Histological Study of the Effect of Isoxicam on Ovary of Albino Mice Mus Musculus

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Abstract

Non-Steroidal Anti Inflammatory Drugs (NSAIDs) are the most prescription as therapeutic drugs, used to treat of rheumatic diseases, due to analgesic, antipyretic and anti-inflammatory activity. Isoxicam is a member of NSAIDs group use to stop inflammation, pain associated with arthritis, osteoarthritis, ankylosing and spondylitis. The goal of the present study is to revealed the effect of different doses of Isoxicam on ovaries tissue in mice. Twenty four female mice are randomly divided into control (n = 6) and experimental (n=18) groups. The experimental groups are subdivides into three groups . Each administrated by (0.0714, 0.1428, 0.71428)mg/kg/day for twenty days, respectively; however the control group just injected by distill water. In twenty day, mice were killed and ovaries tissue was prepared for light microscopic examination. All the experimental animals were injected by drug revealed a hyperplasia of germinal cells on the surface of ovary, tongue like projection of primordial oocytes extend to the medulla, multiple oocytes with disarrangement of follicles and deficient of follicular fluid associated with disappearance of oocytes, vacuolation in the cortical layer of the ovary, compressed premature follicle, hypercellularity of follicular cells, degeneration of germinal layer of cortex surface and hyperplasia of primordial oocytes, therefore it is recommended that using of this drug have many side adverse on female fertility.

Key word : *Histological , Isoxicam , ovary , albino mice .*

Introduction

Isoxicam and Meloxicam these drugs are belonging to the oxicam group. Nonsteroidal anti-inflammatory drugs which display a potent analgesic activity and used for treated rheumatoid arthritis, osteoarthritis and other joint diseases. The pharmacological actions of these oxicam are related to inhibition of cyclo-oxygenase (Cox1,2), an enzyme of prostaglandin biosynthesis at the site of inflammation[1]. Prostaglandin, It have been involved as a regulator of several physiological processes in human body such as inflammatory processes in immune response, vasodilator, vasoconstriction, pain perception and fever. Prostaglandin are produced in every tissue of the body (brain, lung, kidney, intestinal digestive system, male and female reproductive system) [(2)].NSAIDs have many adverse effects of liver, dermis, skin eruption and many physiological disorders in rats' testis [3-6].There are an association between use of prescribed NSAIDs and miscarriage [7]. The modern NSAIDs that belong to tenoxicam, lornoxicam, Piroxicam and Isoxicam which belongs to oxicam family prescribed as inhibitors of both types of Cox [8]. A few research consider these drugs a very good antioxidants [9]. The oxicam family acts on inhibit cyclooxygenases COX-1 and 2, It is also inhibits leucocytes activities.

Fertility in females are affected clearly by COX-2. A study on mice female fertility throughout disruption of COX-2, lead to fails in ovulation, fertilization, implantation, and decasualization. These defects were the direct result of the targeted organ-specific COX-2 deficiency[(10)] .

The aim of this study was elevate effect of Isoxicam on the ovary after application of different concentration on mice ovary .

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Materials and Method

This study was done in medical laboratory department of biology/ Education college/ university of Samarra. Twenty four mature (70 days old) albino Swiss mice Mus musculus Balb/c were employed, weighing (25±3gm) obtained from college of medicine, Tikrit university. They were maintained on 12:12 light: dark bases, and 24 ±2°C with mouse pelleted food and water adlibitum. Female mice were housed in group not bigger than five animals (all from the same experimental group) in plastic cages with metal cover (13*16*30) cm, with wood shavings as bedding material, Twenty four male albino mice were randomly divided into control (n =6) and experimental(n =18) groups. The experimental groups are subdivides into three groups which divided into four groups of mice, each once is injected Intra Peritoneum. with different doses of Isoxicam once daily for 20 days.

Drug administration

Isoxicam ample 200 mg/2ml. Female were injected daily Intra Peritoneum (I.P.) administrated in three doses: Therapeutic dose, over dose1 and over dose2 (0.0714, 0.1428, 0.71428) mg/kg for 20 days respectively [11], and Control group were injected with normal saline 0.9 mg/ L.

Surgical procedure

In twenty one days, the female were anesthetized by chloroform, and the peritoneal cavity was opened through a lower transverse abdominal incision. The ovaries was immediately removed and kept in normal saline. At the end the experimental animals were killed by decapitation.

Histological preparation

The collected tissues Each segments of skin was taken and immersed in 10 % formalin foe 24 hours followed by immersion in graded series of alcohol from 70, 80, 90 and 100 %, then clearing with xylene and embedded in paraffin wax at 60 cº. Blocking of the samples were done and the sectioning were performed using a rotary microtome. The thickness of the sections were 6 micrometer. The tissue sections after application of staining with Hematoxylin and Eosin were mounted on the slides using D.P.X and covered by cover slides [12]. The slides were examined using light microscope and photographed by manipulated camera prepared for this purpose.

Results

Control group

Histological sections of the ovary in the control group show, intact ovarian surface (germinal layer) directly beneath it are tunica albuginea contain numerous primordial follicles, primary oocytes and secondary oocytes, the cells ranged from flat to cuboidal and low columnar cells. Cortex and medulla regions was continuous, Ovarian follicles are various sizes surrounded by theca interna and theca externa as shown. Each follicle contains a single oocyte in the stroma of the cortex. The oocyte within the secondary oocytes was envelop by the zona pellucida and granulosa cells fig (1).

T1 :Therapeutic group

The histological sections of ovarian cortex in this group shows hyperplasia of germinal cells on the surface of ovary, hyper cellularity in follicular cells around follicular cavity, tongue like projection of primordial oocytes extend to the medulla fig (2A), multiple oocytes with disarrangement of follicles and deficient of follicular fluid associated with disappearance of oocytes , hyperplasia of follicular cells primordial oocytes fig (2B).

T2 : Over dose1

This group showed degeneration of germinal cells layer on the surface of cortex with thickening of collagen fibers around premature oocytes, disappearance of oocyte with vacuolation in the cortical layer of the ovary fig (3A). In other sections showed hypercellularity of follicular cells of oocytes in the cortex , degeneration of follicular cells and secondary oocytes with vacuolation of interstitial connective tissue fig (3B). In tertiary follicle increased a multiple layer of follicular cells around oocytes, disappearance of oocytes with decreased amount of follicular fluid and detachment of follicular cells from surrounded connective tissue fig (3C) .other sections of cortex demonstrated compressed premature follicle , degeneration of germinal layer of cortex surface and hyperplasia of primordial oocytes fig (3D).

T3 : Over dose 2

In this group the microscopical examination were show degeneration of germinal epithelium cells of cortex surface, vacuolation in granulosa layers of premature follicle with remnant of oocyte of premature follicle fig (4A). In the surface of cortex showed

disappearance of germinal epithelium ,disarrangement of the other primary follicles with compressed it and partial congestion of blood vessels fig (4B). In other section showed degeneration of follicular cells layer in premature follicle hyper cellularity of connective tissue and vacuolation in between collagen fibers fig (4C). In medulla of ovary showed empty of blood vessels , spaces in between follicular connective tissue, and hypertrophy of the stromal cells fig (4D).

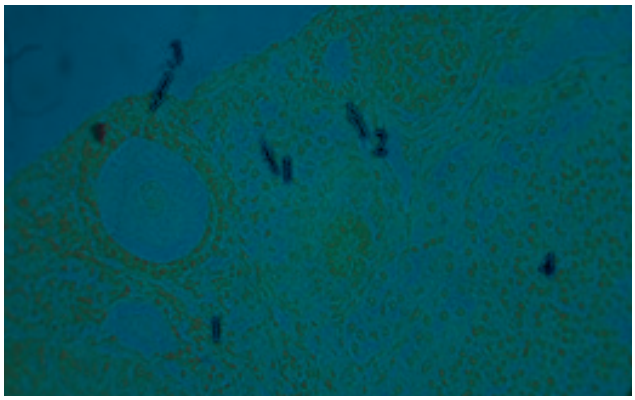

Fig (1) primordial follicles (1), primary oocytes (2) ,secondary oocytes (3) and cortex (4). H&E, 40X .

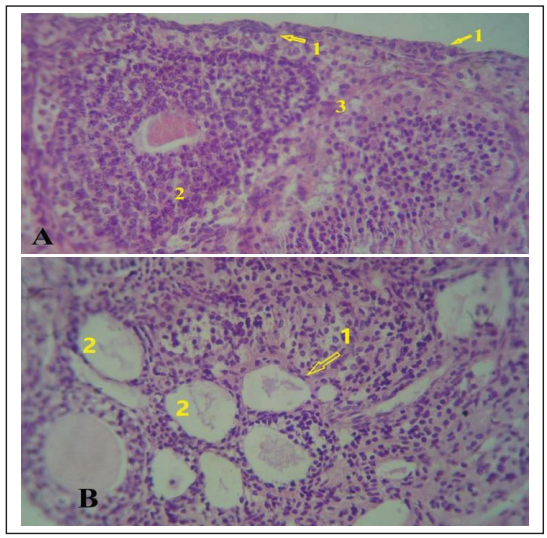

Fig (2): hyperplasia of germinal cells (1), hyper cellularity in follicular cells(2) and tongue like projection of primordial oocytes (3)A. multiple oocytes with disarrangement of follicles with disappearance of oocytes(1), hyperplasia of follicular cells primordial oocytes(2) B. H&E, 40X .

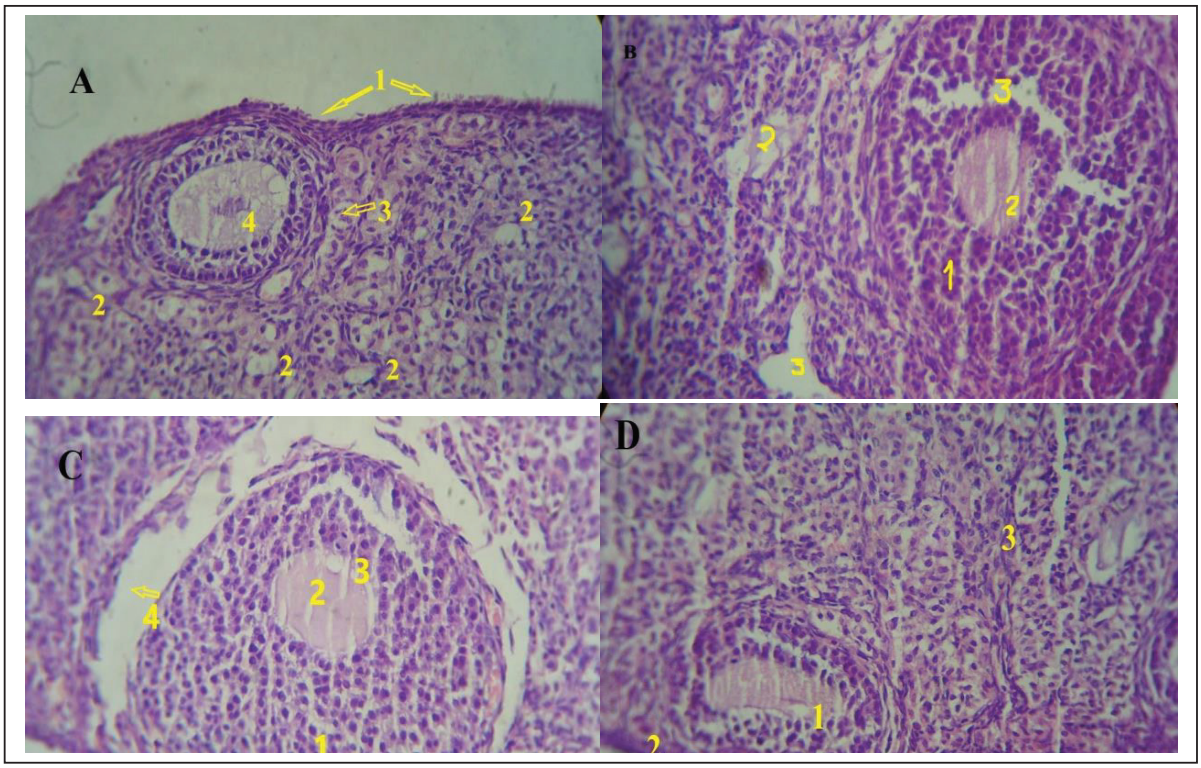

Fig (3) degeneration of germinal cells layer (1), vacuolation in the cortical layer (2), thickening of collagen fibers around premature oocytes (3) disappearance of oocyte (4) A.hypercellularity of follicular cells (1), degeneration of follicular cells (2), vacuolation of interstitial connective tissue (3)B. increased a multiple layer of follicular cells (1), disappearance of oocytes (2), decreased amount of follicular fluid(3) and detachment of follicular cells from surrounded connective tissue(4) C. compressed premature follicle(1), degeneration of germinal layer of cortex surface (2) and hyperplasia of primordial oocytes(3) D.

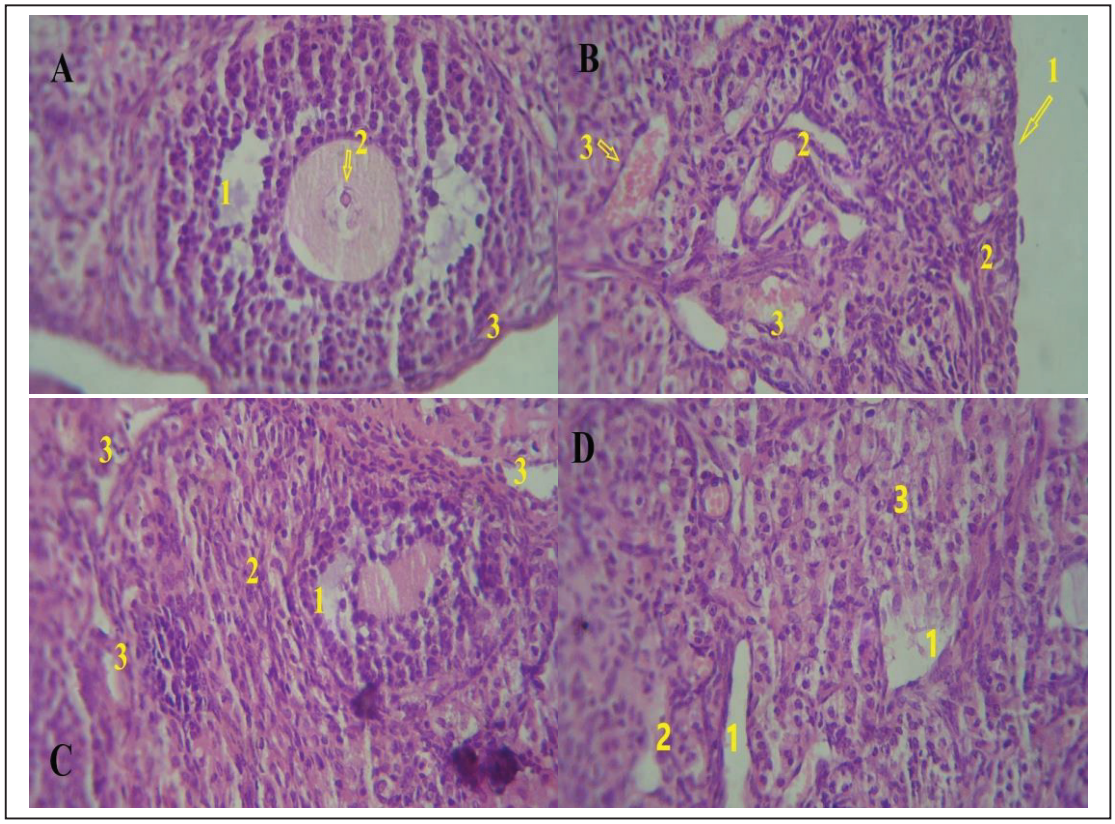

Fig (4): vacuolation in granulosa layers of premature follicle (1), remnant of oocyte of premature follicle(2) and degeneration of germinal epithelium cells of cortex surface(3)A. disappearance of germinal epithelium(1), disarrangement of the other primary follicles with compressed it (2) and partial congestion of blood vessels(3) B. degeneration of follicular cells layer in premature follicle(1), hyper cellularity of connective tissue(2) and vacuolation in between collagen fibers(3) C. In medulla of ovary showed empty of blood vessels(1), spaces in between follicular connective tissue (2) and hypertrophy of the stromal cells(3) D. H&E, 40X.

Discussion

The present study was designed to demonstrate the effect of Isoxicam after application on mice for 21 continuous days, so the Isoxicam was intraperitoneal injection for three different doses The application of this drug demonstrated many histopathological changes in ovaries was affected at different degrees, which means that increasing the concentration lead to severely effect. The female reproductive system is considered to be the most important organ. It characterized by two main functions, synthesis of sex hormones and produce the oocytes [(13)]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the more effectively to reduce pain and inflammation [14].

COX-2 produce prostaglandin which plays a major role in ovulation and fertility. NSAIDS or COX-2 inhibitors which effect on follicle rupture, ovulation, fertilization, luteolysis and parturition when treated rats with indomethacin [15].

Our data was in agreement with [16] who described the effect of ibuprofen at therapeutic dose in mice which induced a histological alteration such as a sequence of events of development and growth of ovarian follicles, increased the number of atretic follicles, Degenerated oocytes of matured follicles with vacuolated stroma. The administration Sodium Metabisulfite on rats which induced histological changes represented by reduced volume of the ovary as well as a decrease in the number of growing follicles, corpus luteum and an increase in the number of atretic follicles. Due to increased lipid peroxidation in the ovarian tissue [17] this study was agreement with our results. The administration of tarragon extract flavonoids reduces cyclooxygenase enzyme and nitric oxide, and thus reduces the amount of prostaglandin which effect on follicle growth [18].Oral administration with indomethacin causes ovulatory dysfunction, represented by the occurred of abnormal follicles at the, with degenerated granulosa cells and reduced follicular fluid of secondary follicle, all these

defect due to COX-2/ prostaglandin synthesis inhibition [19]. Treated rats by atropine sulphate was induced degeneration of granulosa cells and disappearance of antrum the inhibition gonadotrophin release and prostaglandin synthesis[20]. The application of this drug was indicated the insult of Isoxicam in any concentration even therapeutic dose, so our suggestion that this drug must not be used by owner, just used by supervision of the doctors.

Conclusion

The present study revealed that Isoxicam caused a clear histological alterations in ovarian tissue, including hyperplasia of germinal cells on the surface of ovary. hyper cellularity in follicular cells around follicular cavity, tongue like projection of primordial oocytes extend to the medulla, disappearance of oocytes with decreased amount of follicular fluid, vacuolation in granulosa layers of premature follicle with remnant of oocyte of premature follicle. Therefore it is recommended that usage of this drug have harmful side effects on female fertility.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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