Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2022, Issue-1, pp-83-94
Research Paper DOI: 10.21608/bfszu.2022.114270.1108

Biological Activity of Fused Pyrimidine Derivatives on Vital Parameters of Male Albino Rats

Hassan A. El-Sayed[1], Mohamed G. Assy[1], Aly A. EL-Sheakh[2], Weam M. Mahmoud[1,2*]

1 Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt

2 Department of Insect Physiology, Plant Protection Institute, Sharkia Branch, Zagazig, Egypt

***Corresponding author email**: weammahmed87@gmail.com

ABSTRACT : ***The objective of the current study was to evaluate the toxicity of oral administration of three synthesized organic compounds by us; 2-Amino-3-benzyl-6-(benzylthio) pyrimidin-4(3H)-one (amino pyrimidine), 3-Benzyl-7-hydroxy-5-(4- hydroxyphenyl)-2-(phenylamino)pyrido [2,3-d]pyrimidin-4(3H)-one (pyridopyrimidine) and N -{[1-Benzyl-4-(benzylthio)-6-oxo-1,6-dihydropyrimidin-2-yl]carbamothioyl}benzamide (pyrimidine benzamide) for 28 days on some biochemical parameters and hepatic and renal histopathological changes in male albino rats. All the three tested pyrimidine's derivatives showed significant decrease of the body weight, total proteins and significant increase in liver weight and liver index when compared to control group. Additionally, the activities of serum transaminases (ALT and AST) and alkaline phosphate (ALP) were increased significantly in amino pyrimidine, pyridopyrimidine and pyrimidine benzamide treated groups when compared to control group. Moreover, significant inhibition of serum cholinesterase activity (ChE) and hemoglobin (Hb) value were observed with oral administration of the three investigated pyrimidine's derivatives in comparison to control rats. Concerning renal function indicators; serum urea was increased significantly in rats after receiving amino pyrimidine, pyridopyrimidine and pyrimidine benzamide. On the other hand, creatinine levels were increased by administration of amino pyrimidine, pyridopyrimidine and pyrimidine benzamide.The MDA level was detected to be increased significantly and TAC was decreased significantly in rats received amino pyrimidine, pyridopyrimidine, and pyrimidine benzamide. Histopathological examination of rats administered amino pyrimidine, pyridopyrimidine and pyrimidine benzamide, revealed disturbed hepatic and renal architecture that confirm the biochemical results which report hepatic and renal damage. The obtained data demonstrate the toxic effect of the evaluated fused pyrimidine derivatives in male albino rats.***

KEYWORDS: Pyrimidine Benzamide, Liver, Hemoglobin, Renal Damage, Albino Rats.

Date of Submission: 02-01-2021 Date of acceptance: 20-01-2022

I. INTRODUCTION

Pyrimidine's derivatives exhibited rodenticidal activity against rats that had developed resistance against available rodenticides. Pyrimidines and fused pyrimidines are heterocyclic aromatic compounds with a particular interest because they represent a large class of natural and synthetic compounds; many of which have biological and therapeutic applications [1, 2]. Pyrimidine and fused pyrimidines are significant in agriculture chemicals; insecticides [3, 4], rodenticide [5, 6], fungicides [7, 8], and herbicides [9, 10]. Pharmaceutical's preparations are some of the biological activities of fused pyrimidines derivatives [11, 12], e.g., anticancer [13, 14], antimicrobial agents [15, 16] and antibacterial [17]. Resistance developed against pesticides limit their use and required the improvement of new agent [18].The present study focused on the toxicity of three synthesized

compounds by us; Amino-3-benzyl-6-(benzylthio) pyrimidin-4(3H)-one (amino pyrimidine), Benzyl-7-hydroxy-5-(4- hydroxyphenyl)-2-(phenylamino) pyrido [2,3-d]pyrimidin-4(3H)-one (pyridopyrimidine) [19] and 4-(benzylthio)-6-oxo-1,6-dihydropyrimidin-2-yl]carbamothioyl}benzamide (pyrimidine benzamide) [20] on the biochemical parameters, histopathological changes in tissue specimens of different organs in male albino rats.

II. MATERIALS AND METHODS

Chemicals and reagents

Kits for cholinesterase activity was purchased from Abcam (Cambridge, MA, United States). Kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, urea, total proteins and total antioxidant capacity (TAC) were purchased from Bio-Diagnostic CO. (Dokki, Giza, Egypt). While hemoglobin (Hb) level in the rat blood was determined using Rat Hemoglobin (Hb) ELISA Kit Cat No. MBS2607780 that was purchased from My Bio Source (Southern California, San Diego, USA). All the chemicals were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), unless otherwise specified. Three synthesized organic compounds, 2-Amino-3-benzyl-6-(benzylthio)pyrimidin-4(3H)-one (amino pyrimidine), 3-Benzyl-7-hydroxy-5-(4- hydroxyphenyl)-2-(phenylamino)pyrido [2,3-d]pyrimidin-4(3H)-one (pyridopyrimidine)[19] and N-{[1-Benzyl-4-(benzylthio)-6-oxo-1,6-dihydropyrimidin-2-yl]carbamothioyl}benzamide (pyrimidine benzamide) [20].

Experimental animals and protocol

Twenty-four adult male albino rats, weighting 140 - 170 gm. were purchased from the breeding unit of Faculty of Veterinary Medicine (Zagazig University), the experiment and animal handling were designed following the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) ensuring minimal hardship of animals. Rats were housed at suitable atmospheric temperature (22 ± 2 °C) and normal 12 h light/ dark cycle, with free access to water and standard pellet diet. Rats were allowed to acclimatize for 1 week prior to starting the experiment. Rats were randomly allocated into four equal groups: control group, rats were administered water instead of the tested three compounds. Amino pyrimidine group, rats received amino pyrimidine 50 mg/kg. Pyridopyrimidine group, received pyridopyrimidine 50 mg/kg. Pyrimidine benzamide group, received 30 mg/kg pyrimidine benzamide. The used sublethal doses of the freshly prepared tested three compounds were one tenth of LD50 for each of them. Water or the compounds solutions were administered at a fixed volume (0.5 ml) orally by gavage tube for 28 days. LD50 for each of them were obtained by acute toxicity study, in which we administered different doses of the tested compounds and observe lethality for 24 hours and determine the dose which kill 50% of the animals that is termed LD50.

Data and Sample Collection

At the end of the experimental period and after overnight fasting (12 hours), rats were sacrificed by a general anesthetic (Ethyl ether). Blood samples were obtained through heart puncture and divided into 2 parts; one stabilized in heparinized tubes to obtain plasma for detection of Hb concentration. The other part of the blood was collected in glass tubes and retained for 30 min to clot then centrifuged at 1000 xg for 5min to obtain serum for biochemical analysis. After rat's scarification, liver and kidney were excised immediately, cleaned and weighed. The organ index was obtained by dividing organ weight on body weight and multiplies the result by 100 [21].

Assay of serum cholinesterase activity

Cholinesterase (ChE) activity was determined calorimetrically using Acetylcholinesterase Assay Kit (ab138871) according to [22]. Depending on the use of Ellman's Reagent DTNB (5, 5-dithio-bis-(2-nitrobenzoic acid) to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by Acetylcholinesterase (AChE). The absorption intensity of DTNB adduct (410 nm) was used to assess the amount of thiocholine produced, which is proportional to the AChE activity. The kits do not differentiate between AChE and butyrylcholinesterase (BChE) activity as both enzymes can hydrolyze acetylcholine.

Assay of liver function markers in the serum

Following the manufacturer’s instructions that depend on methods prescribed previously [23, 24], the serum activities of transaminases (ALT, AST) and alkaline phosphatase (ALP) were determined calorimetrically respectively.

Assay of kidney function markers in the serum
Urea concentration in serum was estimated by enzymatic colorimetric method [25]. Serum creatinine concentration was determined according to colorimetric method described by [26].

Total serum proteins quantification
Total proteins in serum were determined calorimetrically according to [27].

Assay of hemoglobin concentration (Hb)
Using Rat Hemoglobin (Hb) ELISA Kit, the levels of Hb were measured as described by the manufacturer's instructions.

Assay of serum Malondialdehyde (MDA)
Serum malondialdehyde (MDA) was measured by the method of [28]. Its principle depends on reaction between MDA in the serum with thiobarbituric acid (TBA) provided as a reagent in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be detected at 534 nm.

Assay of the total antioxidant capacity (TAC)
Total antioxidant capacity (TAC) level in the serum was determined spectrophotometrically according to the method described by [29]. Its main principle depends on capacity of all antioxidants in the serum sample to react with defined amount of exogenously provided hydrogen peroxide (H2O2). The antioxidants in the sample eliminate a certain amount of the provided H2O2. The residual H2O2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro –2– hydroxy benzensulphonate to a colored product.

Histopathological examination
Represented part of every rat liver and renal tissue was preserved in 10% neutral buffered formalin, embedded in paraffin for fixation. Paraffin blocks (5 µm thickness) were sliced then stained with hematoxylin and eosin dye (H&E) and examined under a light microscope. Assessment of the injury was done by a pathologist in a blinded manner.

Statistical analysis:

Data are expressed as means ± SEM. The statistical significance of the data was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using Graphpad prism software version 7 (GraphPad Software, Inc. La Jolla, CA, USA). The level of significance was taken as $p< 0.05$.

.III. Results and Discussion

Table (1) illustrates that administration of amino pyrimidine, pyridopyrimidine or pyrimidine benzamide for 28 days significantly ($p<0.05$) decreases the body weight when compared to control group. These results are in accordance with that observed by [30, 31]. It could be concluded that chronic pesticides administration leads to decreased appetite as a simple index of toxic effect which results in decrease of the body weight [32, 33]. In comparison to control rats, amino pyrimidine, pyridopyrimidine and pyrimidine benzamide showed significant ($p<0.05$) increase in liver weight and liver index as illustrated in table (1). These data agree with previously demonstrated results of [31, 34, 33] who reported increased liver weight with other pesticides. [35] attributed this hepatic enlargement to increased nuclear DNA synthesis and mitosis resulting in hyperplasia of hepatic cells that associates with chronic pesticide administration [36]. The results of the current study demonstrate insignificant decrease in kidney weights and kidney index of rats treated with the three tested fused pyrimidines derivatives when compared to control rats as given in table (1). These results were shared by [37] who reported nonsignificant difference in the relative kidney weights of rats treated with dimethoate for 30 days.

Table (1): Effect of oral amino pyrimidine, pyridopyrimidine and pyrimidine benzamide for 28 days on Body weight, Liver weight, Liver index, Kidney weight and Kidney index in male albino rats

	Control	Amino pyrimidine	Pyridopyrimidine	Pyrimidine benzamide
Body weight (gm)	249±3.85	230.7±6.36*	227.3±3.43*	222.8±3.85*
Liver weight(gm)	4.81±0.26	5.93±0.27*	7.01±0.18*	7.36±0.28*
Liver index (%)	1.93	2.57	3	3.3
Kidney weight(gm)	2.23±0.20	1.93±0.18	1.85±0.23	1.95±0.15
Kidney index (%)	0,89	0,83	0.81	0.87

All values are presented as the mean ± SEM (n= 6 rats/group). P value <0.05, * significant compared with control group.

As presented in figure (1), the activities of serum transaminases (ALT and AST) and ALP were increased significantly ($p < 0.05$) in amino pyrimidine, pyridopyrimidine and pyrimidine benzamide treated groups when compared to control group. Many authors reported significant enhancement of activities of serum transaminases and ALP with pesticides administration [30, 38, 35]. The formerly mentioned liver functional enzymes are normally cytosolic enzymes that located inside hepatic cells and their levels in the blood are low, so increase of their serum levels indicates leakage of hepatocytes cell membrane as a consequence of hepatocytes necrosis and cell membrane damage. Moreover, the increased serum level of ALP reflects impairment of its normal excretion in bile that may be due to inflammation of hepatocytes that injure bile canaliculi [39].

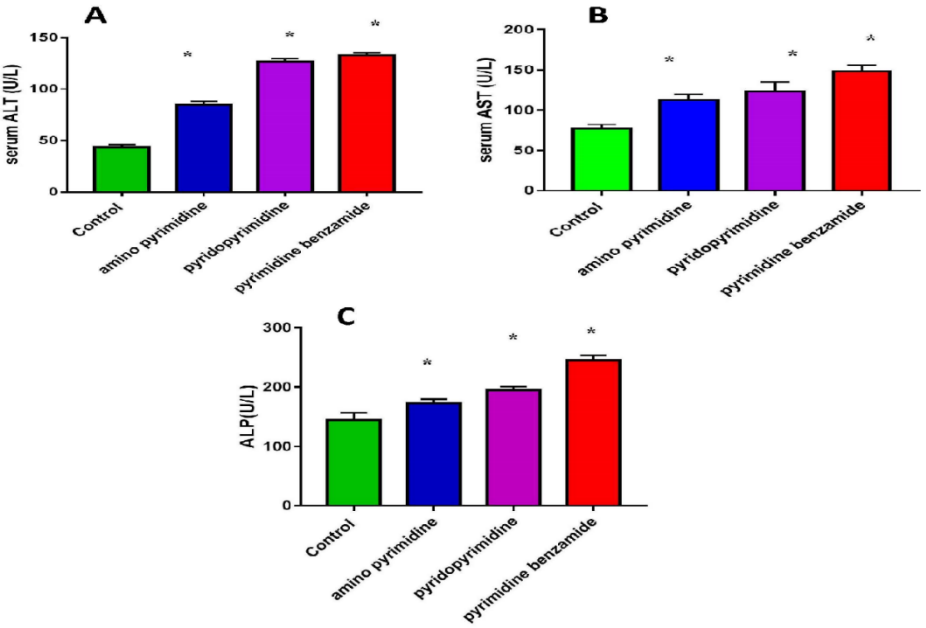

Figure (1): Effect of amino pyrimidine, pyridopyrimidine and pyrimidine benzamide administration for 28 days orally on serum (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), and (C) alkaline phosphatase (ALP) in male rats. Data are illustrated as mean ± SEM (n=6 rats/group). *Indicate significant difference (P<0.05) vs. control.

Serum urea concentration was increased significantly (P<0.05) in groups received amino pyrimidine, pyridopyrimidine and pyrimidine benzamide as compared to control group as presented in figure (2A). Moreover, the creatinine level was increased insignificantly (P<0.05) in rats treated with either amino pyrimidine or pyridopyrimidine and increased significantly (P<0.05) in rats treated with pyrimidine benzamide when compared to control rats as showed in figure (2B). These disturbances in the urea and creatinine suggest renal dysfunction. Previously, renal dysfunction was reported as manifestation to many chemicals administration including the pesticides [30, 31].

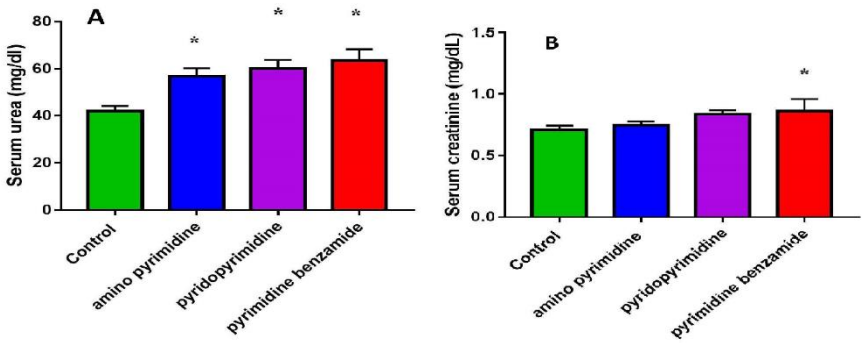

Figure 2: Effect of amino pyrimidine, pyridopyrimidine and pyrimidine benzamide administration for 28 days orally on serum (A) urea and (B) creatinine in male rats. Data are illustrated as mean ± SEM (n=6 rats/group). *Indicate significant difference (P<0.05) vs. control.

Regarding the serum ChE activity as demonstrated in figure (3); amino pyrimidine, pyridopyrimidine and pyrimidine benzamide treated rats showed significant ($p < 0.05$) inhibition of ChE activity in comparison to control rats. ChE activity is considered a standard biomarker of compounds poisoning. Equivalent results carried out by many authors [40, 41, 42] who reported inhibition of ChE activity in multiple animal species after exposure to different pesticide preparations. There are 2 types of cholinesterases; AChE which is termed truecholinesterase and BChE that is called pseudocholinesterase. AChE is present mainly in the synapses where acetylcholine (Ach) is released and also present in red blood cells however, BChE is found in blood plasma, liver, glia, and many other tissues. AChE cleaves Ach to yield choline and acetate in the synaptic cleft while, BChE is characterized by less specificity for Ach and does not contribute to a significant role in the metabolism of Ach in the synapse [43]. One of the main mechanisms of the pesticides is mediated by irreversible inhibition of AChE (the primary target) and BChE. Therefore, inhibition of BChE reflects inhibition of AChE. Inhibition of ChE increases the concentration of endogenous Ach at cholinoceptors [44]. Although Ach is a vital neurotransmitter, excess amount of it is toxic and can results in abnormal muscle contraction followed by muscle paralysis that can be fatal when include respiratory muscles. Additionally excess Ach depress respiratory center and death occur. So, multiple pesticides depend in its mechanism on inhibition of ChE to decrease Ach metabolism [45].

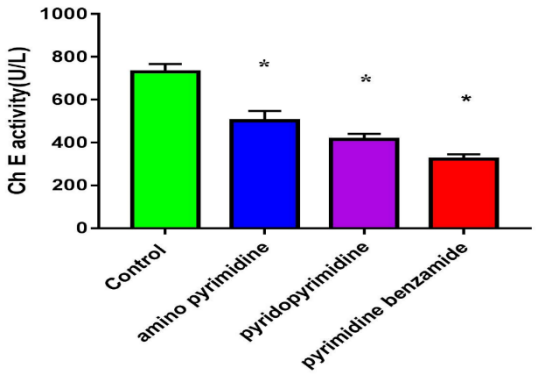

Figure (3): Effect of amino pyrimidine, pyridopyrimidine and pyrimidine benzamide administration for 28 days orally on serum ChE activity in male rats. Data are illustrated as mean ± SEM (n=6 rats/group). *Indicate significant difference (P<0.05) vs. control.

Data presented in figure (4 A) indicates significant (P<0.05) reduction in total proteins levels after oral administration of amino pyrimidine, pyridopyrimidine or pyrimidine benzamide when compared to control rats treated with water. Our data are in line with that published by [46] who reported decrease in serum albumin, globulin and total proteins after administration of pesticides for 4 weeks. This decline in level of total proteins can be explained by impairment of synthesis of proteins by the injured liver (as confirmed in the current work by increased transaminases serum levels and histopathologically) or enhanced its metabolism [39], [47]. Also, as demonstrated in figure (4B) there was a significant (P<0.05) amelioration of the Hb level in groups of rats treated with amino pyrimidine, pyridopyrimidine or pyrimidine benzamide compared to control rats. This result might be attributed to impaired synthesis of heme part of Hb in bone marrow [48] or deficiency of iron needed for formation of Hb. Iron deficiency secondary to pesticide administration may be caused by decreased its intake in dietary sources [49]. Another explanation for decline of Hb level was cited by [50] who attribute this to increase in the rate at which the Hb is destroyed. Concerning the serum MDA levels as an indicator of lipid peroxidation product, it was elevated significantly (p<0.05) in all the three groups treated with the tested compounds (amino pyrimidine, pyridopyrimidine and pyrimidine benzamide) when compared to control group as illustrated in figure (4C). Additionally, the serum TAC was demonstrated to be decreased significantly (p<0.05) in amino pyrimidine, pyridopyrimidine and pyrimidine benzamide treated groups when compared to control group as observed in figure (4D). These observed results point to oxidative stress, older literatures confirmed the role of oxidative stress in pathotoxicity of pesticides on multiple organs including liver, kidney, and brain [51, 33]. Apart from cholinergic toxicity (in the form of convulsion, respiratory failure) pesticides are reported to cause oxidative stress. Pesticides induce generation of excess amount of reactive oxygen species (ROS) e.g., hydrogen peroxide and superoxide anions and deplete endogenous antioxidants. The liberated ROS exert its hazardous effects by triggering oxidative damage to mitochondrial membranes, lipids, proteins and DNA resulting in cellular dysfunction [52]. These can explain the result of the current study in the form of increased polyunsaturated fatty acid lipid peroxidation product (MDA) and decline in the TAC, moreover it explains why liver and renal damage are observed biochemically and histopathologically in this study.

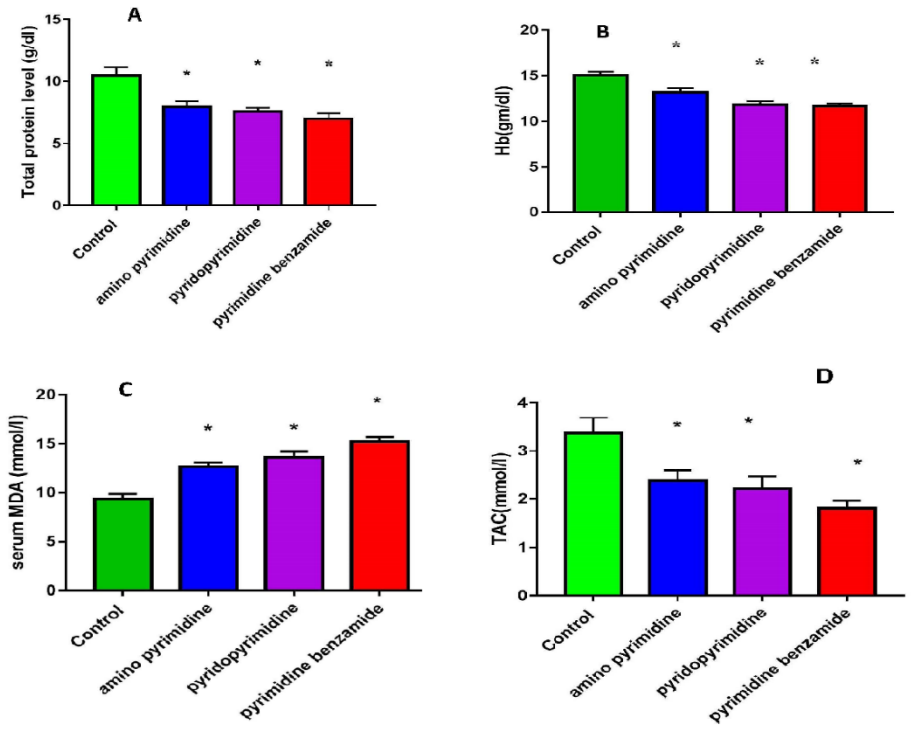

Figure (4): Effect of amino pyrimidine, pyridopyrimidine and pyrimidine benzamide administration for 28 days orally on serum (A) total proteins ;(B) hemoglobin (Hb); (C) malondialdehyde (MDA) and (D) total antioxidant capacity (TAC) in male rats. Data are illustrated as mean ± SEM (n=6 rats/group). *Indicate significant difference (P<0.05) vs. control.

Hepatic sections from control rats displayed normal liver architecture as observed in figure (5A). To the contrary, hepatic sections from rats exposed to oral administration of amino pyrimidine showed mild necrosis of liver tissue as illustrated in figure (5B). Additionally, oral administration of pyridopyrimidine resulted in noticeable lobular necrosis with dilated sinusoids, Portal inflammation and fibrosis as showed in figure (5C). What is more, pyrimidine benzamide administration resulted in a wide area of necrotic liver tissue surrounding a congested central vein as illustrated in figure (5D) These results are in agreement with that reported previously [53], that record histopathological hepatic abnormality in the form of inflammatory cells infiltration, congestion, hydropic degeneration and hepatocellular necrosis after administration of dimethoate, endosulfan and carbaryl in the liver of male rats. Furthermore, [54] observed hepatic severe congestion, lymphocytic infiltration, and inflammation in rats administered Fenitrothion.

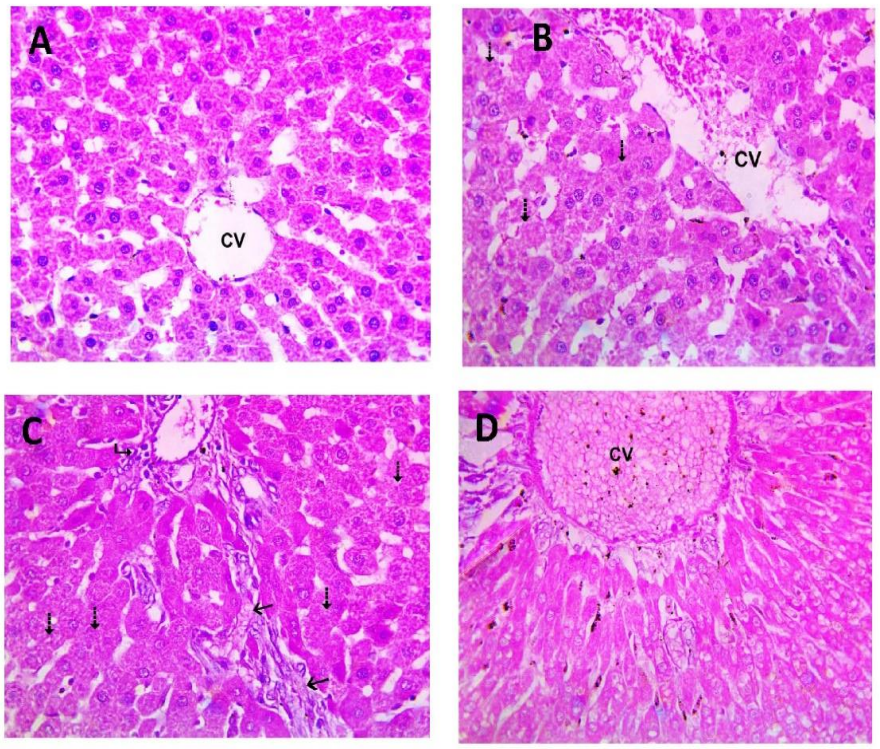

Fig (5): Representative hematoxylin and eosin-stained photomicrographs (H&E ×400) of rat liver sections, (A) Liver tissue section from the control group with a normal hepatic morphology, Central vein (CV). (B) Liver section represent amino pyrimidine treated group showing mild necrosis (dashed arrows) of liver tissue radiating from congested central vein (CV). (C) Liver section of pyridopyrimidine treated rat showing noticeable lobular necrosis (dashed arrows) with dilated sinusoids. Portal inflammation (right sided arrow) and fibrosis (arrows) could be seen. (D) Liver section showing wide area of necrotic liver tissue (ghosts of hepatocytes) surrounding a congested central vein (CV) in rat liver treated with pyrimidine benzamide.

Regarding the renal samples, sections from control group showed normal structure of glomeruli with intact Bowman space and normal tubules as illustrated in figure (6A). Amino pyrimidine treated rats showed mild glomerular hypercellularity with intact Bowman's space, focal tubular necrosis with loss of nuclei and few tubules showed casts as observed in figure (6B). Rats received pyridopyrimidine demonstrated glomerular hypercellularity (glomerular endotheliosis) leaving minimal space (Bowman's space) as showed in figure (6C). Tubules show loss of nuclei and fragmentation of the lining. Group treated with pyrimidine benzamide showed marked tissue destruction as observed in figure (6D). Most tubules showed necrotic changes with loss of cellular details (wide area of necrotic tissue) and other tubules show nuclei loss of the lining epithelial cells. Glomeruli show mild attenuation of Bowman's space. [54] also observed severe renal degenerative changes in rats, when have received Fenitrothion.

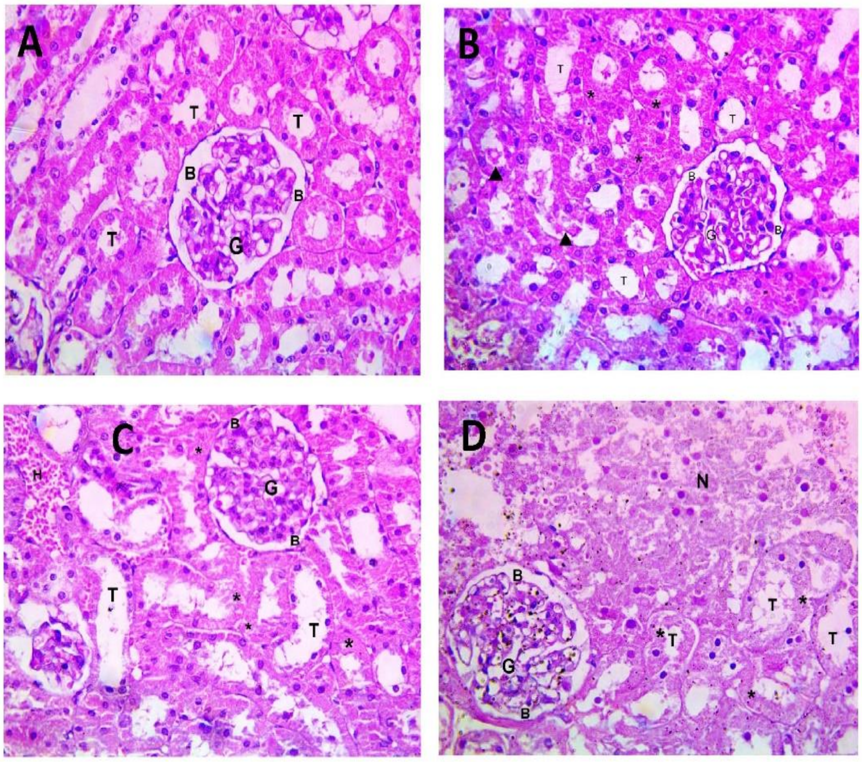

Figure (6): Representative histopathological images of rat's renal tissue stained with hematoxylin and eosin (H& E ×400). (A) Control group showing normal structure of glomeruli (G) with intact Bowman space (B), and normal tubules (T). (B) Group received amino pyrimidine showing mild hypercellularity of glomeruli (G) with intact Bowman's space (B). Focal necrosis with loss of nuclei (asterisks) seen in few tubules (T). Few tubules show casts (arrowheads). (C)Rats treated with pyridopyrimidine showing glomerular hypercellularity (glomerular endotheliosis) (G) leaving minimal space (Bowman's space) (B). Tubules (T) show loss of nuclei (asterisks) and fragmentation of the lining epithelial cells. The interstitial tissue shows areas of hemorrhage (H). (D) Group treated with pyrimidine benzamide showing marked tissue destruction (N). Most tubules (T) show necrotic changes with loss of cellular details (wide area of necrotic tissue) and other tubules show nuclei loss (asterisks) of the lining epithelial cells. Glomeruli (G) show mild attenuation of Bowman's space (B).

The results obtained herein indicate the three tested synthesized pyrimidines derivatives compounds are able to have a toxic effect on male albino rats as they produced both hepatotoxicity, renal damage besides their hazardous effects on the Hb and inhibition of ChE.

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