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EFFECT OF BRADYKININ POTENTIATING FRACTION FROM VENOM OF EGYPTIAN SCORPION, BUTHUS OCCITANUS, ON IMMATURE FEMALE GUINEA PIGS

(With 3 Tables & 3 Fig.)

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تأثير مشتق من سم العقرب المصرى منشط للبراديكينين على اناث الخنزير الغينى غير البالغة

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تضمن البحث تأثير مشتق من سم العقرب المصرى منشط للبراديكينين على وزن الجسم والمبايض والأرحام ومستوى هرمونى الاستراديول والبروجتيرون فى اناث الخنزير الغينى غير البالغة (ثلاثة أسابيع). وقد أسفر حقن هذه الحيوانات بجرعه واحده (١ ميكروجرام جرام من وزن الجسم) من مشتق السم عن زيادة غير معنويه فى وزن الجسم فى الحيوانات المعالجه بالنسبه للحيوانات الضوابط (الغير معالجه). بينما أظهرت مبايض الحيوانات المعالجه نشاطاً واضحاً بما فى ذلك وجود حويصلات جراف الناضجة وقد صاحب ذلك زيادة فى سمك الطبقة الداخلية لأرحام تلك الحيوانات مع زيادة فى عدد وقطر الغدد الرحمية على عكس ماظهر فى مبايض الحيوانات الغير معالجة من حويصلات صغيرة ورقة الغشاء المبطن لأرحامها مع قلة وصغر الغدد الرحمية بها. وعلى العكس من هذا فقد أسفر حقن الحيوانات بخمس جرعات متتالية من مشتق السم عن زيادة معنوية ($P<0.001$) فى وزن الجسم مع نشاط ملحوظ فى الغشاء المبطن للرحم. وقد صاحب ذلك أرتفاع معنوى ($P<0.001$) فى مستوى هرمون الاستراديول وأرتفاع معنوى ($P<0.05$) فى مستوى هرمون البروجيسترون فى مصل الحيوانات التى تم حقنها بمشتق السم عن الحيوانات الضوابط.

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SUMMARY

The effect of an isolated fraction, with bradykinin potentiating activity, from the venom of the Egyptian Scorpion, Buthus Occitanus, on the body weight, overies, uteri, oestradiol and progestexone levels, was studied in immature (3 weeks-old) female guinea pigs. Intraperitoneal injection of a single dose (1 ug/g B.W.) of the venom fraction produced a non-significant increase in the body weight of treated than control animals. However, the ovaries of treated guinea pigs showed different stages of follicular activity including Graafian follicles, together with an increase in the thickness of their endometrium and number and diameter of endometrial glands against primary and growing ovarian follicles, thin endometrium and few narrow-lumen endometrial glands in control animals. On the other hand, 5 successive injections of the venom fraction produced a significant ($P < 0.001$) increase in the body weight of treated animals. Mature follicles and corpora lutea were observed in their ovaries together with marked endometrial activity in their uteri. This was accompanied with a significant elevation of oestradiol Level ($P<0.001$) and progesterone level ($P < 0.05$) in the sera of treated than control guinea pigs.

INTRODUCTION

The mammalian cell growth and differentiation are controlled by a number of bioactive polypeptides, such as insulin epidermal growth factor (EGF), insulin-like growth factors (IGF-IGF_{II}), somatomedins, nerve-growth factor (NGF), fibroblast growth factor (FGF) and EGF-like growth factors (*SALMON and DUVALL, 1970 and KARP, 1984*). Moreover, *JONES* et al.,*(1982)* reported that there are several other bioactive polypeptides in the serum that are involved in cell growth control as 2-macroglobulin, thrombin, transferrin and low-density lipoprotein. *SCHACHTER (1964)* revealed that bradykinin is typical plasma kinin that causes contraction of isolated smooth muscles, vasodilation and increased permeability of capillaries.

UFKES et al., *(1978)* studied the relations between amino acid composition and bradykinin potentiation on isolated fractions

from venom of the Japanese Snake. *MENCONI et al., (1984)* revealed that bradykinin, at low concentrations, activated endothelial cells, fibroblasts and smooth muscle cells in primary and first passage cultures. *STRAUS and PANG (1984)* suggested that bradykinin stimulates (3_H) thymidine incorporation and DNA synthesis in resting serum deprived hamster cells. *LAMBERT et al., (1986)* recorded that bradykinin produced a significant increase in inositol triphosphate (IP_3) in procine aortic endothelial cells grown in culture.

Concerning the involvement of kinins in reproductive function, *ESPEY et al., (1986)* revealed that bradykinin may be involved in the ovulatroy process. They found that the ovarian kinin-generating activity was increased significantly during ovulation. *YOSHIMURA et al., (1988)* reported that kinins are important mediators of the ovulatory process and that bradykinin increased 6 keto-PGF_1 in perfused rabbit ovary. *NASSAR et al., (1990)* studied the effect of an isolated venom fraction with bradykinin potentiating activity from the Egyptian Scorpion, Buthus Occitanus, On the ovaries and endometrium of premature mice. They repoted that 5 days after the injection of a single sublethal dose (1 ug/g), the number of primary, secondary follicles and Graafian follicles was increased in the ovaries of treated animals, together with increased number and size of uterine glands and endometrium.

The present work was conducted to evaluate the growth promoting activity of a bradykinin potentiating fraction isolated from the venom of the Egyptian Scorpion, Buthus Occitanus. This was carried out through the detection of the effect of that fraction on body weight, reptoductive organs and circulating levels of sex hormones (oestradiol and progesterone) of immature female quinea pigs.

MATERIALS AND METHODS

A bradykinin potentiating fraction (BPF) was chemically isolated and purified from the venom of the Egyptian Scorpion, Buthus Occitanus, following the procedure described by *FERREIRA (1965)*. The isolated pelleted fraction was washed 3 times with a mixture of 90% ethanol and ethyl ether (1:4, v:v), dissolved in distilled water, dialyzed and then Lyophilized.

The pharmacological effect of the isolated fraction was demonstrated by the increase of contraction of the guinea pig ileum in presence of synthetic bradykinin (batch No. B-3259 from Sigma Chemical Co., St. Louis, U.S.A.).

The maximum contraction response was recorded by (Oscillograph 400 MD_2C, Plamer Bioscience, Washington, U.S.A.) 2-20 minutes after the addition of 0.3 ug of the venom fraction per ml of Tyrode's solution.

Bradykinin (0.02 ug/ml) was added 50 seconds after the addition of the venom fraction.

For in vivo studies, the experimental design was organized to detect the effect of the BPF on the body weight, reproductive organs and circulating sex hormones of immature female quinea pigs. A total of 32 female quinea pigs (3 weeks-old) with an average body weight of about 180 g, were alloted into 4 groups (G_1, G_2, G_3 and G_4) of 8 animals each and they were kept in 4 separate cages in the animal house. Animals of G_2 were given a single dose of 1 ug/g B.W. of the prepared BPF by the intraperitoneal route of injection. This dose was determined on the basis of the LD_{50} of the venom fraction (*MEIER and THEAKSTON, 1986*). The injection of the animals of G_4 with BPF was repeated every week for 5 successive weeks. Animals of G_1 and G_3 were injected 0.5 ml saline solution instead of BPF and they were kept as control. One week after the injection, the animals of G_1 and G_2 were weighed and sacrificed. Blood samples were collected for radioimmunoassay of oestradiol and progesterone using Coat A-count kits TGE_{21} and $TPKG_1$ (Diagnostic Product Co., Los Angeles, U.S.A.).

Ovaries and small pieces from uteri were collected in 10% buffered formalin, for histological examination according to the techniques of *DRURY and WALLINGTON (1980)*. One week after the fifth injection, animals of G_3 and G_4 were weighed, sacrificed and sampled in the same way as those of G_1 and G_2.

Over the course of the experiment, the animals were fed a concentrate pellet plus green clover and drinking water was allowed adlibitum. Moreover, light, temprature and humidity in the animal house, were under control.

RESULTS

The average values of body weight of the control female guinea pigs and those of BPF-treated animals are presented in table (1). One week after the single injection of BPF, there was a non-significant difference in the body weight of untreated (control, G_1) and treated animals (G_2). On the other hand, a significant ($P < 0.001$) increase was detected in the body weight of BPF-treated female guinea pigs G_4 against that of the untreated (control, G_3) animals, one week after the fifth injection of the venom fraction (Histogram, 1).

The histological examination of the stained sections made from the ovaries and uteri of the untreated (control, G_1) guinea pigs, revealed that the ovarian cortex included numerous primary follicles immediately located under the tunica albugenia and some growing follicles, but no mature or Graafian follicles could be detected (Plate 1). The endometrium of the uteri of those animals was lined with columnar cells having basal oval nuclei and abundant apical pale acidophilic cytoplasm. Endometrial glands were few, with narrow lumen, lined with low columnar cells and containing scanty pale acidophilic secretion (Plate 1). On the other hand, the ovaries of the BPF-treated animals (G_2) included different stages of follicular growth including Graafian follicles. An increase in the thickness of their endometrium was observed, together with an increase in the number and diameter of endometrial glands (Plates 2).

The stained sections of guinea pigs (untreated G_3) showed moderate developmental changes, but those of the BPF-treated animals (G_4) showed very prominent changes. The ovaries included multiple growing and Graafian follicles and large corpora lutea. A marked increase was observed in the thickness of endometrium with increase in height of the lining epithelium. The lamina propria became highly cellular with numerous lymphocytic infiltration. Endometrial glands increased in number, appeared more branched with dilated lumen filled with secretion (Plate 3).

Radioimmunoassay of oestradiol hormone revealed that its level in the sera of the control guinea pigs (untreated G_1) was 5.198 ± 0.438 pg/ml and it was 13.88 ± 0.248 pg/ml in the sera of BPF-treated (G_2) animals, one week after the injection of the venom fraction. Moreover, the hormone level was 47.81 ± 3.124 pg/ml in the sera of BPF-treated (G_4) guinea pigs with a significant ($P < 0.001$) increase than its level in the sera of the control (G_3) animals (26.63 ± 0.89 pg/ml) one week after the fifth injection of the venom fraction.

On the other hand, progesterone level in the sera of control animals (G_1) and those given a single dose of BPF (G_2) was less than 1 ng/ml. One week after the fifth injection of the venom fraction, progesterone level in the sera of injected guinea pigs was 2.136 ± 0.474 ng/ml with a significant ($P < 0.05$) increase than the control animals (G_3).

DISCUSSION

Bradykinin, as a bioactive peptide produced from proteolysis of circulating globulins by the action of a kininogenase enzyme, enhanced cell growth (*BOUCEK and NOBEL, 1973*) and activated endothelial cells and fibroblasts at very low concentrations (*MENCONI et al., 1984*). Moreover, bradykinin poteetiantion has been induced by the action of some snake venom peptide fractions (*FERREIRA et al., 1970 and LABIB et al., 1984*) and by an isolated fraction from the venom of the Egyptian Scorpion, Buthus, Occitanus (*ABDEL-REHIM, 1990 and NASSAR et al., 1990*).

The significant ($P < 0.001$) increase in the body weight of the BPF-treated (G_4) female guinea pigs than that of the untreated (G_3) animals, clarified the growth promoting activity of the venom fraction. This finding coincided with the enhancement of cell growth by bradykinin reported by *BOUCEK and NOBEL (1973)* and the activation of endothelial cells and fibroblasts, recorded by *MENCONI et al., (1984)*. Moreover, the growth promotion activity of such bioactive peptides could be interpretted on the basis of their effects revealed by *SHIMIZU (1984)*. These include stimulation of membrane transport of sugars and amino acids, stimulation of enzymes involved in glycolysis, lipid metabolism and glycogen synthesis. Other delayed effects include stimulation of protein, RNA and DNA synthesis and cell division.

The marked developmental changes observed in the histological sections made from the ovaries and uteri of the BPF-treated guinea pigs (G_2 and G_4), revealed the role of the venom fraction in the enhancement of ovarian follicular growth, ovulation and formation of corpora lutea and uterine growth changes. Similar findings were previously reported by *ABDEL-REHIM (1990) and NASSAR* et al., *(1990)* in immature mice given a single dose (1 ug/g B.W.) of bradykinin potentiating fraction isolated from the venom of the Egyptian Scorpion, Buthus Occitanus. However, they revealed that the ovaries of the treated mice contained Graafian follicle in 50% of the cases, but no corpora lutea were observed, which seems somewhat different than the observed corpora lutea in the ovaries of the BPF-treated guinea pigs in the present work. This may be attributed to the repeated injection of the venom fraction in the present work, which resulted in continuous and progressive ovarian structural growth. Moreover, the detected increase of oestradiol level in the serum of treated female guinea pigs parallel to the ovarian follicular growth, is in accordance with the findings of *ABDEL-REHIM (1990) and NASSAR* et al., *(1990).*

Finally, it can be suggested that the administered venom fraction could potentiate the endogenous bradykinin resulting in the somatic growth promotion, together with the ovarian and uterine developmental changes. Moreover, the future use of such BPF as a growth promoting factor and for induction of ovarian activity and ovulation, might be anticepated.

REFERENCES

Abdel-Rehim, S.A. (1990): Physiological studies of a separated fraction from the venom of an Egyptian Scorpion on the living cellls of some organs. Ph. D. Thesis, Assiut Univ., Egypt.

Boucek, R.J. and Nobel, N.L. (1973): Histamine, norepinephrine and bradykinin stimulation of fibroblast growth and modification for serotonin response. Proc. Soc. Exp. Biol. Med. 144, 929-933.

Drury, R.A.B. and Wallington, E. (1980): Preparation and fixation of tissues. PP. 36-102 in "Carleton's Histological Technique" 1st Ed., Oxford univ. Press.

Espey, L.L.; Miller, D.H. and Margoluis, H.S. (1986): Ovarian increase in kinin-generating capacity in the PHSc/hcg-primed immature rat. Am. J. Physiol. 251, 362-365..

Ferreira, S.H. (1965): A bradykinin potentiating factor (BPF) present in the venom of Bothrops jararaca. Br. J. Pharmac. 24, 163-164.

Ferreira, S.H.; Bartlet, D.C. and Green, L.J. (1970): Isolation of bradykinin potentiating peptides from Bothrops Jararaca venom. Biochemistry 9, 2583-2593.

Jones, P.B.C.; Welsh, T.H. and hsuel, A.J.W. (1982): Regulation of ovarian progestin production by epidermal growht factor in cultured rat granulosa cells. J. Biol. Chem. 257, 11268-11273.

Karp, G. (1984): Cell growth and division. PP 662-715 in "Cell Biology" 1st Ed. (Martin, M.J. Golden, S. and Bardley, J.W. Eds.), McGraw-hill Book Comp. New York.

Labib, R.S.; Camelia, A.; Abdel-Malak, N.; Ziada, A. and Farag, N.W. (1984): Peptides and amino acids of the venoms of Egyptian Vipers, Cerastes cerastes and Cerastes Vipera, Chemical evidence for the presence of bradykinin potentiating peptides. Egyptian J. Biochem. 2, 43-64.

Lambert, T.L.; Kent, R.S. and Whorton, A.R. (1986): Bradykinin stimulation of inositol polyphosphate production in procine aortic endothelial Cells. J. Biochem. 261, 15288-15293.

Meier, J. and Theakston, R.D.C. (1986): Approximate LD_{50} determinations of snake venoms using eight to ten experimental animals. toxicon 24, 345-401.

Menconi, M.; Hahn, G. and Polgar, P. (1984): Prostaglandin synthesis by cells comprising the calf pulmonary artery. J. Cell. Physiol. 120, 163-168.

Nassar, A.Y.; Abu-Sinna, G. and Abdel-Rehim,s. (1990): Effect of a bradykinin potentiating fraction from venom of the Egyptian Scorpion, Buthus Occitanus, on ovaries and endometrium of mice. Toxicon 28, 525-534.

Salmon, W.D. and Duvall, M.R. (1970): A serum fraction with "Sulfation factor activity" stimulates in vitro incorporation of leucine and sulfate into protein-polysaccharide complexes, uridine into RNA and thymidine into DNA into costal cartilage from hypophysectomized rats. Endocrinology 86, 721-727.

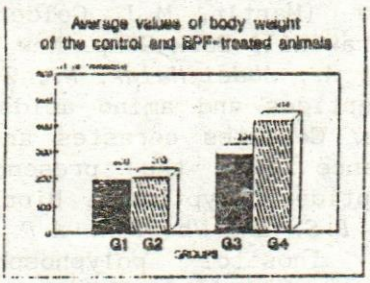
Schachter, M. (1964): Kinins: a group of active peptides. Ann. Rev. Pharmac. 4, 281-292.

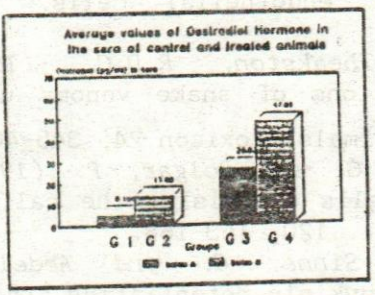
Shimizu, N. (1984): Mechanism of action of the EGF receptor system and control of cell growth. Gan to Tagakn Ryoho 11, 597-606 (cited by Abdel-rehim, 1990).

Straus, D.S. and Pang, K.J.(1984): Effects of bradykinin on DNA synthesis in resting NIL_8 hamster cells and human fibroblasts. Exp. Cell. Res. 151, 87-95.

Ufkes, J.G.R.; Visser, B.J.; Hevver, G. and Van der Meer, C. (1978): Structure-activity relationships of bradykinin potentiating peptides. European J. Pharmac. 50, 119-122.

Yoshimura, y., Espey, y.; Hosoi, T.; Adachi, S.J.; Atlas, R.B.; Ghodgaonkar, N.H. and Wallach, E.E. (1988): The effects of bradykinin on ovulation and prostaglandin production by the perfused rabbit ovary. Endocrinology 122, 2540-2546.





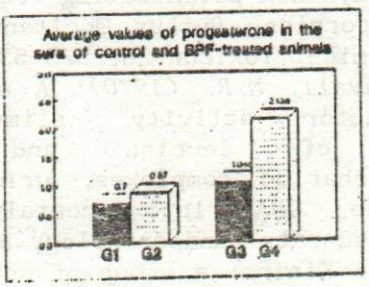


Table (1): Average values of body weight of control and BPF-treated female guinea pigs.

Group of animals	Treatment	Average Body weight(g)
G_1	Control	200 ± 5.659
G_2	Given a single dose of BPF	203 ± 6.402
G_3	Control	291 ± 9.099
G_4	Given 5 doses of BPF	414 ± 0.375 ***

*** = Significant (P<0.001)

Table (2): Average values of oestradiol hormone in the sera of control and BPF-treated female guinea pigs.

Group of animals	Treatment	Average values of oestradiol (Pg/ml serum)
G_1	Control	5.198 ± 0.470
G_2	Given a single dose of BPF	13.88 ± 0.267 ***
G_3	Control	26.63 ± 0.955
G_4	Given 5 doses of BPF	47.81 ± 3.322 ***

*** = Significant (P<0.001)

Table (3): Average values of progesterone hormone in the sera of control and BPF-treated guinea pigs.

Group of animals	Treatment	Average values of progesterone (ng/ml serum)
G_1	Control	> 0.7
G_2	Given a single dose of BPF	0.87 ± 0.07
G_3	Control	1.048 ± 0.13
G_4	Given 5 doses of BPF	2.136 ± 0.474 *

* Significant (P < 0.05)

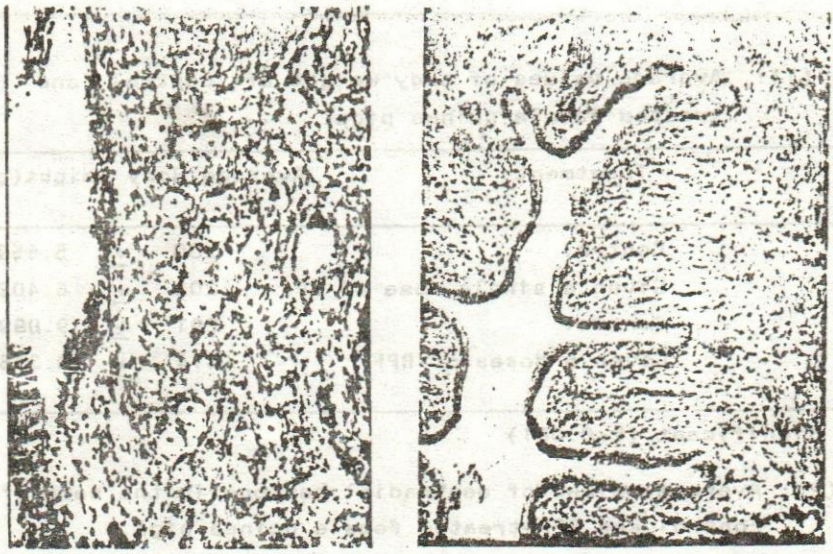


Plate (1): Ovary (left) and uterus (Right) of control animals (G_1) showing small primary follicles in ovarian cortex and few small endometrial glands (H & E X 125)

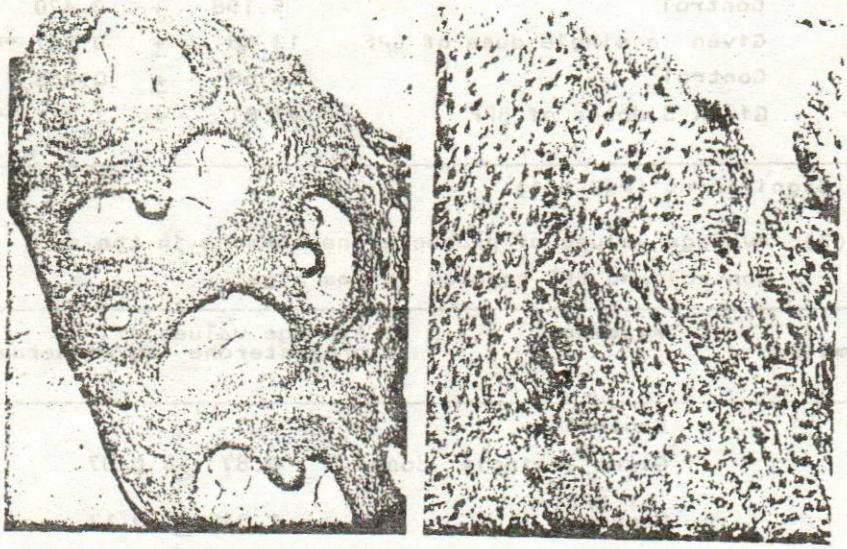


Plate (2): Ovary (left) and uterus (Right) of guinea pigs injected with single dose of DPF showing marked follicular growth, increased endometrial thickness, number and diameter of endometrial glands (H & E X 125 & 250)

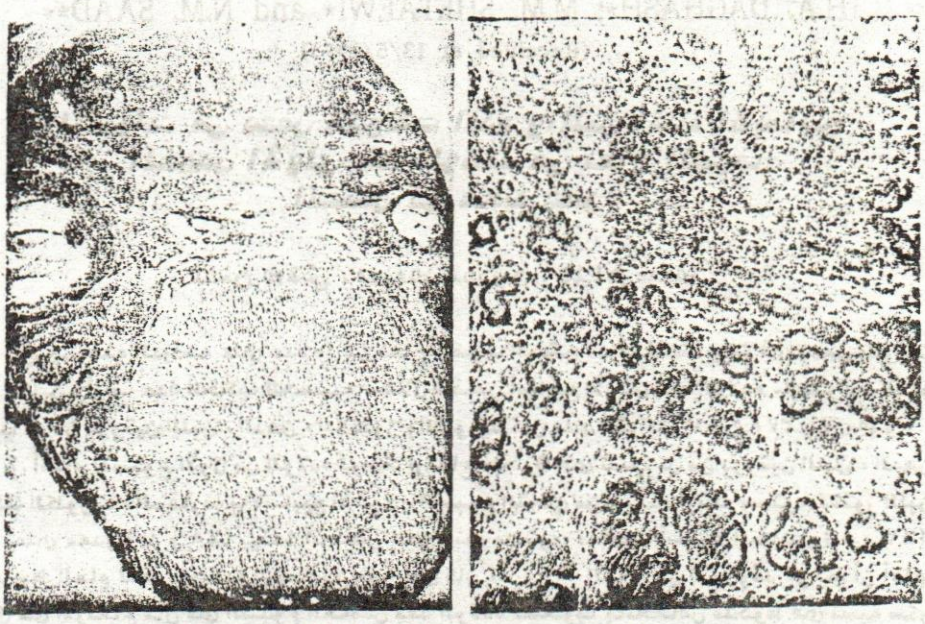


Plate (3): Ovary (left) and uterus (Right) of guinea pigs injected with 5 doses of BPF showing mature ovarian follicles and large corpus luteum together with marked endometrial growth, numerous branched endometrial glands and lymphocytic infiltration (H & E X 125)