Egypt. J. Plant Prot. Res. Inst. (2020), 3 (4): 1183-1194

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Toxicity of apricot seeds extracts as a rodenticide for climb rats *Rattus rattus* (Rodentia: Muridae)

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ARTICLE INFO

Article History

Received: 9 / 11 /2020

Accepted: 22 / 12 /2020

Keywords

Apricot seeds extracts, *Rattus rattus*, toxicity and histopathological changes.

Abstract:

This study investigated the efficacy of the apricot seeds extracts in *Rattus rattus* L. (Rodentia: Muridae). The used of 28 rats has been selected to study acute and chronic toxicity to start a trial in the non-choice test. three animals for each of acetone, ethanol, hot water, cold water extract , and powder seeds. Five animals were used for hexane extract twice LD_{50} apricot seeds crushed maize bait and seven animals were used for LD_{50} apricot seeds hexane extract crushed maize bait. The concentration used for LD_{50} and twice LD_{50} were calculated 2.14 and 4.28%, respectively. The experiment in a non-choice laboratory test, results indicated for an experiment that LD_{50} of hexane apricot seeds extract highest toxicity treatment after 48 hrs was recorded 71.43% and acceptability 48.87%, twice LD_{50} of apricot seeds hexane extract bait recorded mortality 60% and acceptability 41.47%. The most acceptable bait was cold water apricot extract recorded 92.76%. Histopathological changes after 28 days for twice hexane extract and seeds powder in kidney showing membranoproliferative glomerulonephritis and the renal tubules some renal tubules showing costs, liver showing widely dilated hepatic sinusoids, ballooning degeneration and disorganization of hepatocytes. Testis showing undulation and buckling of basal laminae of the seminiferous tubules, there is maturation arrest at various stages of spermatogenesis. Ovary showing without any cellular content and atrophied.

Introduction

Many plant extracts show a broad spectrum of activity against pests and such products have long been touted as attractive alternatives to synthetic chemical pesticides for pest management because they pose little threat to the environment, the Among these extracts are bitter apricot seeds (Cho *et al*., 2013). The cyanogenic compound is present mainly as glycoside in more than 2650 plant species. Apricot kernel, peach kernel, cassava, almond, bamboo shoot, sorghum, Japanese apricot, flaxseed among others have been consumed by humans worldwide either as food or as herbal medicine. Cyanogenic glycosides are natural plant toxicants (Bolarinwa *et al*., 2015). *Hydrogen cyanide* (HCN) is a powerful and rapidly acting poison. There are various forms of cyanogenic compounds that release hydrogen cyanide upon breakdown. Amygdalin is one of the nitriloside, natural cyanide-containing

substances abundant in the seeds of plants of the prunasin family (Chang *et al*., 2006). It is a major component of apricot kernels, bitter almonds, peach, plum, pear, and apple seeds (Conn, 1974 and Tanyildizi, 1997). It is widely distributed in plants, especially in the rosaceous plant seed, for example, apricot, peach, cherry, plum. Side effects of amygdalin ingestion in humans mirror symptoms of cyanide poisoning which includes nausea, vomiting, headache, dizziness, bluish coloration of the skin, liver damage, hypotension, nerve damage, fever, mental confusion, coma, and death (Howard-Reuben and Miller,1984). Rodents are one of the most dangerous pests in Egypt. Among the common types of rodents (Climb and Norway rats). Rodents were also considered a serious pest on crops and stored goods and have behavioral phenomena such as high intelligence and a strong sense of smell, which makes it difficult to control.

The repeated use of chemical rodenticides, in agriculture pest control programs, led to the development of many serious problems affecting the control success. These problems are pest resistance to these rodenticides and the pollution of the environment. Therefore, there is a growing need to find new natural extractions, which have a strong effect against pests but safe and friendly to the environment to ensure the future safety of agricultural food required for the world population without destroying the ecosystems.

The aim of this study developing a method to control the rats by using natural plant extracts environmentally safe that has no side effect on non-target (Human, animal, and plant) in the environment.

Materials and methods

1. Collection of apricot seed samples:

Apricot seeds are self-collected from apricot available on the market from different varieties. Seeds were washed with water to remove contaminating materials then left the seeds in the air to dry completely until they are suitable for grinding. Vetter (2000) recorded; apricot seeds contain approximately 20 – 80 μmol/g and 100 μmol/g of amygdalin, respectively. Amygdalin is also called bitter apricot, laetrile, almond; it is a cyanogenic compound and belongs to the aromatic cyanogenic glycoside group (Holzbecher *et al.*, 1984 and Santos Pimenta *et al*., 2014). Molecular formula is: C20H27NO11, the molecular weight is 457.42. The chemical structure is D-mandelonitrile-β-D-glucoside-6-β-glucoside.

2. Preparation of seed water extracts:

The method of Metspalu *et al* . (2001) was adopted for the preparation of water extracts 200 gm. of apricot fruit powder soak about 400 ml water in a 1000 ml flask . The powder is mixed with water in the electric mixer; the solution is covered and left for 72 hours. The solution is then filtered and used to obtain dry raw materials. The same method was followed with the remaining seed extracts but by replacing the cold water with boiling water. Then, test the raw materials to see the effect of cold water and boiled water extracts on rats *R. rattus.*

3. Solvent extracts:

The extracts were prepared by grinding apricot seeds until obtaining a fine powder, weighing 250 g of powder sock about 400 ml,

and placing it in a 1000 ml flask. The powder is soaked in a solvent (Ethanol – Hexane – Acetone) for 72 hours. The solution is then filtered and used to obtain dry raw materials .The solution extracted by a rotary evaporator is evaporated to full dryness, then weighed and dissolved in a certain amount of ethanol to prepare the stock solution.

4. Animals test:

Healthy animals elected climbing rat's *R. rattus*, from Abou-Rawash District, Giza governorate. . Rats were individually caged in wire cages (50 x 30 x 20 cm), acclimatized for at least 2 weeks. Food (Dried bread) and water were provided *ad libitum.* Inactive (Unhealthy) rats were excluded. Choice of 28 rats has been selected to start the trial. Three animals for each treatment of extract acetone, ethanol, hot water, cold water, and powder seed twice LD_{50} and used 5,7 animals of twice LD_{50} and LD_{50} hexane extract. Studies were conducted on rats to test the effect of apricot seeds extract toxicity/bait formulations and its histopathological effect. Studies were conducted on rats to test the effect of apricot seeds extract toxicity/bait formulations and its histopathological effect.

5. Study the effect of climb rats in non-choice feeding tests:

A known amount (25g) of crushed maize was placed in a buttery dish in pre-treatment and a known amount (Twice $LD_{50,}$ LD_{50}) of amygdalin apricot seed extract bait mixing was provided daily to each of 3,5,7 caged rats for 4 days in the treatment test. The concentration used from the extract of apricot seeds and seed powder twice $LD_{50,}$ LD50 (0.15% / rat) were 4.28 and 2.14% respectively. Water was provided to rats *ad libitum*. Consumption has been, a number of dead rats were daily recorded. Rats of each treatment were sacrificed by an overdose of chloroform, kidney, liver, testis and ovary and, were dissected out for gross as well as for histopathological examination. Males and females of the group were sacrificed after 28 days of treatment.

The acceptability and mortality of rats were calculated. Finally, observation to period 4week with plain bait (Crushed maize) *ad libitum.* The acceptability and mortality of rats were calculated using the following equation (Mason *et al.*, 1989).

$$Acceptability\ \% = \frac{Average\ daily\ \ consumption\ \ of\ treated\ food\ (g)}{Total\ average\ daily\ \ consumption\ \ of\ (treated + untreated)\ food\ (g)}\ X\ 100$$

6. Histopathological examination:

For histopathological examination, the collected specimens were fixed in 10% neutral buffered formalin for at least 24 hours and then routinely processed by conventional method and finally stained with heamatoxylene and Eosin (Suvarna *et al*., 2013).

The preparations were examined by the light microscope for identifying the presence of histopathological changes for kidney, liver testis, and ovary for different treatments of apricot seeds extract.

7. Statistical analysis:

The results were statistical analyses using the standard statistical methods LSD- test was applied in the analysis by **SAS (2003).**

Results and discussion

1. The effect of climb rats in non-choice feeding tests:

The mean lethal dose (LD_{50}) of amygdalin in rats was reported to be 880 mg/kg body weight (BW) by oral administration (Adewusi and Oke, 1985 and Park *et al.*, 2005).

The number of cyanogenic glycosides in plants varies with plant species and environmental effects (Conn, 1979). After oral administration, amygdalin is hydrolyzed by ruminal microorganisms and released as benzaldehyde, glucose, and cyanide. Both glycosides and released cyanide have toxic effects on animals (Majak, *et al*., 1990 and Tanyildizi, 1997).

This glycoside is absorbed unmetabolized in the jejunum of the rat via the transport system of glucose to the blood and is then concentrated in the spleen, liver, kidney, stomach, and intestines (Strugala *et al*., 1995 and Adewusi and Oke, 1985). Action by endogenous plant enzymes can release hydrogen cyanide causing potential toxicity issues for animals including humans (Bolarinwa *et al*., 2015) including cell death by blocking cytochrome oxidase and the arrest of ATP production.

The acute toxicity experiments of amygdalin have proved that the toxicity of the oral administration route is far greater than the intravenous route (Oyewole and Olayinka 2009). Oral administration of amygdalin at its therapeutic dose to rats for 14 days resulted in death and toxicity due to cyanide poisoning. Co-administration of amygdalin with hydroxocobalamin prevented mortality and significantly reduced amygdalin toxicity in the blood and liver of rats. Therefore, conclude that co-administration of amygdalin with hydroxocobalamin effectively reduced the extent of cyanide poisoning arising from oral amygdalin ingestion in rats.

The results indicated in Table (1) that the high acceptability of rats was recorded 92.76% of cold water extract bait high than hot water, power seed, ethanol, acetone, and hexane extracts bait. The results are recorded 72.25, 55.43, 48.74, 42 and 39.22% apricot seeds extract crushed maize baits, respectively. Rats mortality was observed during the treatment and after 96 hrs.of treatment in the case of hexane extract bait recorded 60% death rates did not appear in the remaining treatments.

That the average daily consumption significant change between the pre-treatment and treatment test for rats in hexane extract bait was 8.71 and 6.17g., acetone extract 9.49 and 6.87g., hot water extract 4.96 and 6.90g, and powder seed apricot bait 5.44 and 6.98g., respectively. Non-significant change between the average daily consumption in pre-treatment and treatment test for rats in ethanol extract bait 8.51 and 8.09g., cold water extract bait 6.36g and 6.86g respectively.

Table (1): Effect of average for apricot seed extracts crush maize bait 4.28% for climb rats daily consumption (g±SD), acceptability, and mortality under laboratory conditions.

Treatment twice (LD_{50})	R	Average daily consumption g/rat										M%	A%
		Pre-treatment					Treatment						
		1st day	2nd day	3rd day	4th day	A	1st day	2nd day	3rd day	4th day	A		
Hexane extract	5	9.25±1.21	8.74± 0.81	8.59± 0.87	8.28± 1.10	8.71± 0.19 ^{A}	6.16±1.10	6.21±0.78	6.05±1.41	6.27± 1.29	6.17± 0.28 ^{B}	60	41.47
Acetone Extract	3	9.66±1.36	9.34± 1.05^{A}	8.92± 1.52	10.05±0.82	9.49±0.31^{A}	6.95±0.06	6.73±0.07	6.85±0.26	6.96±0.07	6.87± 0.10^{B}	0	42
Ethanol	3	9.86±0.81	9.48±0.53	7.28±2.60	7.40±2.74	8.51±1.16^{A}	6.17±2.92	7.86±4.20	7.21±5.34	11.12±7.18	8.09± 1.81^{A}	0	48.74
Hot water extract	3	5.05±0.47	4.83±0.28	5.04±0.54	4.91±0.21	4.96±0.16^{B}	6.94±0.05	6.83±0.15	6.88±0.19	6.93±0.07	6.90±0.07^{A}	0	72.25
Cold water extract	3	6.36±1.02	6.04±1.12	6.86±11.13	6.36±1.07	6.36±0.05^{A}	6.88±0.12	6.78±0.13	6.83±0.25	6.94±0.08	6.86±0.07^{A}	0	92.76
Powder seed	3	5.56±0.12	4.75±0.25	5.85±0.54	5.72±0.10	5.47±0.20^{B}	7.61±1.21	6.73±0.20	6.80±0.35	6.77±0.39	6.98±0.45^{A}	0	55.34

The vertical columns marked with the same letters are not significantly different by SAS (2003).
R: Replicate A: Average M%: Mortality A%: Acceptability.

Table (2) showed that the average daily consumption and acceptability of hexane extract crush maize bait 2.14% of rats (LD_{50}), was 48.87%. Rats mortality was recorded at 71.43% during the treatment after 48h and 72hrs.of treatment. Non-significant change between the average daily consumption in pre-treatment and treatment test for rats of hexane apricot seeds extract 7.02g and 6.70g respectively with 1.8 fold in crushed maize. From the data mention above the acceptability increased when decreased treatment and increased mortality of in case hexane from LD_{50} from twice LD_{50} hexane extract bait. Amygdalin itself is non-toxic, but its production of HCN decomposed by some enzymes is a poisonous substance (Suchard *et al*., 1998). Both glycosides and released cyanide have toxic effects on animals (Strugala *et al*., 1995 and Adewusi and Oke, 1985). Action by endogenous plant enzymes can release hydrogen cyanide causing potential toxicity issues for animals including humans after the incubation of amygdalin, the Amygdalin and laetrile (a synthetic form of amygdalin) are commonly used as complementary or alternative medicine (CAM) for the treatment of cancer. Vitamin C is known to increase the in vitro conversion of amygdalin to cyanide and reduce body stores of cysteine, which is used to detoxify cyanide. Amygdalin has been used for decades by patients with cancer who are seeking alternative therapies, and severe reactions have not been reported with this dose. An interaction with vitamin C is a plausible explanation for this life-threatening response. The use of the drug was discouraged when it was demonstrated that amygdalin is metabolized in the body to release a significant amount of cyanide thus leading to cyanide poisoning (Bromley *et al*., 2005; Chandler *et al*., 1984).

Table (2): Effect of average hexane apricot seed extract crush maize bait 2.14% for climb rats daily consumption (g+SD), acceptability, and mortality under laboratory conditions.

Treatment (LD_{50})	R	Average daily consumption g/rat										M%	A%
		Pre-treatment					Treatment						
		1st day	2nd day	3rd day	4th day	A	1st day	2nd day	3rd day	4th day	A		
Hexane extract	7	7.06± 2.20	7.05± 2.48	7.07± 2.94	6.89± 2.44	7.02±0 .31^{A}	6.14± 1.14	7.02± 2.14	6.51± 1.13	7.14± 0.97	6.70± 0.53^{A}	71.43	48.87

The vertical columns marked with the same letters are not significantly different by SAS (2003).
R: Replicate A: Average M%: Mortality A%: Acceptability.

2. Histological studies:

Histopathological changes in liver, kidney, testis, and ovary in survived rat fed apricot seeds extract crushed maize bait during 4 days in non-choice test after 28 days of treatment in the following.

2.1. The kidney of the rat was treated with apricot seeds hexane extract and powder seeds 4.28% apricot crush maize bait:

The kidney of rat treated with apricot seeds extraction with hexane Figure (1), showing membranoproliferative glomerulonephritis and the tubules illustrating necrobiotic changes of the renal tubular epithelium where the cells revealing deeply eosinophilic

cytoplasm with pyknotic nuclei and some renal tubules showing renal costs .The kidney of rat treated with apricot seeds power Figure (2), the glomeruli showing membranoproliferative glomerulonephritis and the renal tubules showing necrobiotic changes of renal tubular epithelium, some renal tubules showing costs. Normal of kidney Figure (3) showed no alteration observed and normal histological structure of the glomeruli and renal tubules in cortex. Results of our previous apricot seeds/amygdalin studies described variety of effects a slight increase in renal parenchyma dystrophy of rabbits fed apricot seeds (Kolesárová *et al.*, 2017) Anyway, consumption of bitter apricot seeds (60 mg/kg bw) for 42 days had a significant effect on amount of calcium excreted in human urine and significant changes were also observed in phosphorus levels in urine after apricot seed ingestion. In another study, we observed decrease in calcium in human urine after 84 days of apricot seeds consumption. The same study showed decreasing tendency in urine urea (Tušimová, Kováčik *et al*., 2017).

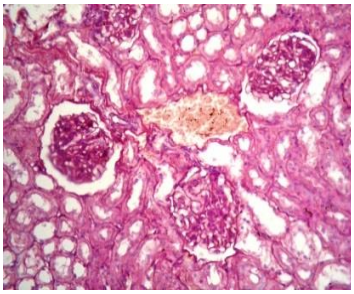

Figure (1): Kidney apricot seeds extract with hexane (H and E X400).

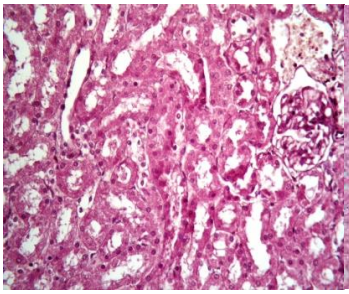

Figure (2) : Kidney apricot seeds power (H and E X400)

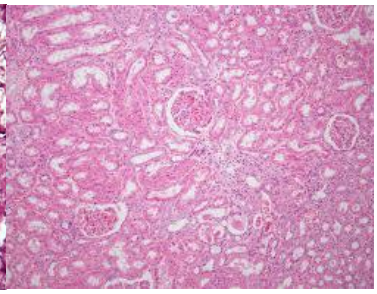

Figure (3): Normal kidney (H and E X100).

2.2. Liver of the rat was treated with apricot seeds hexane extract and powder seeds apricot 4.28% apricot crush maize bait:

Liver of rat treated with apricot seeds extraction with hexane Figure (4). Showing widely dilated hepatic sinusoids and most of the hepatocytes showing ballooning degeneration and others showing granular degeneration, also the hepatocytes showing disorganization. Liver of rat treated with apricot seeds power Figure (5). The hepatocytes showing ballooning degeneration and disorganization of hepatocytes with focal area individual cellular necrosis Figure (6) The normal histological structure of liver with no alteration observed and normal histological structure of the center veins, hepatic cords and sinusoids.Some reports indicate a toxicity problem after consumption of apricot and other fruit seeds represented by elevated liver chemistry tests (Seghers *et al*., 2013). Contrary, Abdel-Rahman (2011) suggested amygdalin to be a possible option in hepatic fibrosis prevention. Increase in serum ALT indicates liver damage more specifically than AST. It has been reported.

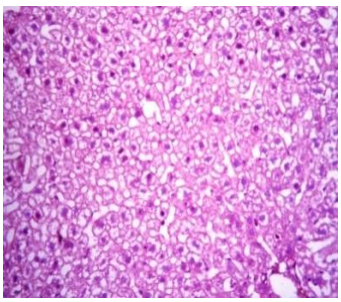

Figure (4): Liver apricot seeds extract with hexane (H & E X400)

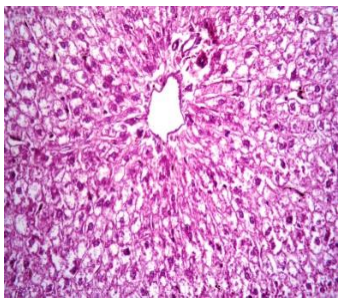

Figure (5): Liver apricot seeds power (H & E X400)

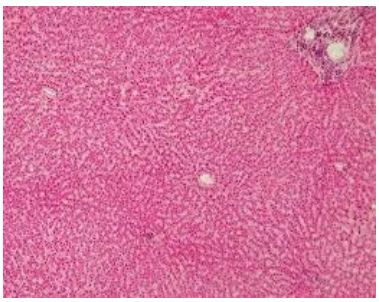

Figure (6); Normal liver (H & E X100)

2.3. The testis of rat treated with apricot seeds powder crush maize 4.28%:

The testis of rat treated with apricot seeds power Figure (8) showing undulation and buckling of basal laminae of the seminiferous tubules with interstitial edema decreased the number of germinal epithelium and also there is maturation arrest at various stages of spermatogenesis. The testis of control showed Figure (7) normal histological structure of the active mature seminiferous tubules and interstitial leydig cells. (Eduard *et al.*, 2015). Describe the characteristics, metabolism, and possible effects of amygdalin on reproductive processes. Previous studies describe the effects of natural compound amygdalin on female and male reproductive systems focused on the process of steroidogenesis, spermatozoa motility, and morphological abnormalities of spermatozoa. In accordance to the previous studies on amygdalin, its benefit is controversial. The resulting sperm are malformed and are morphologically similar to abnormal sperm seen in some cases of human male infertility. The spermatozoa motility decreased very significantly ($P<0.001$) in a dose-dependent manner and all spermatozoa were immobile at 10 min. Besides, the percentages of morphological abnormalities did not change in comparison with the control group. The control values were between 4.21% and 6.87% (Tanyildizi and Bozkurt, 2004). It is not known whether the amygdalin crosses the blood-testes barrier. It has been reported that the hyaluronidase enzyme plays an important role in supporting spermatozoa penetration into the cumulus oophorus matrix (Meyers and Rosenberger, 1999). Hyaluronidase activities were inhibited significantly by amygdalin ($P<0.01$) (0.4 to 2 μM). The inhibition of spermatozoa hyaluronidase activity and spermatozoa motility showed that these compounds have deleterious effects on bull spermatozoa in vitro (Tanyildizi and Bozkurt, 2004).

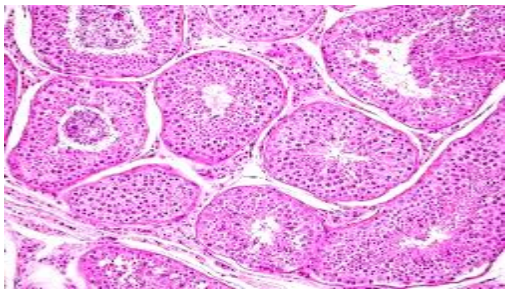

Figure (7): Normal testis (H & E X400)

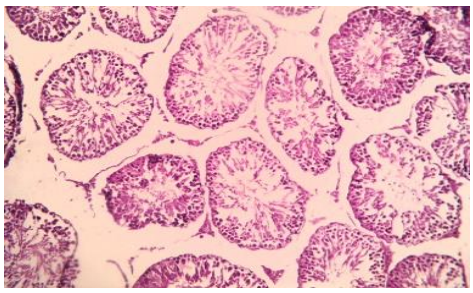

Figure (8): Testis treated apricot seeds powder (H & E X400)

2.4. The ovary of the rat was treated with apricot seeds hexane extract crush maize bait 4.28%:

Ovary of female rat treated with apricot seeds extraction with hexane Figure (9) showing the graafian follicle without any cellular content and atrophied high power and necrosis of ova of ovary. Figure (10) cycling active ovary female rat showing mature ovarian follicle secondary follicle with oocyst ant its nucleus in the center and antrum antrum. Previous studies examined the effects of natural compound amygdalin on female reproductive system concentrated on secretion activity of porcine ovarian granulosa cells (GC) in vitro (Halenár *et al*., 2013a). The release of steroid hormone progesterone by granulosa cells from cyclic and non-cyclic porcine ovaries was not affected by the amygdalin addition (1, 10, 100, 1000, 10 000 μg/mL). But on the other hand, amygdalin (at 10 000 but not at 1, 10, 100, 1000 μg/mL) combined with mycotoxin deoxynivalenol (DON) (1000 ng/mL) significantly ($P \leq 0.05$) stimulated the release of steroid hormones progesterone and estradiol by granulosa cells from non-cyclic porcine ovaries (Halenár *et al*., 2013b). Richard *et al.* (2000) impaired reproductive function accompanies chronic renal insufficiency (uremia) in the experimental animal. Clinical hypogonadism occurs in both genders anti-ovulatory effects of uremia in the female rat were recorded.

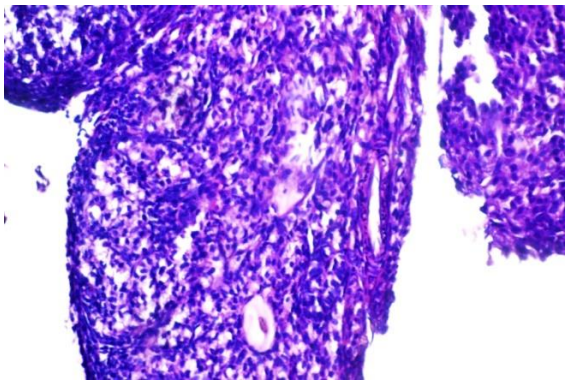

Figure (9): Ovary treated apricot seeds power (H&EX400).

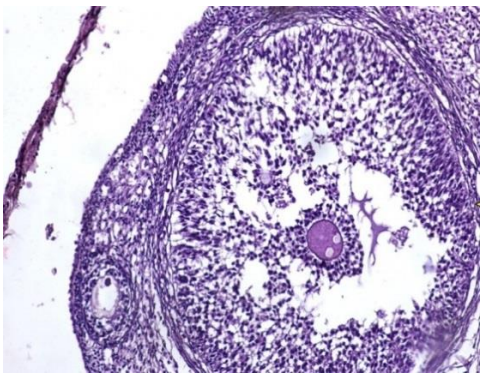

Figure (10): Normal ovary (H&EX400).

This study indicates that Amygdalin itself is non-toxic, but HCN production decomposed by some enzymes is a toxic substance. The possible effects of natural compound amygdalin on reproduction were shown in previous studies. The mechanism of action of amygdalin is unknown. The toxic effect of amygdalin or its benefit is controversial, and the realization of in vivo and vitro experiments is necessary.

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