Clinical, biochemical and histopathological alterations referred to chlorfenapyr residues in male albino rats

By

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SUMMARY

In order to investigate the toxic effect of chlorfenapyr on some haematological and biochemical parameters as well as estimation of its residues in different body organs with reference to the pathological alteration induced. A total of 60 adult male albino rats were divided into 3 groups. The first group were given chlorfenapyr in a dose of 0.45 mg/Kg b.wt. by gastric intubulation daily for 28 days, the second group was given a dose as the first one then kept for 15 days as a recovery period and the third group of non treated rats was left as a control group. The results revealed a significant decrease in Hb concentration, PCV%, RBCes and WBCs of treated rats of both groups compared with the control one. Chlorfenapyr induced a significant elevation in ALT, AST, ALP, cholesterol, urea and creatinine, while a significant decrease was noticed in total protein, albumin and globulin in all treated rats. Residual analysis verified the presence of chlorfenapyr in brain, heart, lung, liver, kidneys, spleen and testes in both treated groups. Although these residues in the treated group after recovery period were less than the only treated group, values of both treated groups exceeded the limit of the American Cynamide Company (1998) and Federal Register (2002). Histopathological examination of both treated groups revealed neuropathy (leukoencephalomyelopathy), hepatopathy, nephropathy and infertility. These results may indicate that the body may need a recovery time more than 15 days or that the drastic aggressive toxic effect of chlorfenapyr was irreversible.

INTRODUCTION

Wide-spread application of pesticides in agricultural and veterinary field constitutes a major role in contamination of the environment. The presence of pesticide residues in human food even in extremely small quantities is considered a potential risk to human health. Pesticides are among the most widely used chemical in agriculture. Nowadays, the use of pesticides to eliminate pests or to regulate crop growth had led to pesticides residues in soils, air, water, stored grain-
ns, crops and plants at concentration levels which exceed the legal limits (Susse and Muller, 1996 and Hala and Elbadry, 2006). The great hazards caused by pesticides on the livestocks are due to their accidental exposure to these pesticides either by inhalation, skin absorption and/or ingestion (Yamanaka et al., 1996).

Chlorfenapyr is the first commercial pesticide to be derived from a class compounds produced by bacteria and known as halogenated pyrroles (Black, 1994; Albers et al., 2006). Synthesized in 1988 from a naturally produced chlorinated pyrrole, chlorfenapyr technical (CL 303630; 4-bromo-2-[4-chloropheyl]-1-[ethoxymethyl]-5-[Trifluoromethyl]-1H-pyrrole-3-carbonitrile) (Fig., 1) is used in more than 30 countries all over the body (Albers, 2002).

The other commercial names of chlorfenapyr are Pirate, Alert, Sunfire, Citrex, Interpid, Kotetsu, Pyonga and Stalker (USEPA, 1998a). Chlorfenapyr acquires insecticidal properties after metabolic activation. The parent compound is converted to a metabolite which functions as uncoupler of oxidative phosphorylation in mitochondria (Black et al., 1994).

Chlorfenapyr is a novel broad-spectrum insecticide-miticide for the control of various insects and mite pests on cotton, ornamentals and a number of vegetable crops (Rand, 2004). It has been found efficacious against several crop pests (Mascrenhas and Boethel, 1997) as well as the horny fly (Haematobia irritans) (Sheppard and Joyce, 1998), in controlling natural infestation of lice on cattle (Phillip et al., 2001), western subterranean mite (Reticuliter mesheperus) (Rust and Saran, 2006) and Egg plant flea (Efuscula) (Mcleod et al., 2002).

Chlorfenapyr has low volatility and water solubility, it is lipophilic and binds strongly to soil particles. It degrades slowly in soil (average half-life of 1.1 year) (Federal Register, 2002); Sediment (average half-life of 0.8 years) (American Cyanamid, 1998 and Albers et al., 2002).

Biological evidence presented by the manufacturer indicates that chlorfenapyr is rapidly metabolized and excreted by mammals, birds and fish, hence it is unlikely to bioaccumulate in individual organisms or biomagnify between trophic levels (American Cyanamid Company, 1998).

Chlorfenapyr residues are found in avian food items including weed seeds, insects and foliage. Levels of chlorfenapyr in avian diets may be as much as 68 times higher than the EPA threshold for
reproductive effects, EPA states that these toxicological threshold may be exceeded for up to five weeks after initial application to cotton crops (Kelley, 1999). There were 19 tolerances for residues of chlorfenapyr in/on cotton, milk, cattle, hog, sheep, horse and goat (Albers et al., 2002). In Egypt, Chlorfenapyr is the active ingredient (36% insecticide and acaricide challenger) registered on May 27, 2001 (Company BASF, AGRO, S.A.C., France. Local Company BASF Limited-Egypt).

Because the registrant is expected to try again to register chlorfenapyr for use on cotton and other outdoor uses, it is important to increase our knowledge on the biological effects of this compound on our animals. The aim of the present work was to study the hematological, biochemical, the residual and pathological effects of chlorfenapyr toxicity on rats.

MATERIALS AND METHODS

1. Insecticide uses in the experiment:

Challenger 36% (chlorfenapyr), Technical: CL 303630; 4-bromo-2-[4-chlorophenyl]-1-[ethoxymethyl]-5-[Trifluoromethyl]-1H-pyrrole-3-carbonitrile. M.f. C_{15}H_{11}BrCLF_{3}N_{2}O of chemical formula shown below was used.

![Molecular structure of chlorfenapyr](image)

Fig. (1): Molecular structure of chlorfenapyr.

2. Experimental design:

A number of 60 adult male albino rats of an average of weight 150±10 g were used. The animals were obtained from the Farm of General Organization of Serum and Vaccine (Helwan Farm). The experimental animals were acclimatized for 15 days before the beginning of experiment. They were housed in plastic cages at room temperature and uniform light, fed on a balanced diet and fresh water ad-libitum. The experimental animals were divided into the following groups:

Group (1): 20 rats were kept as control rats.

Group (2): 20 rats were given challenger 36% SC in a dose equals the recommended Sprayed dose by gastric intubation daily (0.45
mg/Kg b. wt) for 28 days (American Cyanamid Company, 1998).

Group (3) 20 rats were given challenger 36% SC in a dose equals the recommended Sprayed dose by gastric intubation daily (0.45 mg/Kg b. wt) for 28 days and after that kept without given anything for recovery (15 days).

All rats were observed daily throughout the study, at the end of the experiment, the animals were sacrificed to collect blood samples for hematological and biochemical studies. The tissues samples were collected for residual and pathological studies.

3. Samples:
   a. Blood samples:

   Blood samples were collected in 2 separated vials, the first contained anticoagulant (EDTA) and used for haematological examination. The second vial was used without anticoagulant, blood was left to clot at room temperature, samples were centrifuged at 3000 r.p.m for 20 minutes to obtain serum which were stored at – 18°C till performing the biochemical analysis.

   b. Tissues samples:

   Tissues samples from the brain, heart, lung, liver, kidney, spleen and testes were obtained for residual analysis. Another tissues specimens of all internal organs were obtained and kept in neutral 10% buffered formalin for pathological studies.

4. Haematological studies:

   The anticoagulated blood previously collected were examined for Hemoglobin (Hb) according to Crosby et al. (1954). Packed cell volume (PCV) was determined by the microhaemtocrit method of Schalm (1986); the total erythrocytes (RBCs) and total leucocytes (WBCs) were counted according to Thompson (1980).

5. Biochemical assay:

   Serum samples were analysed serum transaminases including aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) according to Retiman and Frankel (1957); alkaline phosphatase (Estman and Bixter, 1977), cholesterol (Richmond, 1973). Total protein was determined according to Weichselbaum (1946); albumin (Dumas et al., 1971); creatinine (Henry, 1974) and urea (Patton and Crouch, 1977).

6. Residual analysis:

   Tissue samples of the internal organs (brain, heart, lung, liver, kidney, spleen and testes) were processed and extraction were obtained for residual analysis according to Leon et al. (1990), using HPLC [HPLC instrument was used with the following
condition: a) DV detector, b) C18 column, c) mobile phase was 40% methanol and 60% acetonitril, d) flow rate was 0.9 ml/min and detection limiting µg/gm. Values were calculated as apparent compound by direct comparison of sample peak area with that of an external standard as recorded by Cao et al. (2005) and Watanable et al. (2005).

7. Histopathological studies:
Tissue specimens were collected on neutral 10% buffered formalin, were processed to obtain five micron thick paraffin sections, stained with haematoxylin and eosin according to Bancroft et al. (1996) and used for histopathological examination. Prussion Blue stain for demonstration of haemosidrin pigment was performed according to Clayden (1971).

8. Statistical analysis:
The obtained results were statistically analyzed according to Petrie and Watson (1999).

RESULTS AND DISCUSSION
The clinical signs observed on rats treated with chlorfenapyr during the experimental period showed decrease in activity, salivation, ataxia, hyperthermia and death. The post-mortem examination revealed congested as well as enlargement of the lung, liver, kidneys, spleen and testes. These findings were in agreement with USEPA (1998a).

The haematological analysis (Table, 1) demonstrated that chlorfenapyr elicited a significant decrease (P < 0.001) in values of hemoglobin (Hb), haematocrit as well as red and white blood cells among the rats of the treated and treated with recovery groups as compared to control one. These results means that the rats were suffering from hemolytic anemia and leucopenia revealing that chlorfenapyr had depressant effect on haemopoetic system. Similar results were recorded by Moore (1995) and American Cyanamid Company (1998). Many instances of a plastic anemia and related blood dyscrasias were associated with pesticides as chronic effect (Fatma and Menha, 1993).

The results shown in table (2) revealed a significant increase (P< 0.001) in AST and ALT and ALP in both groups treated with chlorfenapyr as compared with the control. The elevation of these liver enzymes may be attributed to the drastic condition caused by the pesticide and/or severe hepatocellular damage as confirmed by the pathological alteration. These results were in agreement with previously recorded with Benjamin (1984) and Hala and Elbadry (2006). Kao (1993) reported that chlorfe-
napyr and its metabolites are present in high concentration in liver. Also, Albers et al. (2006) stated that liver is the preferred tissues for chemical confirmation of exposure to chlorfenapyr. Moreover, Moore (1995) found that chlorfenapyr induced an increase in ALP in rats treated with a diet containing 600 ppm for 90 days or diet containing 1600 ppm for 28 days.

A significant increase (P<0.001) in total cholesterol levels (Table, 2) was noticed in rats treated with chlorfenapyr. Hypercholesterolemia may be due to impairment of liver and kidney as confirmed by microscopic examination. These findings were in concurrent with those of USEPA (2001) who reported increased cholesterol value associated with changes in liver chemistry and morphology (Structure and form) when chlorfenapyr was applied to the shaved skin rabbits at a dose level of 400 mg/mg for 6 hours/day and 5 days/week for 4 weeks. Hala & El badry (2006) suggested that hypothyroidism occurred in rats received 0.22 mg/kg b.wt chlorfenapyr may decrease cholesterol catabolism which in turn increase the serum cholesterol level.

There were a significant decrease (P< 0.001) in the levels of total proteins, albumin as well as globulin (Table, 2) either in the treated or after recovery groups compared with control one. Less protein bio-synthetic activity by liver revealed the metablolic decrease in protein levels in serum in response to the toxicity by the pesticide (Joan and Pannall, 1981). Also, the reduction of albumin levels may be due to inhibition of protein synthesis in liver as a result of toxication (Morgaonkar et al., 1993). Moreover, the reduction in globulin level could be attributed to renal and hepatic inflammation or hemolytic anemia as a result of chlorfenapyr toxicati- (Kaneko et al., 1997).

A significant increase (P<0.001) were found in urea and creatinine among the rats of the both groups treated by chlorfenapyr. This elevation in urea levels coincided with the results reported by Moore (1995) who recorded an elevation in blood urea nitrogen level in rats fed chlorfenapyr in concentration of 44.9 mg/Kg/day in diet for 90 days. This may be due to the faulty excretion as occurs in renal failure (Hood, 1980). While the increased of creatinine levels may occur due to renal impairment or reduction of glomerular filtration as confirmed by the microscopic examination. Also, the elevation of creatinine levels could be attributed to the significant loss of muscle mass in treated rats (Lees et al., 1994). This elevated blood creatinine levels could simply reflect the injuries indu-
ced by chlorfenapyr in muscles tissues (Paulino et al., 1996 and Hala and Elbadery, 2006).

Analysis of individual tissues of brain, heart, lung, liver, kidney, spleen and testes of treated rats with chlorfenapyr and treated rats with recovery period indicated that chlorfenapyr residues had accumulated in these tissues (Table 3). Although the recovery group had less residual values than the only treated one, both values were higher than the limit of quantification (0.05 ppm) in the liver and kidney according to American Cyanamid Company (1998).

The mean residue levels in brain, heart, lung, spleen and testes of rates of both treated groups (Table, 3) were higher than the limit of quantification (0.01 ppm) according to Federal register (2002) who stated that the metabolic pathway of chlorfenapyr in the laying hen and lactating goat was also similar to that in laboratory rats. No limits of quantification are recommended by WHO or USEPA. Biological evidence presented by manufacture claims that chlorfenapyr is rapidly metabolized and excreted by mammals, birds and fish and hence it is unlikely for this compound to bio-accumulate in individual organisms or biomagnify between trophic levels (American Cyanamid Company, 1998). However, USEPA (2001) believes that the “Still yield dietary exposure estimates may exceed chronic toxicity threshold” which support this result. It was noticed that the residue levels of all organs among rats of the recovery group were less than the treated group but it still higher than quantification limit of each organ according to American Cyanamid Company (1998) and Federal Register (2002). These results indicate that rats may need more chance and longer recovery time to get rid the residual accumulation of the pesticide from their bodies.

Histopathological examination of different organs of rats of treated group and recovery group, showed nearly the same results. The brain of both groups showed vacuolation of the white matter (spongyform myelopathy) with swelling of the myelin sheath i.e. axonal degeneration (myelinopathic alterations in the central nervous system) (Figs. 2 &3). Neurophagia, perivascular and perineural edema (Fig., 4) as well as malasia of the brain with mild gliosis (Fig., 5) were seen. This pesticide contains both bromine and fluorine; a combination that has the potential effect to produce severe adverse effects - particularly to the brain (Federal Register, 2003a), and also due to the accumulation of its residues in the brain tissues. These findings coincided with that of USEPA
in mice; USEPA (2000) in mice and rats, California EPA (2001a) in albino rats and Federal Register (2001b) who performed an inhalation studies in rats, dogs, rabbits and mice as well as Federal Register (2003a) in male rats, who recorded that the brain was the major target organ of this pesticide toxicity.

The muscle of heart of rats in both treated groups, showed mild degenerative changes with accumulation of heamosidrin pigment which stained blue by brussion blue (Figs. 6 A & B). This alteration may be due to the toxic effect of the pesticide and its accumulated residues in the heart muscles which exceed the permissible limits (USEPA, 2001).

Microscopic examination of the lungs showed proliferation of the cells lining the bronchioles, infiltration of mononuclear inflammatory cells (Fig., 7), accumulation of haemosidrin pigment as well as atelectasis with compensatory emphysema (Fig., 8 A). The haemosidrin pigment appears blue when stained by brussion blue stain (Fig., 8 B). These lesions may be due to direct and indirect toxicity of the pesticide and its residual accumulation (Federal register, 2001a).

Concerning the liver, the alteration revealed vacuolar degenerative changes in hepatocytes (Fig., 9) as well as necrobiotic changes, newly formed bile ductules, dilated and congested blood vessels, infiltration of mononuclear inflammatory cells specially around the portal area and activation of kupffer cells (Fig., 10). This drastic changes may be due to it is the organ responsible to detoxication of the pesticide so it receives massive amount of its metabolites and also due to the residual accumulation of it as mentioned by (Black et al., 1994) who mentioned that Chlorfenapyr acquires insecticidal properties after metabolic activation, i.e. the parent compound is converted to a metabolite which function as uncoupler of oxidative phosphorylation in mitochondria. These results were in agreement with California EPA (2001b). Federal Register (2003b) added that adenomas and combined adenomas/ carcinomas may be showed due the toxicity of this pesticide.

The spleen showed mild depletion of the cellular elements of white pulp as well as accumulation of the haemosidrin pigment (Fig., 11 A) which stained blue by Prussian blue stain (Fig., 11 B). The accumulation of the haemosidrin pigment in the heart, lung and spleen may indicate that this pesticide has a haemolytic effect on the red blood cells confirmed by the estimated anemia in rats of both treated
groups as previously reported by Fatma and Menha (1993) and Hala and Elbadry (2006).

Microscopic examination of kidneys of both groups revealed necrosis of some cells lining the renal tubules, periglomerular edema, shrinkage of some glomeruli, hemorrhage (Fig. 12). Thickening of the wall of blood vessels as well as infiltration of mononuclear inflammatory cells, some renal tubules showed the formation of the renal cast inside their lumens (Fig., 13). These findings may be attributed to the fact that the kidneys is the main responsible organs for excretion and also due to the residual accumulation of the pesticide in the kidneys tissue as mentioned by California EPA (2001a). These results were confirmed by disturbances in the level of urea and creatinine as reported by Moore (1995) and Hala and Elbadry (2006). Also, the infiltration of mononuclear inflammatory cells in many organs such as lung, liver and kidneys explained leucopenia in rats of both treated groups as recorded by Davis (1981).

Testes showed thickening of the capsule as well as congestion of the blood vessels. Some spermatoocyte series showed degenerative changes and necrosis. Some seminiferous tubules were devoid of any sperms (Fig., 14), others showed only debris of necrotic cells in their lumen (Fig., 15). Moreover, thickening in the seminefrous septal spaces was related to infiltrated mononuclear inflammatory cells accompanied with proliferated interstitial cells (Figs., 16-18). Presence of interstitial cells with more than one nuclei and their proliferation may be the beginning formation of testicular interstitial cell tumors as previously recorded by USEPA (1998b) and Federal Register (2003a) who studied the endocrine effect of chlorfenapyron male rats. Albers et al. (2006) also, stated that Chlorfenapyr has high acute, sub-acute, and chronic (reproductive) toxicity in birds.

From the pathological studies, it was noticed that the recovery period (15 days) was not enough that all cells of different body organs return to its normal structure or/and function. Some pathological alterations may be overcome with a longer recovery period, others never overcome due to drastic effect of pesticide toxicity even after removal of the causative agent and/or after the disappearance of the pesticide residues from these organs. This in fact, may be an indication that the pesticides can be translocated, bio-concentrated or converted into more dangerous chemicals (Matsumura, 1985). This may explain the disturbances observed in the haematological parameter and
the biochemical analysis after recovery period (15 days) which mainly confirmed by the pathological lesion.

It is concluded that the technical chlorfenapyr induced some haematological, biochemical indices changes after exposure of adult male albino rats to 0.45 mg/Kg b.wt. for 28 days as well as histopathological alterations. The most toxic effect consisted of neuropathy (leukoencephalo-myelopathy), hepatopathy, nephropathy and may lead to infertility. The body may need a longer recovery period (more than 15 days) to overcome the toxicity effect of the pesticide and return to its normal physiological state.

Table (1): Effect of chlorfenapyr on haematological assay of treated and treated with recovery rats (Means ± S.E).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>treated</th>
<th>Treated with recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb g/dl</td>
<td>14.867 ±0.13</td>
<td>11.90 ±0.27**</td>
<td>12.79 ±0.23**</td>
</tr>
<tr>
<td>PCV%</td>
<td>48.06 ±0.15</td>
<td>37.65 ±1.5**</td>
<td>40.13 ±0.16**</td>
</tr>
<tr>
<td>RBCs x 10^6 mm^3</td>
<td>6.58 ±0.06</td>
<td>4.37 ±0.17**</td>
<td>5.01 ±0.05**</td>
</tr>
<tr>
<td>WBCs x 10^3 mm^3</td>
<td>8.72 ±0.033</td>
<td>5.39 ±0.24**</td>
<td>6.54 ±0.09**</td>
</tr>
</tbody>
</table>

**= Significant at P < 0.001.
Table (2): Effect of chlorfenapyr on some biochemical assay of treated and treated with recovery rats (Means ± S.E).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>treated</th>
<th>Treated with recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST U/L</td>
<td>36.81±2.3</td>
<td>97.0 ±3.8**</td>
<td>78.5 ±2.6**</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>28.31 ±1.8</td>
<td>92.41 ±2.1**</td>
<td>87.1 ±3.1**</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>33.45 ±0.57</td>
<td>65.72 ±1.43**</td>
<td>61.5 ±1.33**</td>
</tr>
<tr>
<td>Cholesterol gm/L</td>
<td>0.91 ±0.02</td>
<td>1.36 ±0.01**</td>
<td>1.33 ±0.006**</td>
</tr>
<tr>
<td>Total protein g/dl</td>
<td>6.33 ±0.132</td>
<td>5.20 ±0.02**</td>
<td>5.12 ±0.01**</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>2.43 ±0.05</td>
<td>1.94 ±0.03**</td>
<td>1.5 ±0.04**</td>
</tr>
<tr>
<td>Globulin g/dl</td>
<td>3.90 ±0.02</td>
<td>3.26 ±0.04**</td>
<td>3.51 ±0.03**</td>
</tr>
<tr>
<td>Urea mg/dl</td>
<td>14.37 ±0.42</td>
<td>43.49 ±0.8**</td>
<td>39.5 ±0.7**</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>1.16 ±0.02</td>
<td>1.91 ±0.06**</td>
<td>1.85 ±0.53**</td>
</tr>
</tbody>
</table>

**= Significant at P < 0.001.

Table (3): Residues of chlorfenapyr (ppm) in different organs of treated and treated with recovery rats (Means ±S.E).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>organs</th>
<th>Brain</th>
<th>Heart</th>
<th>Lung</th>
<th>Liver</th>
<th>Kidney</th>
<th>spleen</th>
<th>testes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45 mg/Kg b.wt</td>
<td></td>
<td>0.015*</td>
<td>0.014*</td>
<td>0.014*</td>
<td>0.056*</td>
<td>0.55*</td>
<td>0.054*</td>
<td>0.016*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.001</td>
<td>±0.0001</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.0001</td>
<td>±0.003</td>
<td>±0.0001</td>
</tr>
<tr>
<td>0.45 mg/Kg b.wt</td>
<td></td>
<td>0.013*</td>
<td>0.012*</td>
<td>0.013*</td>
<td>0.052*</td>
<td>0.53*</td>
<td>0.051*</td>
<td>0.013*</td>
</tr>
<tr>
<td>with recovery</td>
<td></td>
<td>±0.003</td>
<td>±0.001</td>
<td>±0.002</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.001</td>
</tr>
<tr>
<td>Permissible Limit</td>
<td></td>
<td>0.01</td>
<td>0.1</td>
<td>0.01</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

According to

- Fedral Register (2002)
- American Cyanamid Company (1998)
- Fedral Register (2002)

* = Exceed the permissible limit
Fig. (2, 3): Brain of rats treated by 0.45 mg/Kg b.wt chlorfenapyr showing vacuolation of the white matter as well as welling of the myelin sheath (axonal degeneration). (H &E, x 200).

Fig. (4): Brain of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing malasia and mild gliosis. (H&E, x 200).

Fig. (5): Brain of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing neurophagia as well as perivascular and perineural edema. (H & E, x 400 & 200).
Fig. (6 A & B): Heart of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing mild degenerative changes in the muscles with accumulation of haemosidrin pigment. (H & E, x 400). B: The haemosidrin pigment stained blue (Prussion Blue x 400).

Fig. (7): Lung of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing proliferation of cells lining the bronchiols with infiltration of mononuclear inflammatory cells. (H &E, x 200).

Fig. (8 A): Lung of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing A: severe accumulation of haemosidrin pigment. (H & E, x 100).
Fig. (8 B): Lung of previous picture, the haemosidrin pigment stained blue. (Prussian Blue x 100).

Figs. (9 & 10): Liver of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing vacuolar degenerative change of the hepatocytes as well as necrobiotic changes of the nucleus, newly formed bile ductules, dilated and congested blood vessels and infiltration of mononuclear inflammatory cells mainly in the portal area. (H & E, x 400).

Fig. (11 A): Spleen of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing A: mild deplesion of the white pulp as well as accumulation of haemosidrin pigments. (H&E, x 100).
Fig. (11 B): Spleen of pervious picture, the haemosidrin pigment stained blue. (Prussion Blue, x 100).

Fig. (12): Kidney of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing necrosis of some cells lining the renal tubules, haemorrhage. (H&E, x 200).

Fig. (13): Kidneys of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing renal cast formation inside the lumen of some renal tubules as well as infiltration of mononuclear inflammatory cells. (H & E, x 200).

Fig. (14): Testes of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing devoid of some seminefrous tubules from any sperms formation. (H & E, x 400).
Fig. (15): Testes of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing debris of necrotic cells in their lumen. (H & E, x 400).

Fig. (16, 17 & 18): Testes of treated rats by 0.45 mg/Kg b.wt chlorfenapyr showing thickening in the seminiferous septal space include infiltrated mononuclear inflammatory cells accompanied with proliferated interstitial cells. (H& E, x 400 and 1000).
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