Assiut Vet. Med. J. Vol. 39 No. 78, July 1998.

Dept. of Forensic Med. and Toxicology,
Faculty of Vet. Medicine, Assiut University
Head of Dept. Prof. Dr. A.A. Shaaban.

EFFECT OF NUVACRON® ON REPRODUCTIVE SYSTEM OF MALE ALBINO RATS

(With 1 Table and 13 Figures)

By

D.A. SALEM and S.KH. ABD ELGHAFFAR*

*Dept. of Pathology and Clinical Pathology

(Received at 25/6/1998)

تأثير النوفاكرون على الجهاز التناسلى فى ذكور الفئران البيضاء

ضيفى أحمد سالم ، سارى خليل عبد الغفار

تم دراسة التأثير تحت الحاد للنوفاكرون (مبيد حشرى فسفورى عضوى) على ذكور الفئران البيضاء. تم استخدام ستون (٦٠) فأراً أبيضاً (ذكور) قسمت الى ثلاث مجموعات متساويه. أعطيت المجموعه الأولى والثانيه ١/١٠ ، ١/٢٠ من الجرعه النصف مميته للنوفاكرون يومياً لمدة أربع أسابيع بينما استخدمت المجموعه الثالثه كمجموعه ضابطه. بعد أسبوعين تم ذبح خمس حيوانات من كل مجموعه أسبوعياً و ١٠ حيوانات من كل مجموعه فى نهاية التجربه. تم أخذ عينات سيرم لقياس الهرمونات وعينات من أنسجة الجهاز التناسلى الذكرى والكبد للفحص الباثولوجى. وقد تبين وجود زياده فى مستوى هرمون التستوستيرون زياده معنويه جداً فى المجموعتين المعاملتين بينما ازداد مستوى هرمون الأستروجين فى المجموعه الأولى فقط وقد أظهر الفحص الباثولوجى للخصيه احتقان فى الأوعيه الدمويه، أوديما فى النسيج ما بين أنابيب الخصيه وتكاثر لخلايا "لايدج" واستحالات فى الخلايا المنويه لأنابيب الخصيه تبعه ضمور فى أنابيب الخصيه خاصة فى المجموعه الأولى. وقد لوحظ ان الوعاء المنوى فى حيوانات المجموعه الأولى والثانيه خالى من الحيوانات المنويه مع وجود استحالات فى الخلايا المبطنه له. وقد نوقشت هذه النتائج فى ضوء التغيرات الهرمونيه. وقد أظهرت الدراسه أن النوفاكرون اما أن يؤثر على أنابيب الخصيه تأثيراً مباشراً على الخلايا المنويه أو تأثير غير مباشر من خلال تأثيره على الأوعيه الدمويه والنسيج البين خلوى والكبد.

SUMMARY

The subacute toxopathological effects of monocrotophos (Nuvacron®) organophosphate insecticides was studied on male albino rats. Sixty male albino rats were devided into three equal groups. The first and the second groups were received 1/10 and 1/20 LD_{50} of Nuvacron®, daily for four weeks. The third group was kept as control. After two weeks, 5 animals from each group were sacrificed weekly and ten animals at the end of the treatment.. Tissue specimens were taken for histopathology, while serum samples were taken for hormonal assay only at the end of the treatment. Testosterone levels showed a highly significant increase in both the first and second groups in comparison to control group. The average levels of testosteron were 6049.8±33.1, 3394.6±25.6 and 1080.6±18.1 (pq/ml) in the first, second and control group, respectively. Estrogen levels were increased significantly in the first group. The average levels of estrogen were 17.9±0.02, 10.78±0.73 and 11.05±0.06 (pq/ml) in the three groups, respectively. Pathological examination of the testes revealed congestion of the blood vessels, intertubular oedema, fibroblastic proliferation followed by leydig cell hyperplasia, and hypertrophy. Degeneration of spermatogenic cells, atrophy of semineferous tubules and degeneration of vessel walls were noticed at the end of the experiment specially in the first group. The epididymis of the treated groups were empty from sperms and showed degeneration of their epithelium. The liver showed, degeneration and necrosis of the hepatic parenchyma. Our results concluded that, the effect of Nuvacron® on the testis was both directly on the spermatogenic cells and testicular blood vessel and indirectly through its effect on the intertubular tissue as well as on the liver

Key Words: *Male rats-Reproductive System-Nuvacron.*

INTRODUCTION

Pesticides occupy a rather unique position among the many chemicals that man and animals encounters daily, in that they are deliberately added to the enviroment for many purposes. Over the years, vas quantities of pesticides have been used in Egypt for crop protection and control of disease transmitting insects.

Organophosphorus insecticides (OP.) are of general importance because of their extensive use in combating the disease vector and agriculture pests after the problems of organochlorine residues in the environment. Their serous effect on animal heath and reproduction were reported (Lein, et al. 1982 and Sanders et al., 1985). Nuvacron® (monocrotophos) is a systemic insecticide with contact and systemic action. It is used for control of a broad sepectrum pests, including (sucking, chewing and boring insects and spider mits on cotton, citrus, olives, rice, maize, sorghum, sugar cane, sugar beet, peanuts, potatoes, soya beans, vegetables, etc. It classified by WHO as lb (highly hazardous) (Worthing and Hance, 1991).

Some pathological effect of OP. on the reproductive system of experimental animals were recorded by many authers, (Abd-Elghaffar, 1989; Afifi, 1991; Salem et al. 1992 and Abou Salem et al., 1997).

This study was conducted to investigate the subacute toxopathological effect of monocrotophos (Nuvacron®) insecticide on male reproductive system of albino rats.

MATERIALS and METHODS

I- Experimental animals:

Sixty male albino rats, one month age, were obtained from experimental animal house of Assiut University. The animals were kept under hygenic measures, provided with commercial ration and fresh water for about 60 days until they reached puberty and their weight became 120-150 gm.

II- Insecticide:

Monocrotophos [dimethyl (E) -1- methyl-2- (methyl carbamoyl) vinyl phosphate], commercially named Nuvacron® 40. It is a number of a new group of OP. produced by Ciba Geigy limitted, Basal switzerland. It is an emulsifiable concentrate containing 400g monocrotophos/liter. Acute oral LD_{50} for rats was 20 mg/kg B.W (Worthing and Hance, 1991).

Methods:

1- Experimental design:

The animals were devided into three groups. Each group consisted of 20 rats and subjected to the following treatment:

-First group: given daily 2 mg monocrotophos/kg B.W, (1/10 LD_{50})

dissolved in tap water, orally by stomach tube.
-Second group: given daily 1 mg monocrotophos/kg B.W. (1/20 LD_{50}) dissolved in tap water, orally by stomach tube.
-Control group: given the same quantities of tap water by stomach tube.

To induce subacute toxicity, these doses were given daily for four weeks (Wallace, 1994). After two weeks of treatment, five animals were sacrificed weakly from each group and 10 animals from each group sacrificed after four weaks of treatment . Blood samples were collected from the last sacrificed animals and serum was seperated and stored at (-20°) for testosterone and estrogen assay.

2- Pathological study:

After postmortem examination of sacrificed rats, tissue specimens were taken from testes, head of the epididymis and liver. The tissue specimens fixed in 10% neutral buffer formalin and in Bowan's fixative. The fixed samples were dehydrated in alchols, processed and embedded in paraffin blocks. Sections of 5-7 μ were prepared. The sections were stained with heamatoxelin and eosin stain (Banchroft and Stevens 1982).

3- Testosterone assay:

Testosterone was measured by using Ridoscrean® testosteron ELISA kits, obtained from R-Biopharm GmbH, Darmstadt, Germany. Anthos Ht II (program included) microtiter plate photometer Reader (450 nm) and Anthos plate autowasher were used. Testosterone was extracted and measured according to kit procedures based on the methods of Rattenberger and Matzk (1989).

4- Estrogen assay:

Serum sample were analysed for total unconjugated estrogen by solid phase ^{125}I radioimmunoassay techniques using kits supplied by diagnostic products corporation 5700 west 96th street, Loss Angelos (A 90045-5597), based on the method of Bergquist et al. (1983)

RESULTS

Gross pathology:

Postmortem examination of the sacrificed animals showed congestion of the parenchymatous organs and brain with presence of echimotic haemorhages on their surface. Meanwhile there were grayish white foci in the liver of the rats in the first group. The testes were congested and became small in size at the end of experiment. The

epididymis were also smaller in size in comparison with that in the control group.

Histopathology:

Testis:

The normal histological picture of the testes of the control rats were showen in fig.1.

After two weeks,the testes in the second group (1/20 LD_{50}), showed widening of the intertubular tissue, occuluded with a faint pink transudate(Fig. 2). In the first group, there was an increase in the number of interstatial cells in addition to the oedema of the intertubular tissue (Fig. 3). The spermatogenic cells in the seminferous tubules showed mild degenerative changes in the 2 ry spermatocytes and spermatids.

After three weeks, the testes in the second group showed extensive oedema in the interstitial tissue associated with multiple fibroblastic cellular proliferation (Fig. 4). The seminiferous tubules showed degenerative changes, with presence of spermatid gaint cells and few sperms in the center (Fig. 5). In the testes of the first group, there were obvious hypertrophy and hyperplasia of the laydig cells in the intertubular tissue. Laydig cells have ovoid large nuclei with dotted nuclear chromatin (Fig. 6). The seminiferous tubules showed extensive necrosis of the spermatogenic cells, only sertoli cells and few spermatogenia could be seen in some cases (Fig. 7).

After four weeks the testes of the second group showed extensive necrosis in some seminferous tubules which appeared empty from sperms, only sertoli cells and the basal layer remained intact (Fig. 8). The testes of the first group showed complete disorganization of the normal histological architecture of the testes. The seminiferous tubules were irregular in shape and in size and were reduced in diameter. The spermatogenic layers showed marked destructive changes while the basal layer remained more or less intact. The blood vessels were congested and showed fibrinoid degeneration in their walls (Fig. 9).

Epididymis:

Microscopic examination of the epididymal head of the control rats was normal and filled with sperms (Fig. 10). The pathological changes manifested only after three weeks of treatment as the epididymal ducts were empty from sperms. After four weeks the epidydmal ducts of the second group were empty from sperms and contained acidophilic depries (Fig. 11). In the first group, the epidydmal duct were irregular in

shape and in size, contained no sperms and their epithelial lining showed vacular degeneration. There was also an increase in the interstatial tissue (Fig. 12).

Liver:

The liver of the treated groups showed congestion and varying degree of degenerative changes begained from the second week of treatment reached to focal areas of necrosis specially in the first group (Fig. 13).

Hormonal assay:

The obtained results are recorded in table (1). Testosterone mean values were 1080.6±18.1, 3394.6±25.6 and 6049.8±33.1 pq/ml in control, 1/20 and 1/10 LD_{50} treated groups, respectively. The averages of esterogen were 11.05±0.06, 10.78±0.73 and 17.9±0.02 pq/ml in the same groups, respectively

DISCUSSION

Increasing animal reproduction is of high economic importane specially in our countery. So study the toxicity of pesticides which lowered the animal fertility is recommended due to the excessive use of many types of them in compating the agriculture and animal pests. However, pesticides also affect the fertility of human which exposed to them (Whorton, et al. 1977 and Taylor, et al. 1978)

Mammalin reproduction is a highly coordinated process in which almost all of the biologic resources are mobilized to achieve this critical function. So any toxic insults to the testes can result in a multiplicity of effects. Since the testis is compartmentalized into spermatogenic (seminferous tubules) and steroidogenic (Leydig cells, interstitial) components, such effects can occur individually or in combination. Our results indicated that ingestion of Nuvacron® either in 1/10 or 1/20 LD_{50} to male albino rat could affect both components of the testis.

The ingestion of 1/20 LD_{50} to male albino rats (subacut toxicity) resulted in oedema, fibroblastic proliferation with leydig cells hyperplasia in the interstatium tissue of the testis associated with degeneration of spermatogenic cells in the seminferous tubules. Only germinal layer and sertoli cells were seen at the end of the experiment. This pathological changes associated with marked increase in the testosteron levels in a highly significant manner. In the group which received 1/10 LD_{50} ,the

testis showed the same above lesions in a sever manner in addition to fibrinoid degeneration of the vessel walls and atrophy of the seminiferous tubules. This indicated that, the effect of Nuvacron® on the testis was dose related. The hormonal changes in this group showed increase both testosteron and estrogen levels.

The increase of the testosterone levels in both treated group is attributed to the hyperplasia and hypertrophy of the leydig cells in the testes, as the primary function of the leydig cell is the biosynthesis and secretion of the testosteron hormone (Mooradian et al., 1987). Increase of testosterone level may be incriminated to have an indirect bad effects on the spermatogenesis (Meineck and Mcdonald 1961), both authers cited that a highly increase in the testosterone level caused depression of semen quality and testicular size in bulls. In spite of testosterone play an important role in the support of sexual behavior and maintanance of spermatogenes (Mooradian et al. 1987), its abnormal increase will lowered the fertility of males.

Increase of the estrogen level in the first group is attributed to the hepatic damage, as the liver is the main organ of estrogen detoxification. This estrogenic action of Nuvacron® is incrimenated as indirect cause of testicular toxicity, through fiminization process. The indirect testicular toxicity of OP through its estrogenic action resulted from primary hepatic damage was mentioned by (Wayland, 1975 and Lofts and Murtan 1973).

The hyperplasia of the ledyig cells was explained in two mechanisms. The first is postulated by Onyango et al. (1993) who mentioned that sertoli cells in case of germ cells damage appear to be responsible for the production of a sertoli cell factor that stimulate proliferation of smooth endoplasmic reticulum in leydig cells. A similar results was experimentally obtained by Aoki and Hawcett (1978) who induced degeneration of the germ cells in rat testis by injection of cyproterone acetate, which dose not affect sertoli cells. After 30 days, the ledyig cell population showed hypertrophy and hyperplasia.

The second mechanism is that, the undifferentiated fibroblasts are the normal precursors of the leydig cells (Friendloder et al., 1984, Warbel et al. 1988; Zayed et al. 1995), they added that this assumption is further supported by the presence of intermediate form between fibroblast and leydig cells.

Accordingly, in our results the testicular reaction due to administration of Nuvacron® was began by interstatial oedema which

intiate fibroblastic proliferation. The fibroblast cells were differentiated to leydig cells. This ended by hyperplasia and hypertrophy of the leydig cells under the effect of sertoli cell factors.

Thus the testicular toxicity due to Nuvacron® was attributed to both direct and indirect action. The direct cytotoxic effect on the spermatogenic cells and interstitial blood vessel walls. The indirect effect through its estrogenic action resulted from primery hepatic damage and/or highly increase of testosterone level due to leydig cell hyperplasia.

The epididymal lesions share also in lowering the fertility of the animals. The empty of the epididymmal duct from sperms in the treated groups confirm the toxic effect of nuvacron on the spermatognesis

From these results, we can concluded that:

-Monocrotphos (Nuvacron®) OP.insecticide have marked testicular toxicity through both direct and indirect effects

REFERENCES

Abd Elghaffar, S.Kh. (1989): Pathological studies on the effect of some organophosphorus compounds "phosfolan and mephosfolan" on albino rats. M.V.Sc. Thesis, Fac. of Vet. Med. Assiut University.

Abou Salem, M.E.; El-Mashad, A.I. and Moustafa, S.A. (1997): Pathological male reproductivity and residues of Dimethoate toxicity in albino rats. Alex. J. Vet. Sci., Vol. 13, No. 2: 119-140.

Afifi, N.; Ramadan., A.; Abd El-Aziz, M. and Saki, E. (1991): Influence of diamethoate on testicular and epidydimal organs, testosterone plasma level and their residues in rat. DTW. D Tsch-Tierarzti-Wochensche, Vol. 98 (11): 419-423.

Aoki, A. and Hawcett, D.W. (1978): Is there is alocal feed back from seminiferous tubules affecting activity of the leydige cells?. Biol. Reprod., 19: 144-158.

Bancroft, J.D. and Stevans, A. (1982): Theory and practic of Histologic Techniques, 2nd edition. Long Man Group Limited. pp. 113.

Bergquist, C.; Nillus, S.G. and Wide, L. (1983): Human gonadotropin therapy. 1- Serum estradiol and progestrone pattern during conceptual cycles. Fertil. and Steril., 39: 761-765.

Friendloder, M.; Rosenstrach, A. and Bedrak, E. (1984): Leydig cell differentiation during the reproductive cycle of seasonal breeder Camelus dromedarium: An ultrastructural analysis. Gen. Cemp. Endocrinol. 55: 1-11.

Lein, D.H.; Mglin, G.A.; Hillman, R.B. and Reb Hun, W.C. (1982): Chloropyrifos (Durspan 44r), Toxicity in dairy bulls. Cornell Vet. 72 (supple 3): 58-

Lofts, B. and Murtan, R.K. (1973): Reproduction in birds, in Avain Biology, Academic Press. New York.

Meineck, C.F. and Mcdonald, L.E. (1961): The effect of exogenous testosterone on spermatogenesis of bulls. Am. J.Vet. Res. 22: 209-216.

Mooradian, A.D.; Morley, J.E. and Korenman, S.G. (1987): Biological action of androgens. Endocrine Re. 8: 1-27.

Onyango, D.W.; Doduor-Okel and Otianga-Owiti, G.E. (1993): Ultrastructural study of the testis of non breeding naked-male rat (Heterocephalus Ruppell). Ann. Anat., 175: 447-452.

Rattenberger, E. and Matzk, P. (1989): Nachweiseiner illegalen Anabolikabehandlung mit den Karperingenen Hormonen Testosteron und Ostradiol. Arch. Fur Lebensmittethyg. 112.

Salem, H.A.; El-Ballal, S.S. and Seddek, A.Sh. (1992): The effect of AD_3 E vitamin mixture on experimentally induced mephospholan short term toxicity in mic. Assiut Vet. Med. J. 28, 55: 158-173.

Sanders, D.E.; Delhunts, A.; Cummijs, J.F. and Sanders, J.A. (1985): Progressive paresis in sheep due to delayed neurotoxicity of trianyl phosphates. Cornell Vet., 75, 493-504.

Taylor, J.R.; Selhorst, J.B.; Hauff, S.A. and Martinez, A.J. (1978): Chlordecone intoxication in man. 1. Clinical observations. Neurology, 28: 626-630.

Wallance, A.H. (1994): Principles and methods of toxicology. Third edition. Revan Press, New York.

Wayland,J. Hoys (1975): Toxicology of pesticides. The Williams&Wilkans Company.

Whorton, D.; Krauss, R.M.; Marshall, S. and Milby, T.H. (1977): Infertility in male pesticide worker. Lancet, 2: 1259-1261.

Worbel, K.II; Postal, S. and Schimmel, M. (1988): Post natal depelopment of tubular lamina propria and intertubular tissue in bovin testis. Cell Tissue Res., 252: 639-653.

Worthing, C.R. and Hance, R.J. (1991): The pesticide Manual, A wrold compendium, ninth edition British Crop Protection Council.

Zayed, A.E.; Hifny, A.; Abou-Elmagd, A. and Worbel, K.H. (1995): Seasonal changes in the intertubular tissue of camel testis (camelus dromedarius). Ann. Anat. 177: 199-212.

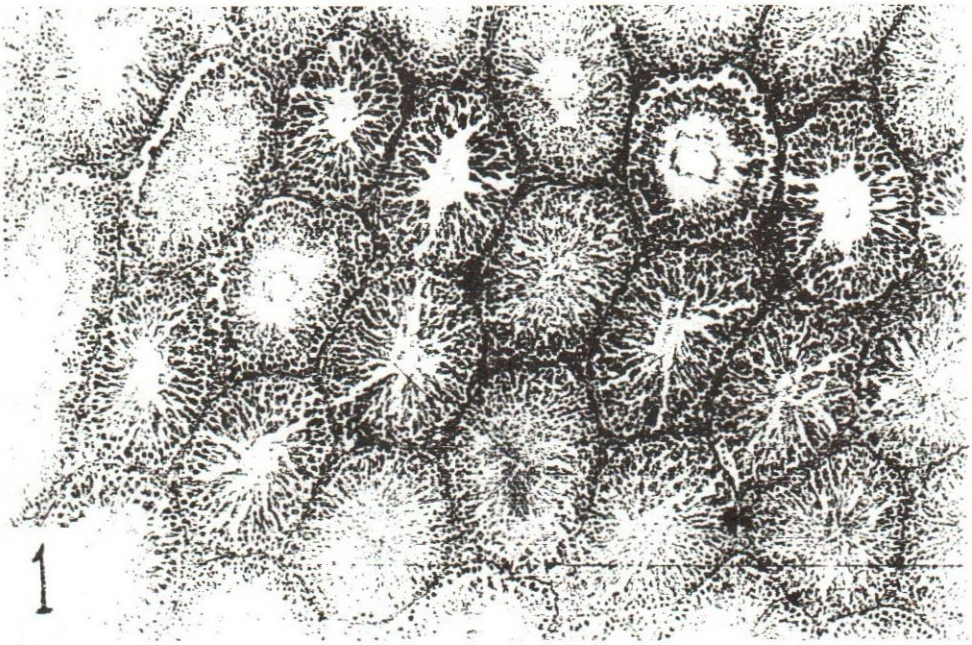
Table 1: Serum testosterone and estrogen level in male albino rats treated with Nuvacron® insecticide.

Treatment	Testosterone pq/ml (mean ± SE)	Estrogen pq/ml* (mean ± SE)
Control	1080.6±18.1	11.05±0.06
1/20 LD_{50}	3394.6±25.6**	10.78±0.73
1/10 LD_{50}	6049.8±33.1**	17.9±0.02*

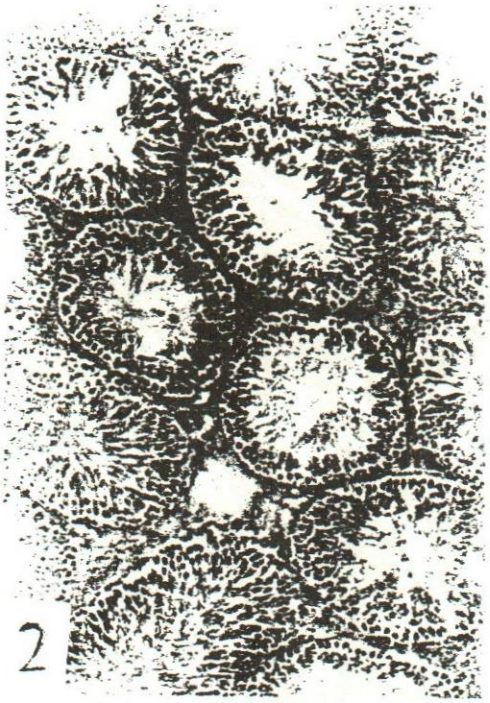
* Significant increase.

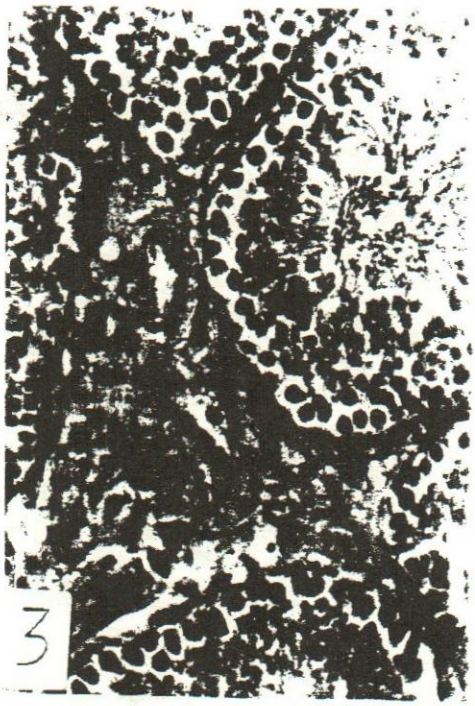
** Highly significant increase

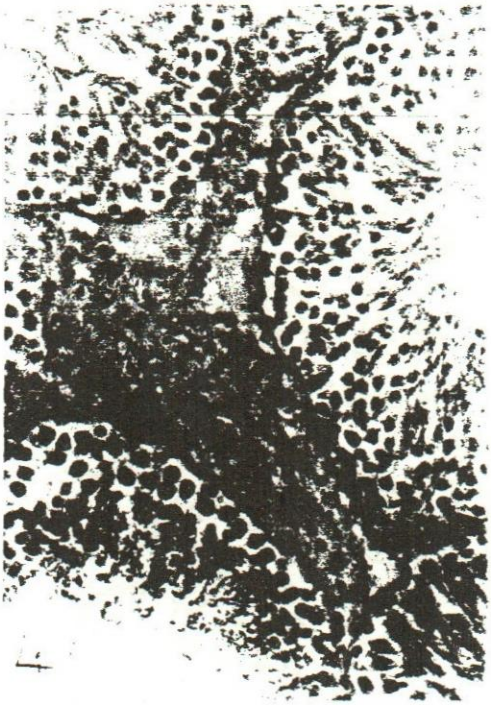
LIST OF FIGURES



1


2


3


4


5