68 <sup>ns</sup>	11.04±0.34 <sup>ns</sup>	16.14±0.46 <sup>ns</sup>	16.14±0.37 <sup>ns</sup>	6.14±0.37*	2.46±0.17 <sup>ns</sup>	4.77±0.25 <sup>ns</sup>	3.96±0.05 <sup>ns</sup>	4.57±0.73 <sup>ns</sup>
Group-C 200mg/kg	324.41±14.28*	278.49±9.57*	282.19±6.56*	151.09±5.50 <sup>ns</sup>	9.47±0.91 <sup>ns</sup>	16.25±1.71 <sup>ns</sup>	16.25±1.59 <sup>ns</sup>	6.35±0.10*	2.11±0.24*	4.38±0.19 <sup>ns</sup>	3.59±0.17 <sup>ns</sup>	4.51±0.55 <sup>ns</sup>

Data exposed as Mean ± S.E, ns = non-significant, \* Significant ( $p \leq 0.01$ ), \*\* Highly Significant ( $p \leq 0.001$ ).

### 3.8 Histological observations

Photomicrograph of control rats (Group-A) testes showing well-developed structure of highly convoluted seminiferous tubules lined by a stratified germinal epithelium, which contained all spermatogenic cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid and mature spermatozoa) and sertoli cell with their distinctive nuclei present in the basal lamina. The spermatogonia can see close to the basal lamina. The seminiferous tubules are well developed and supported by loose connective tissue containing leydig cells, blood vessels and nerves. Photomicrograph of testis of rats treated at the dose

level of 100mg/kg body weight of methanol extract of the stem of *Nerium oleander* show testicular lesions and degenerative changes in germinal epithelium of seminiferous tubules, number of spermatids and spermatozoa was reduced and lumen is devoid of mature sperms. At the dose level of 200mg/kg body weight of methanol extract of the stem of *Nerium oleander* treatment, spermatogenesis was completely arrested and atrophied in treated rats. Cellular debris appears in the lumen is normal, seminiferous tubules were reduced and intertubular in between seminiferous tubules space increase (Group-B and C) (PLATE-1).



PLATE 1: Testis. Fig. 1: Photomicrograph shows normal histoarchitecture of testis, seminiferous tubules contain all the stages of spermatogenesis from spermatogonia to spermatozoa. Spermatozoa were clearly visible in the lumen. Sertoli cell in tubules in interstitial tissue and leydig cell also seen (200 X HE).

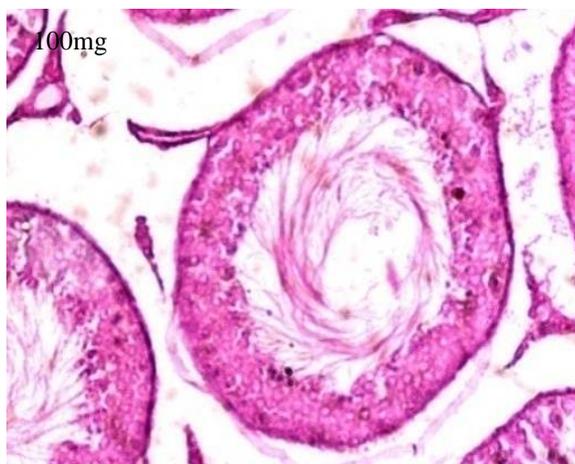


PLATE 1: Testis. Fig. 2: Photomicrograph of testis of rats treated with *Nerium oleander* stem at 100mg/kg body weight for 60 days shows degenerative changes in spermatogenesis, inhibition of primary spermatocyte stage and spermatozoa (200 X HE).

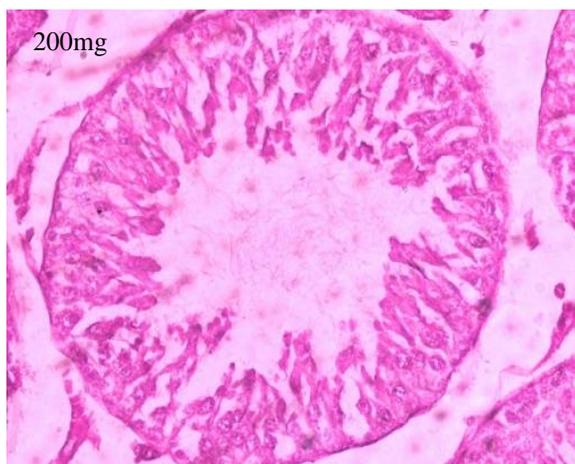


PLATE 1: Testis. Fig. 3: Photomicrograph of testis of rats treated with *Nerium oleander* stem at 200mg/kg body weight for 60 days shows reduced number of spermatids and spermatozoa, clearly visible cellular debris in lumen and lumen was devoid of sperm (200 X HE).

#### 4. Discussion

This study was conducted to elucidate the effect of the association of biological activities with triterpenoids from *Nerium oleander* on male reproductive system. The result of this investigation demonstrated that the triterpenoids interfere with the structure and function of major elements of male fertility as reflected by a marked decrease in the rate of fertility. The plant-based contraceptive, inhibit male fertility after administration of (200mg/kg body weight) methanolic extract of *Nerium oleander* exhibited a marked reduction in counts and motility of cauda epididymal sperms in dose-dependent manner. The reduction of sperm density is confirmed by histological and hormonal investigation of testis and serum of the animals. The testes of the treated animals revealed the arrest of spermatogenesis. Size of seminiferous tubules appeared to be reduced. Vacuolization was observed in the sertoli cells, spermatogonia and spermatocytes. The significant decrease in sperm motility, density, weight of testis, epididymis and seminal vesicle may occur due to significant reduction of testosterone, luteinizing, and follicle-stimulating hormone. After treatment of methanolic extract of *Nerium oleander*, there is no significant change was observed in serum biochemistry and hematological parameters of treated group of animal which showed triterpenoids are free from side effects. However, the treatment resulted in the reduction of fertility and number of pups, indicating the fertilization might be due to stored epididymal sperms. It is histologically evident that the treatment arrested spermatogenesis.

#### 5. Conclusion

The oral administration of triterpenoids from *Nerium oleander* at 100 and 200mg/kg body weight in male albino rats did produce antifertility effects. The results revealed that oral administration of methanol extract of the stem of *Nerium oleander* affected male fertility in two ways, antispermatogenic and antiandrogenic action. From the foregoing account, it can be conjectured that methanol extract of the stem of *Nerium oleander* could be a scope of developing an ideal male contraceptive, due to its antispermatogenic effects and no untoward side effect on the general health of the animal.

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**Conflicts of Interest:** The author declares no conflict of interest.

#### References

- [1]. Ma, W.K. & Ramaswamy, S.B. (1987). Histological changes during ovarian maturation in the tarnished plant bug, *Lygus lineolaris* (Palisot de beauvois) (Hemiptera: Miridae). *Int. J. Insect Morphol. Embryol.*, 16: 309-322. [https://doi.org/10.1016/0020-7322\(87\)90003-1](https://doi.org/10.1016/0020-7322(87)90003-1).
- [2]. Kamal, R., Gupta, R.S. & Lohiya, N.K. (2003). Plants for male fertility regulation. *Phytother. Res.*, 17(6): 579–590. <https://doi.org/10.1002/ptr.1320>.
- [3]. Hussain, M.A. & Gorski, M.S. (2004). Antimicrobial Activity of *Nerium oleander* Linn. *Asian J. Plant Sci.*, 3: 177-180. <https://doi.org/10.3923/ajps.2004.177.180>.
- [4]. Zhao, M., Zhang, S., Fu, L., Li, N., Bai, J., Sakai, J., Wang, L., Tang, W., Hasegawa, T., Ogura, H., Kataoka, T., Oka, S., Kiuch, M., Hirose, K. & Ando, M. (2006). Taraxasterane- and ursane-type triterpenes from *Nerium oleander* and their biological activities. *J. Nat. Prod.*, 69(8): 1164–1167. doi: 10.1021/np0680073.
- [5]. Siddiqui, S., Hafeez, F., Begum, S. & Siddiqui, B.S. (1988). Oleanderol, a New Pentacyclic Triterpene from the Leaves of *Nerium oleander*. *J. Nat. Prod.*, 51(2): 229–233. doi: 10.1021/np50056a006.
- [6]. Kang, S.S. (1987). <sup>13</sup>C-NMR spectroscopy of amyrins. *Korean J. Pharmacogn.*, 18: 151–167.
- [7]. Siddiqui, B.S., Sultana, R., Begum, S., Zia, A. & Suria, A. (1997). Cardenolides from the methanolic extract of *Nerium oleander* leaves possessing central nervous system depressant activity in mice. *J. Nat. Prod.*, 60(6): 540–544. doi: 10.1021/np960679d.
- [8]. Ding, K., Fang, J.-N., Dong, T., Tsim, K. W.-K. & Wu, H. (2003). Characterization of a Rhamnogalacturonan and a Xyloglucan from *Nerium indicum* and their activities on PC12 Pheochromocytoma cells. *J. Nat. Prod.*, 66(1): 7–10. doi: 10.1021/np020118o.
- [9]. Jeong, S.E., Lee, Y., Hwang, J.H. & Knipple, D.C. (2001). Effects of the sap of the common oleander *Nerium indicum* (Apocyanaceae) on male fertility and spermatogenesis in the oriental tobacco budworm *Helicoverpa assulta* (Lepidoptera, Noctuidae). *J. Exp. Biol.*, 204(22): 3935–3942.
- [10]. CPCSEA (2006). Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi.
- [11]. WHO (1983a). Protocol CG-04: Preparation of alcoholic extract for bioassay and phytochemical

- studies (APJF/IP, 1001A). Geneva, World Health Organization.
- [12]. WHO (1983). Protocol MB-50: A method for examining the effect of the plant extracts administration orally on the fertility of male rats (APF/IP, 99914E). Geneva, World Health Organization.
- [13]. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193(1): 265–275.
- [14]. Zlatkis, A., Zak, B. & Boyle, A.J. (1953). A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med.*, 41(3): 486–492.
- [15]. Zilversmit, D.B., Davis, A.K., Memphis, B.S. & Tenn, N. (1950). Estimation of phospholipids in biological fluids. *J. Lab. Clin. Med.*, 35: 155-60.
- [16]. Fiske, C.H. & Subbarow, Y. (1925). The Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, 66: 375-400.
- [17]. Cabaud, P.G. & Wroblewski, F. (1958). Colorimetric measurement of lactic dehydrogenase activity of body fluids. *Am. J. Clin. Pathol.*, 30(3): 234-236. doi: 10.1093/ajcp/30.3.234.
- [18]. WHO (1999). Annual Technical Report 1998, Special programme of research, development and research training in human reproduction. Geneva, World Health Organization. p. 111.
- [19]. Varley, H. (1969). *Practical Clinical Biochemistry*. 4th ed., Whitebriers, London, pp. 200-275.
- [20]. Asatoor, A.M. & King, E.J. (1954). Simplified colorimetric blood sugar method. *Biochem. J.*, 56: 44.
- [21]. Lynch, M.J., Raphel, S.S., Mellor, L.D., Spare, P.D. & Inwood, M.J.H. (1969). *Medical Laboratory Technology and Clinical Pathology*. 2nd Ed., W.B. Saunders Co., London, U.K.
- [22]. Abercrombie, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.*, 94: 239–247. doi: 10.1002/ar.1090940210.
- [23]. Gupta, S. (1978). Sampling and test of significance. In: Gupta, S. (Ed.), *Statistical Methods*. Sultan Chand and Sons Publishers, New Delhi, pp. 58–76.