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Original Research Article

Effect of liver diseases on hormonal and biochemical parameters in Wistar albino rat

Amira, H. Mohamed[1], Kamal, H.H.[2], Walaa, M.S. ahmed[2] and Hanan, E. Saeed [2].

1 Departement of Clinical Pathology, Faculty of Veterinary Medicine, Cairo Univerisity.

2 Departement of Clinical Pathology, Faculty of Veterinary Medicine, Beni- Seuf Univerisity.

ABSTRACT

The current study was performed to evaluate the effect of acute and chronic hepatotoxicity induced by paracetamol and thioacetamide respectively on serum hormonal levels and biochemical parameters. Female Wistar albino rats were divided into 3 equal groups (C), (P) and (T). Group (C) were kept as control, group (P) were received paracetamol orally (500 mg/kg b.wt) daily for 15 days and those of group (T) were injected thioacetamide (200 mg/kg b.wt) intraperitonialy twice/ week for 90 days. In P group, results revealed significant elevation in liver enzyme activities (ALT, AST and ALP), T4, insulin (7^{th} day), estrogen (7^{th} and 15^{th} days), triglycerides (7^{th} day) and cholesterol levels throughout the experiment while serum proteins and T4 (15^{th} day) showed significant decreased values. Whereas, at 90^{th} days of chronic intoxicated group (T) resulted in significant elevation in liver enzyme activities (ALT, AST and ALP), bilirubin, estrogen, T4, triglycerides (60^{th} and 90^{th} days) and T3 (120^{th} day). While the levels of T4 and cortisol (60^{th} day), serum total protein, albumin, globulin (90^{th} day) and insulin (120^{th} day) showed significant decreased values when compared to control group. In conclusion, both paracetamol and thioacetamide cause different degrees of damage in liver of rats leading to clear changes in their hormonal and biochemical profiles.

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*Corresponding author. *Departement of Clinical Pathology*, Faculty of Veterinary Medicine, Cairo Univerisity, Egypt. Email: Damira115@yahoo.com

1. Introduction

Hepatotoxicity is associated with impaired liver function caused by exposure to a drug or another non-infectious agent **(Navarro and Senior, 2006)**. Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions. Nevertheless, chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide) are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions **(Kucera et al., 2006, Domenicali et al., 2009 and Rousar et al., 2010)**.

Liver cirrhosis is frequently associated with endocrine dysfunctions (**Gonzales et al., 2007)**. Chronic liver disease may be accompanied by signs of apparent hormonal imbalance. An overdose of paracetamol, analgesic drug, causes severe hepatotoxicity and necrosis in both humans and experimental animals **(Kaplowitz 2005 and Jaeschke, et al., 2010)**. Paracetomol is a powerful inducer of cytochrome P450. The action of the P450 system on paracetomol produces a highly reactive quinoneimine that combines to the sulfhydryl groups of proteins. The toxicity occurs because of its reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI**)**. NAPQI exerts inactivation of proteins leads to death of liver cells **(Hzai et al., 2002).** Thioacetamide (TAA) is a hepatotoxin and hepatocarcinogenic when administered in the diet of experimental animals, and is widely used as a model of acute and chronic liver disease (**Ramaiah et al., 2000).**

2. Materials and Methods

2.1. Chemicals:

Paracetamol was obtained from El-Nasr pharmaceutical chemicals company Abu Zaabal, Egypt. TAA was obtained from Oxford Lab Chem, India. Diagnostic kits for serum enzymes (ALT, AST and ALP), total protein, albumin, cholesterol, triglycerides, phospholipids and bilirubin were supplied by Biodiagnostic Company, Egypt. Enzyme immune assay test kits supplied by Immunospec Pharmaceutical Chemicals Co., Egypt and the Egyptian American Corporation for Laboratory Services were used for determination of serum insulin, thyroxine (T4), tri-iodothyronine (T3), estrogen and cortisol.

2.2. Animals and treatment.

Seventy five Wistar albino female rats weighing between 80-120 g. They were housed in plastic cages under constant healthy environmental conditions. Water and diet were provided ad libitum.

Rats were randomly divided into three groups, each of which with 25 rats as follows:

Group C: kept as control and rats were received basal diet for 120 days.

Group P: rats were received paracetamol orally (500 mg/kg b.wt) daily for 15 days.

Group T: rats were injected thioacetamide intraperitoneal (200 mg/kg b.wt) twice/ week for 90 consecutive days.

The period of experiment extended to 120 days through which the weighting of rats and collection of samples were done. Blood samples were collected from the retro-orbital venous plexus of each rat after the 7^{th} , 15^{th} , 21^{th} and 30^{th} in P group while in T group after the 30^{th} , 60^{th} , 90^{th} and 120^{th} day of the experiment. Whole blood samples were used for leucocytic examination. Serum samples were obtained and stored at -20°C until used for biochemical and hormonal tests. Specimens of liver tissue were collected and fixed in neutral formalin 10% for histopathological examination.

2.3. Leucocyte examination

WBCs and differential leucocytic count were done according to **Feldman et al., (2000)**.

2.4. Serum biochemical assays:

2.4.1. Liver function tests

Activities of ALT, AST and ALP enzymes were estimated according to **Reitman and Frankel,**

(1957) and **Belfield and Golgberg, (1971)** respectively. Serum Bilirubin was estimated according to **Walter and Gerade, (1970**).

2.4.2. Serum proteins

Serum total protein and albumin levels were determined according to **Peters, (1968**) and **Dumas and Biggs, (1972)** respectively. Serum globulins were calculated by subtracting the obtained albumin values from the total proteins.

2.4.3. Lipid profile

Serum cholesterol, triglycerides, and phospholipids were determined according to **Allain et al., (1974)**, **Fossati and Prencipe, (1982)** and **Zilversmit and Davies, (1950)** respectively.

2.5. Hormonal assay

Serum thyroxine (T4) and triiodothyronine (T3) levels were estimated according to **Schuurs and Van Weeman, (1977) and Wisdom, (1976)** respectively**.** Serum insulin, estrogen and cortisol values were estimated according to **Flier et al., (1979), Tsang et al., (1980) and Burtis and Ashweed, (1994)** respectively**.**

2.6. Histopathological examination:

Histopathological examination of liver tissue of control and treated groups were performed according to **Bancroft and Gamble, (2008).**

2.7. Statistical analysis:

The obtained data were expressed as mean±SE and analyzed using the statistical program SPSS v17.0 for Windows (SPSS Inc., Chicago, IL). Statistical differences were determined using the unpaired Student's *t*-test throughout the experiment except at the 30^{th} day was examined with f-test.

3. Results:

3.1. Leucogram

In paracetamol group, the leucogram revealed non- significant leucopenia at the 7^{th} and 15^{th} days and significant leukocytosis at the 30^{th} day. These changes were accompanied by neutropenia the 7^{th} and 15^{th} days and neutrophilia revealed at the 30^{th} day as compared to control group. In thioacetamide group, at the 30^{th}, 60^{th} and 90^{th} days, there was leucocytosis associated with lymphocytosis at the 60^{th} and 90^{th} days, neutrophilia and eosinophilia at the 30^{th} and 90^{th} days when compared to control group (Table-1).

3.2. Hormonal levels

In paracetamol group, there was non-significant change in T3 and T4 hormone level along the experiment except significant increase in T4 hormonal level at the 7^{th} day and significant decrease at the 15^{th} day. There was significant increase in insulin level at the 7^{th} day, significant increase in estrogen hormone level at the 7^{th} and 15^{th} days and there was non-significant change in cortisol hormone level along the experimental period in comparison with control group. In thioacetamide group, there was significant increase in T3 at the 120^{th} day whereas T4 hormonal level was significant decrease at the 60^{th} day and significant increase at the 90^{th} day. There was significant decrease in insulin level only at the 120^{th} day. Estrogen level showed no significant change except at the 90^{th} day significantly increased when compared to control group. Non-significant change was observed in cortisol hormone level along the experimental period except at the 60^{th} day, there was significant decrease in comparison with control group (Table-2).

3.3. Serum biochemical parameters

3.3.1. Liver enzymes

In paracetamol group, there was non-significant change in ALT activity along the experimental period except at the 7^{th} day, its activity was elevated. The obtained results of AST activities were elevated at the 7^{th}, 15^{th}, 21^{st} and 30^{th} days and ALP activity showed highly significant increase at the 15^{th}, 21^{st} and 30^{th} days in comparison with control group. In

thioacetamide group, ALT activity showed non-significant change along the experimental period only at the 90th day, its activity was elevated, AST activity was elevated at the 30th and 90th days and ALP activity showed elevation at the 30th and 90thdays in comparison with control group (Table-3).

3.3.2. Serum bilirubin

In paracetamol group, serum bilirubin concentration at the 15th and 21st days showed significant increased values when compared to control group. In thioacetamide group, at the 60th and 90th days, there was hyperbilirubinemia in comparable to control group (Table-3).

3.3.3. Serum proteins

In paracetamol group, values of total protein and albumin showed non- significant change allover the experiment except hypoproteinemia and hypoalbuminemia showed at the 15th and 30th days and hypoglobulinemia showed at the 15th day. In thioacetamide group, the results showed hypoproteinemia and hypoalbuminemia at the 30th and 90th days, hypoglobulinemia was occurred at the 90th day and percentage of A/G ratio showed significant decrease at the 60th day and significant increases at the 90th day in comparison with control group (Table -4).

3.3.4. Lipid profile

In paracetamol group, the levels of serum triglycerides and phospholipids revealed non-significant change throughout the experiment, only hypertriglyceridemia showed at the 7th day while the cholesterol level showed significant increase throughout the experiment in comparison with control group. In thioacetamide group, hypocholesterolemia and hypophospholipidemia showed at the 60th day and hypertriglyceridemia observed at the 60th and 90th days (Table -5).

3.4. Histopathological results

The hepatic parenchymal appeared more or less histologically normal (fig.1). Liver of rats in paracetamol group: at the 7th day**,** the hepatocytes showed moderate degenerative changes; hydropic degeneration and cloudy swelling at the centrolobular areas and extended to throughout the hepatic lobules (fig.2). The blood vessels were dilated and congested especially central veins. The sinusoids were mildly dilated with active proliferation of Van kuppfer cells (fig.3). In some cases, leucocytic infiltrations were focally found in the vicinity of necrotic areas (fig.4). The connective tissue was slightly proliferated in the portal areas (fig.5). At the 15th day**,** the histopathological changes were more progressed comparing to the previous groups. Fatty changes were mildly seen within the hepatocytes (fig.6). The blood vessels and sinusoids were congested (fig.7). Some of the hepatocytes appeared necrotic; some of nuclei were pyknotic. The kupffer cells were proliferated. Early active proliferation of connective tissue in the portal areas could be detected (fig.8). After 21 days, the liver was very mildly affected with minor degenerative changes were seen in some hepatocytes (fig.9). But after 30 days, the liver appeared more or less normal, indicating histologic regeneration, with very mild congestion and proliferation of Kupffer cells. Also, the fibrous tissue proliferation was reduced compared with the previous period (fig.10).While liver of thioacetamide group: at the 30th day, the hepatocytes appeared mildly vacuolated with centrally located nuclei especially at the areas surrounding the central veins and extended in some cases throughout the hepatic lobules towards the periphery (fig.11). The central veins were slightly congested in some areas. Slight fibroplasia was found in the portal areas. No lecuocytic infiltration could be seen. No hepatic nodulation could be seen in this group although signs of chronicity were established. Parenchymal nodules without fibrous tissue surrounding them were seen in a few cases (fig.12). At the 60th day, at the same manner, pathologically, the liver had marked hisopathological changes which appeared as

congestion of some of blood vessels, early necrotic changes (pyknosis) (fig.13).Marked fibrous connective tissue proliferation in the portal areas (fig.14). At the 90th and 120th days, the most common alterations were in the form of mild vacuolation of hepatocytes (fig.15), and very mild congestion of veins and sinusoids. No connective tissue proliferation or leucocytic infiltration was seen.

Table (1): Means ±SE of WBCs and differential leucocytic count of different experimental groups.

Time (day)	Group	WBCs (x 10^3/ul)	Lymophocyte (x 10^3/ul)	Neutrophiles (x 10^3/ul)	Monocytes (x 10^3/ul)	Eosinophils (x 10^3/ul)
7	C	8.57± 0.87 ^{a}	5.44± 0.81^{a}	1.97 ± 0.14^{a}	0.92± 0.17 ^{a}	0.20 ± 0.03^{a}
	P	7.90 ± 0.96 ^{a}	5.75 ± 0.72 ^{a}	1.25 ± 0.25^{b}	0.84± 0.07 ^{a}	0.07 ± 0.03^{a}
15	C	6.73± 1.90^{a}	4.80 ± 1.01^{a}	1.44 ± 0.33^{a}	0.82±0.19 ^{a}	0.21 ± 0.11^{a}
	P	5.83 ± 0.64^{a}	4.25 ± 0.54^{a}	0.95 ± 0.22 ^{b}	0.56 ± 0.04^{a}	0.11± 0.03 ^{a}
21	C	6.33 ± 0.33^{a}	4.18 ± 0.23^{a}	1.21 ± 0.11^{a}	0.68 ± 0.07 ^{a}	0.11 ± 0.06^{a}
	P	6.03 ± 0.44^{a}	4.78 ± 0.24^{a}	1.49 ±0.33 ^{a}	0.83 ± 0.22^{a}	0.13 ± 0.07 ^{a}
30	C	5.97 ± 0.26^{a}	4.32 ± 0.21^{a}	1.08 ± 0.12^{a}	0.51 ± 0.10^{a}	0.04 ± 0.19^{a}
	P	6.23 ±0.32^{b}	4.04 ± 0.50^{a}	1.61 ± 0.47^{ab}	0.56 ± 0.14^{a}	0.04 ± 0.04 ^{a}
	T	7.57± 0.39^{c}	4.44 ± 0.26^{a}	2.15 ± 0.22 ^{c}	0.75 ± 0.25 ^{a}	0.18 ± 0.03 ^{b}
60	C	6.73± 0.79^{a}	4.96 ± 0.59^{a}	1.96± 0.60 ^{a}	0.40 ±0.07 ^{a}	0.25 ± 0.13 ^{a}
	T	8.13± 0.13 ^{b}	5.72± 0.08^{b}	1.62 ± 0.07^{a}	0.52 ± 0.06 ^{a}	0.27 ± 0.10 ^{a}
90	C	7.00 ± 0.81^{a}	4.75± 0.59 ^{a}	1.50 ± 0.21^{a}	0.53 ± 0.10 ^{a}	0.20 ± 0.03 ^{a}
	T	11.00±0.51^{b}	6.40± 0.50 ^{b}	3.43 ± 0.08 ^{b}	0.69±0.07 ^{a}	0.44 ± 0.07 ^{b}
120	C	6.80 ± 0.51^{a}	4.56 ± 0.31^{a}	1.66 ± 0.32 ^{a}	0.40 ± 0.03 ^{a}	0.18 ± 0.07^{a}
	T	7.39 ± 0.90^{a}	3.87 ± 0.13 ^{a}	1.31 ± 0.08^{a}	0.36 ± 0.13 ^{a}	0.28 ± 0.06^{a}

Group (C): represents the control group, group (P): represents the paracetamol group and group (T): represents the thioacetamide group. Means±SE with different superscript letters (^{a,b,c}) are significant at $p \leq 0.05$.

Table (2): Means ±SE of serum hormonal levels of different experimental groups

Time (day)	Group	T3 (ng/ml)	T4 (ug/ml)	Insulin (ng/ml)	Estrogen (pg/ml)	Cortisol (µg/dl)
7	C	1.50± 0.72^{a}	6.92±0.08^{a}	3.93± 0.15^{a}	371.1± 6.15^{a}	11.26± 0.23^{a}
	P	2.15± 0.16^{a}	8.33±0.20^{b}	6.49±0.06^{b}	394.2± 5.10^{b}	15.67± 2.26^{a}
15	C	2.73±0.29^{a}	8.75 ±0.06^{a}	6.94±0.38^{a}	343.5±9.40^{a}	10.96± 1.29^{a}
	P	2.88± 0.17^{a}	7.32± 0.09^{b}	6.35±0.21^{a}	377.4± 7.82^{b}	12.75 ± 1.56^{a}
21	C	2.62± 0.12^{a}	5.76 ±0.55^{a}	6.81±0.27^{a}	348.2±2.95^{a}	11.56± 0.25^{a}
	P	2.51± 0.20^{a}	4.90±0.30^{a}	6.20 ±0.12^{a}	347.4± 3.62^{a}	10.63 ± 1.01^{a}
30	C	2.82±0.28^{a}	6.74± 0.60^{a}	5.47±0.61^{a}	353.5± 9.20^{a}	11.71± 0.10^{a}
	P	2.88±0.32^{a}	6.91± 0.77^{a}	5.58±0.39^{a}	344.4± 6.78^{a}	12.95 ± 0.45^{a}
	T	2.70 ±0.59^{a}	7.56± 0.16^{a}	5.19 ±1.38^{a}	355.0± 0.61^{a}	13.20 ± 0.62^{a}
60	C	2.74 ±0.20^{a}	7.90± 0.34^{a}	6.25±0.21^{a}	268.4± 2.85^{a}	11.44 ± 0.24^{a}
	T	2.68 ± 0.15^{a}	5.89± 0.64^{b}	6.40±1.19^{a}	287.9 ± 9.25^{a}	9.16 ± 0.57^{b}
90	C	2.44 ±0.12^{a}	5.46± 0.37^{a}	4.58± 0.12^{a}	258.1± 6.19^{a}	11.50±0.23^{a}
	T	2.85 ± 0.26^{a}	7.62± 0.45^{b}	4.26 ± 0.24^{a}	287.1± 6.03^{b}	11.95± 0.35^{a}
120	C	2.45 ± 0.30^{a}	7.67± 0.22^{a}	3.50 ± 0.04^{a}	252.2± 3.30^{a}	10.99 ±0.61^{a}
	T	3.42 ± 0.19^{b}	6.62± 1.30^{a}	3.23 ± 0.03^{b}	241.1±1.94^{a}	11.41± 0.61^{a}

Group (C): represents the control group, group (P): represents the paracetamol group and group (T): represents the thioacetamide group. Means±SE with different superscript letters (^{a,b,c}) are significant at $p \leq 0.05$.

Table (3): Means ±SE of serum liver enzymes activities, bilirubin level of different experimental groups.

Time (day)	Group	ALT (u/L)	AST (u/L)	ALP (u/L)	Bilirubin (mg/dl)
7	C	46.44 ±0.48^{a}	28.43 ± 0.74^{a}	198.7 ± 19.72^{a}	0.63± 0.03^{a}
	P	50.83 ±1.01^{b}	47.43 ± 0.50^{b}	207.0 ± 16.05^{a}	0.60 ± 0.01^{a}
15	C	44.26 ±1.46^{a}	26.00 ± 1.53^{a}	128.5 ± 8.81^{a}	0.26 ± 0.02^{a}
	P	47.37 ±1.70^{a}	46.40 ± 2.75^{b}	416.3 ± 23.14^{b}	0.59 ± 0.01^{b}
21	C	46.62 ±2.18^{a}	28.37 ± 8.67^{a}	282.6 ± 3.99^{a}	0.08 ± 0.07^{a}
	P	50.63 ± 2.17^{a}	61.00 ± 8.39^{b}	545.8 ± 2.87^{b}	0.35 ± 0.08^{b}
30	C	46.14 ± 1.54^{a}	20.32 ± 0.89^{a}	255.2 ± 47.90^{a}	0.07 ± 0.00^{a}
	P	45.58 ± 0.73^{a}	72.67 ±2.45^{b}	477.6 ± 42.45^{b}	0.11 ± 0.02^{a}
	T	47.66 ± 1.72^{a}	24.87 ±0.72^{ac}	513.4 ± 9.27^{cb}	0.14 ±0.03^{a}
60	C	48.55 ± 0.35^{a}	29.70 ± 2.77^{a}	212.8 ± 19.60^{a}	0.27 ± 0.06^{a}
	T	47.74 ± 1.02^{a}	29.43 ± 4.75^{a}	289.1 ± 25.75^{b}	0.51 ± 0.10^{b}
90	C	47.91 ± 0.97^{a}	24.56 ± 0.56^{a}	220.3 ± 18.80^{a}	0.16 ± 0.02^{a}
	T	63.11 ± 2.16^{b}	35.87 ± 1.95^{b}	457.3 ± 14.80^{b}	0.34 ± 0.02^{b}
120	C	42.31 ± 0.73^{a}	28.89 ± 1.78^{a}	129.4 ± 12.72^{a}	0.31 ± 0.01^{a}
	T	40.12 ± 0.52^{a}	34.40 ± 5.45^{a}	101.2 ± 7.10^{a}	0.31 ± 0.09^{a}

Group (C): represents the control group, group (P): represents the paracetamol group and group (T): represents the thioacetamide group. Means±SE with different superscript letters (^{a,b,c}) are significant at $p \leq 0.05$.

Table (4): Means ±SE of serum total protein, albumin, globulins and A/G ratio of different experimental groups (C, P and T)

Time (day)	Group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
7	C	6.26 ± 0.45^{a}	4.98 ± 0.46^{a}	1.28 ± 0.34^{a}	4.68 ± 1.45^{a}
	P	6.16 ± 0.71^{a}	3.97 ± 0.57^{a}	2.19 ± 1.27^{a}	3.47 ± 1.44^{a}
15	C	5.61 ± 0.69^{a}	4.34 ± 0.41^{a}	1.27 ± 0.29^{a}	3.74 ± 0.71^{a}
	P	4.75 ± 0.05^{b}	3.59 ±0.05^{b}	1.16 ± 0.10^{b}	3.15 ± 0.29^{a}
21	C	4.80 ± 0.81^{a}	3.98±0.73^{a}	0.21±0.13^{a}	18.95± 5.67^{a}
	P	4.34 ± 0.05^{a}	3.98 ± 0.09^{a}	0.36 ± 0.09^{a}	12.29 ±2.95^{a}
30	C	5.00 ± 0.18^{a}	4.17 ± 0.33^{a}	0.83 ± 0.50^{a}	5.02 ± 0.66^{a}
	P	3.58 ± 0.18^{b}	3.24 ± 0.05^{b}	0.34 ± 0.17^{a}	9.20 ± 0.29^{a}
	T	3.23 ± 0.48^{c}	3.05 ± 0.44^{c}	0.18 ± 0.07^{a}	16.92± 6.15^{a}
60	C	4.04 ± 0.28^{a}	3.88 ± 0.25^{a}	0.16 ± 0.09^{a}	24.10± 2.83^{a}
	T	4.19 ± 0.51^{a}	3.65 ± 0.38^{a}	1.17 ± 0.58^{a}	3.20 ± 0.50^{b}
90	C	5.05 ± 0.27^{a}	4.56 ± 0.05^{a}	0.49 ± 0.23^{a}	9.32 ± 0.22^{a}
	T	3.64 ± 0.23^{b}	3.49 ± 0.18^{b}	0.15 ± 0.05^{b}	27.72± 3.00^{b}
120	C	4.19 ± 0.33^{a}	3.40 ± 0.12^{a}	0.80 ± 0.22^{a}	4.78 ± 0.96^{a}
	T	4.19 ± 0.41^{a}	3.22 ± 0.46^{a}	0.97± 0.23^{a}	3.78 ± 0.98^{a}

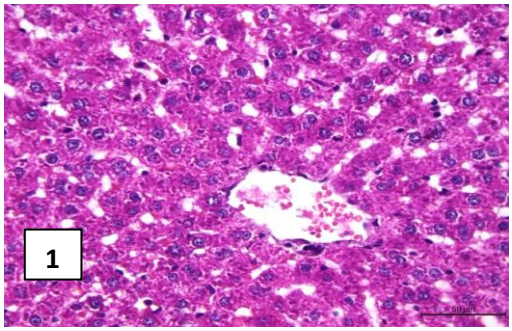
Group (C): represents the control group, group (P): represents the paracetamol group and group (T): represents the thioacetamide group. Means±SE with different superscript letters (^{a,b,c}) are significant at $p \leq 0.05$.

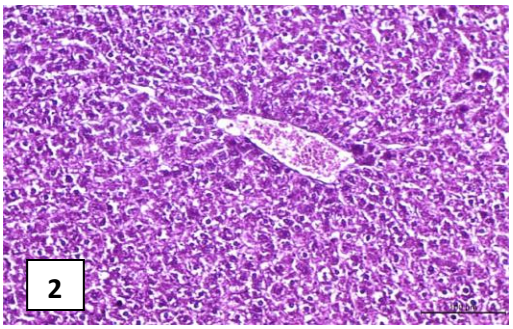
Table (5): Means ±SE of serum triglycerides, cholesterol and phospholipids of different experimental groups

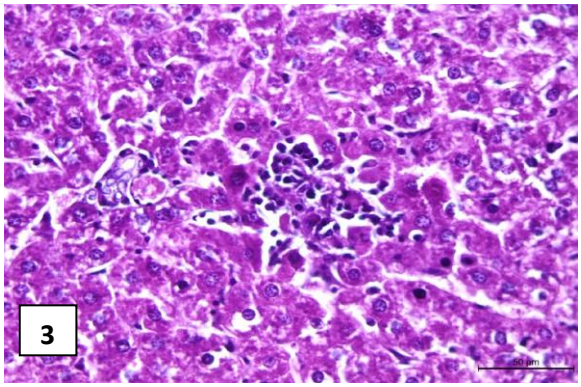
Time (day)	Group	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Phospholipids (mg/dl)
7	C	50.19 ± 3.86^{a}	57.86 ± 0.53^{a}	3.95 ± 0.53^{a}
	P	135.4 ±11.50^{b}	71.66 ± 1.91^{b}	3.12 ± 0.36^{a}
15	C	55.81 ±10.12^{a}	70.78 ± 2.79^{a}	3.35 ± 0.63^{a}
	P	54.28 ± 7.54^{a}	96.04 ± 7.45^{b}	2.46 ± 0.08^{a}
21	C	35.89 ± 8.99^{a}	49.03± 6.34^{a}	4.89 ± 0.08^{a}
	P	26.99 ± 3.66^{a}	152.3 ± 18.98^{b}	4.24 ± 1.05^{a}
30	C	38.38 ± 5.19^{a}	51.10± 7.77^{a}	3.76 ± 0.10^{a}
	P	43.81±7.70^{a}	72.33± 7.97^{b}	3.83 ± 0.24^{a}
	T	31.53± 1.35^{a}	55.70 ± 1.49^{a}	3.59 ± 0.11^{a}
60	C	51.71 ± 6.04^{a}	62.14± 3.36^{a}	4.77 ± 0.06^{a}
	T	84.78 ± 9.07^{b}	34.36 ± 0.88^{b}	3.99 ± 0.05^{b}
90	C	28.68 ± 4.18^{a}	59.18 ± 1.31^{a}	4.01 ± 0.02^{a}
	T	126.3 ± 5.57^{b}	61.23 ± 2.43^{a}	4.28 ± 0.18^{a}
120	C	28.61 ± 5.50^{a}	68.28 ± 10.97^{a}	3.92± 0.21^{a}
	T	24.27 ± 3.38^{a}	50.44 ± 8.40^{a}	4.09 ± 0.29^{a}

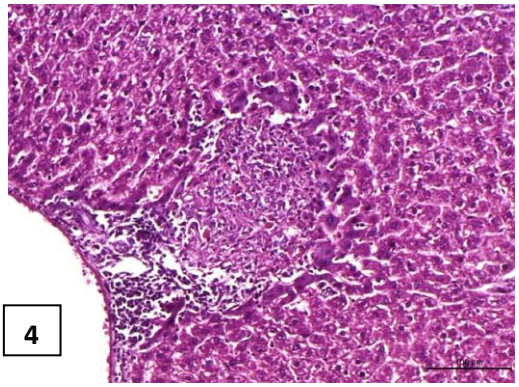
Group (C): represents the control group, group (P): represents the paracetamol group and group (T): represents the thioacetamide group. Means±SE with different superscript letters (^{a,b,c}) are significant at $p \leq 0.05$.

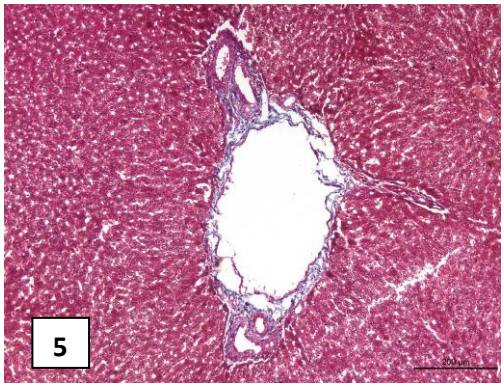
Histopathological changes in liver of different experimental groups

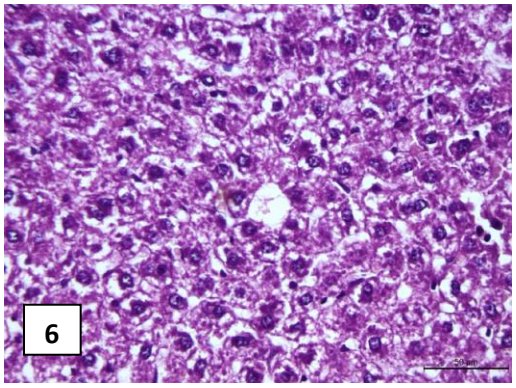


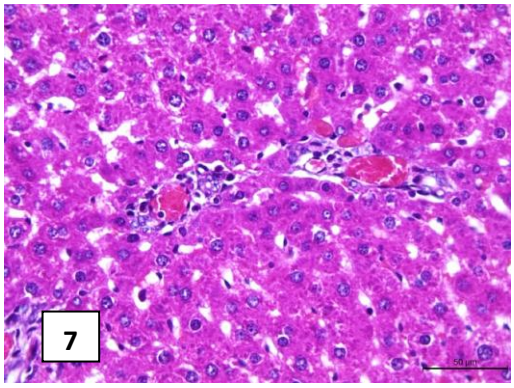


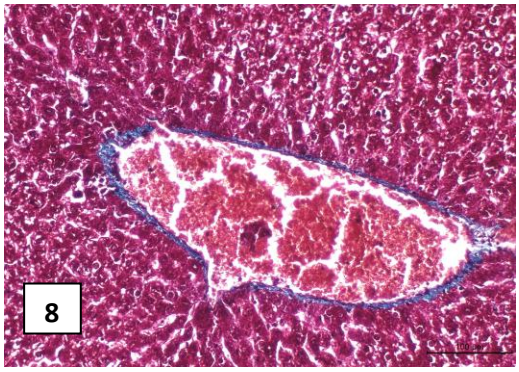


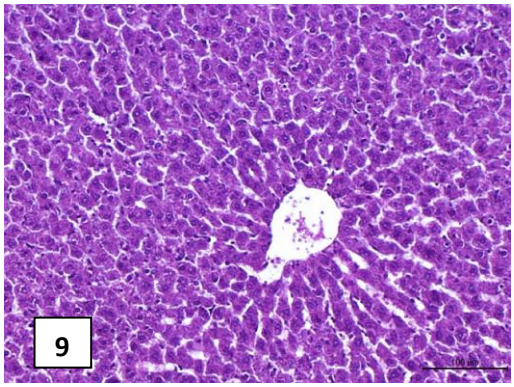








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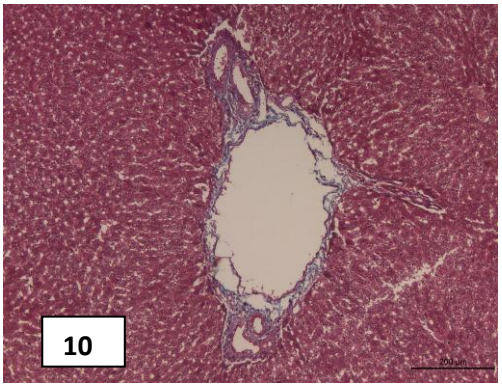
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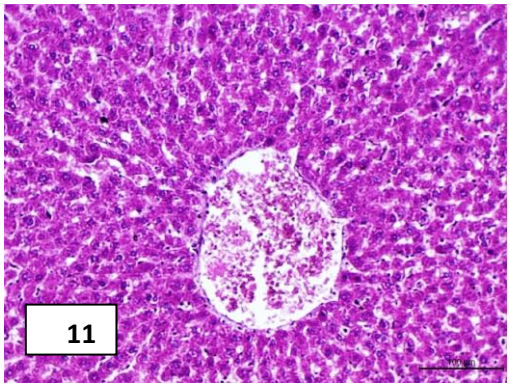
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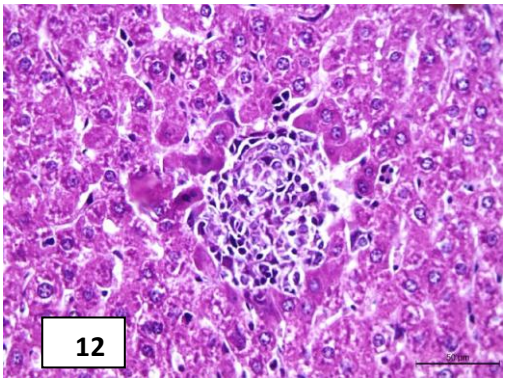
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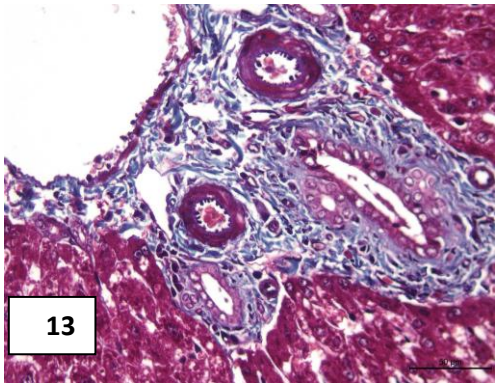
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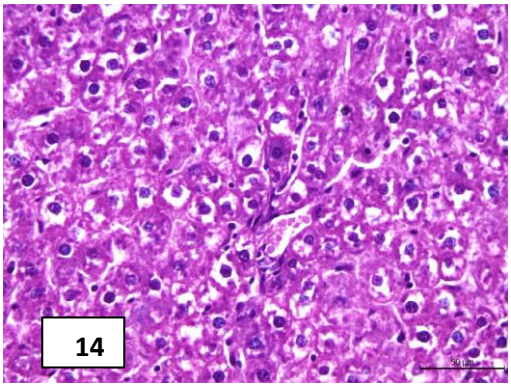
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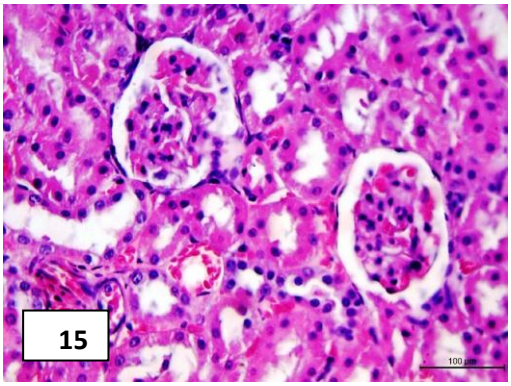
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(1): normal control liver, **at the7th day** (2): liver of paracetamol group: showing moderate degenerative changes, (3): dilatation and congestion of the central blood vein, (4): leucocytic infiltrations were focally found in the vicinity of necrotic areas, (5): The connective tissue was slightly proliferated in the portal areas**. At the 15th day:** (6): Fatty changes were mildly seen within the hepatocytes. (7): The blood vessels and sinusoids were congested. (8): Some of the hepatocytes appeared necrotic; with pyknotic nuclei. Early active proliferation of connective tissue in the portal areas could be detected. (9): **At 21 days**, the liver was very mild affected with minor degenerative changes were seen in some hepatocytes. (10): **after 30 days**, in P group: the liver appeared more or less normal, indicating histologic regeneration. In T group, (11): the hepatocytes appeared mildly vacuolated, (12): The central veins were slightly congested in some areas. Slight fibroplasia was found in the portal areas. (13): **at the 60th**, congestion of some of blood vessels, early necrotic changes (pyknosis), (14) marked fibrous connective tissue proliferation in the portal areas. (15): **At the 120th day**, the most common alterations were in the form of mild vacuolation of hepatocytes, and very mild congestion and sinusoids. No connective tissue proliferation or leucocytic infiltration was seen.

4. Discussion

The liver is responsible for detoxifying the chemical substances in the blood and in this process it is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury (**Arun Sam et al., 2007**). Nevertheless, chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide) are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions (**Kucera et al., 2006, Domenicali et al., 2009 and Rousar et al., 2010**). Liver diseases remain to be serious health problems and the management of liver disease is still a challenge to the modern medicine. Paracetamol (N-acetyl-p-aminophenol; APAP), a highly popular analgesic and antipyretic drug. It is safe at therapeutic doses; however the overdose following accidental ingestion or suicidal attempt causes a toxic response leading to the centrilobular necrosis in liver. APAP overdose, either alone, or in combination with other drugs, is account for 60% of cases of acute liver failure and leading to orthotopic liver transplant in the United States of America and United Kingdom **(Sweetman, 2009 and Mohit et al., 2011).** Thioacetamide (TAA) is known to be a hepatotoxic and carcinogenic compound in animals and most likely in humans as well, although no studies have shown these effects clearly. The cirrhosis model induced by TAA in rats produces histopathological changes that are similar to those found in humans and animals and is considered as a valid model **(Laleman et al., 2006).**

Paracetamol showed non- significant change in leucogram except significant neutropenia at the 7th, 15th days, this could be attributed to a rare case of hematologic side effects called neutropenia and thrombocytopenia often associated with antipyretic drug overdose like paracetamol **(El-Habib et al., 2007),** and significant neutrophilia at the 30th day due to Neutrophils and its derived cytokines play a crucial role in the development and manifestation of inflammation. The stimulation of neutrophils can lead to the production of oxygen-derived free radicals also called reactive oxygen species (ROS) that cause further cellular damage **(Bartosz, 2000)**. The formation of free radicals and cytotoxic oxygen metabolites probably impart a key role in various types of tissue degeneration and pathology such as aging, cancer and retinal degeneration **(Shapiro et al., (2006), Reznick et al., (2006) and Hussain and Harris, (2007)**.

Thioacetmide treatment accombined with leucocytosis, neutrophilia, lymphocytosis, and eosinophilia that agrees with **Abbasi et al., (2013) and Mani et al., (2014**) who reported

that leucocytosis occurred in case of chronic toxicity might be due to tissue necrosis or infarction and suggested that leucocytosis occurs in response to exposure to certain chemicals including steroids. In the present study, after intraperitonial injection of TAA, neutrophilia occured may be due to the free radicals resulting from TAA metabolism which caused liver injury and a proportion of these free radicals librated into the blood may also affect the circulating cells and induced a significant increase in their number. This significant neutrophilia might reflect its involvement in inflammation by forming various reactive oxygen species (ROS), inflammatory disorders and tissue necrosis **(Doi et el., 1991and Sheikh et al., 2006).**

T4 hormonal level was significantly decreased at the 7th day (in P group) and at the 60th day (in T group) and significantly increased at the 15th day (in P group) and at the 90th day (in T group). While results of T3 revealed non-significant change in both group. Results of T3 and T4 hormones in accordance with **Greg Kelly, (2000**) who suggested that the liver, and to a lesser extent kidneys, have primary influence on the circulating levels of thyroid hormone metabolites, the health and function of these organs play a critical and under-appreciated role in thyroid hormone function. Deiodination of T4 to form T3 or rT3 and the subsequent disposal of rT3 occurs in the liver and kidneys. The deiodination enzymes are also responsible for formation and elimination of T2 and T1 isomers. So in liver damage lead to decrease T3 formation and T4 still normal or increased.

Insulin hormonal level was significantly increased at the 7th day by paracetamol effect and TAA cause significant decrease in insulin hormonal level at the 120th day. This agree with **Siegel et al., (2000)** who suggested that hyperinsulinemia is caused by increased insulin secretion and diminished insulin degradation by the liver and the metabolic disturbances of glucose and insulin homeostasis have been intensively investigated in both humans and animal models. In addition to the liver cirrhosis are intolerant to oral glucose, even when their fasting blood glucose concentration is normal. Impaired regulation of carbohydrate metabolism includes glucose intolerance, hyperinsulinemia and hyperglucagonemia. Also agree with **Ming et al., (2006)** who reported that the experiments were conducted in a rat model of acute liver injury induced by thioacetamide (TAA), which has been demonstrated to cause liver damage through oxidative stress. The liver damage was assessed by a rapid insulin sensitivity test (RIST) to evaluate the ability of the liver to produce hepatic insulin-sensitizing substance (HISS). HISS is responsible for approximately 55% of the glucose disposal effect of an injection of insulin in the fed state and this portion of insulin response is termed HISS-dependent insulin action. It has been demonstrated that the ability of the liver to release HISS is severely impaired in situations such as hepatic parasympathetic dysfunction and chronic bile duct ligation. The insulin resistance caused by bile duct ligation is due to absence of HISS action; mimicking the normal parasympathetic nerve function with intraportal acetylcholine restores insulin action to normal levels.

The present study reported that paracetamol revealed elevation in estrogen level at the 7th and 15th days while thioacetamide cause elevation in estrogen at the 90th day in comparing to C group. This is in agreement with **Rupp et al., (1951) and Humm et al., (1951**) indicate an increase in total estrogen levels and decreased levels of neutral 17-ketosteroids in some patients with chronic liver disease, in so far as blood levels can be inferred from urinary excretion values. Although the most severe grade of liver disease was associated with the highest total estrogen and the lowest 17-ketosteroid excretion. Somewhat smaller increases in total estrogens and decreases in 17-ketosteroids have also been found in the acute stages of hepatitis, subsiding as the patient

improved **(Gilder and Hoagland, 1946).**Chronic liver disease may be accompanied by signs of apparent hormonal imbalance. Estrogens, such as estradiol, may be converted to estriol and estrone and then conjugated with glucuronic acid or sulfate. So liver cirrhosis lead to increase level of estradiol. **Yang et al., (1999)** indicated that almost any kind of toxin causes the liver to be less efficient at excreting other substances, including hormones. In malnutrition, sickness, and in aging, there is a tendency for higher levels of estrogen to remain circulating in the blood.

Cortisol hormones reported non-significant change in both treated groups (P &T) except significant decrease at the 60th day in T group. **Yokoyama et al., (2007**) studied that the liver is a key player in this interplay, in addition to being the site of steroid hormone metabolism, the liver is responsive to sex hormones. So when liver damage or cirrhosis induced by chemicals substances lead to imbalance in level of steroidal hormones such as cortisol and estrogen.

In the apparent study, paracetamol and TAA toxicity resulted in elevation of serum ALT, AST and ALP activities which supported by histopathological picture of liver. Paracetamol causes increased hepatocyte permeability and necrosis which in turn elevates activity of ALT, AST and ALP in agree with **Kalantari and salehi (2001), Guven et al., (2008**) and **Ramachandran et al., (2010)** who suggested that increase in the activities of serum AST and ALT indicated occurrence of hepatic dysfunction, necrosis and degenerative changes. Also this agrees with **Fatma et al., (2013), Wafaa et al., (2013) and Ahmed et al., (2014).**

Eraslan et al., (2008) interpreted the elevated activities of AST, ALT and ALP in thioacetamide group as a result of the hepatocytes damage or alterations in the membrane permeability leading to the leakage of these enzymes. Also this is in accordance to **Schrawat et al., (2006**) who mentioned that when the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. Their estimation in the serum is a useful quantitative marker for the extend and type of hepatocellular damage.

The significant increase in serum bilirubin in both treated (P&T) groups in according to **Kanchana and Sadiq, (2010)** who revealed that the hyperbilirubinemia that observed in the treated APAP-intoxicated group may be attributed to blockage of the biliary tract and occurance of a mass inhibition of the hepatic conjugation mechanism. In most instances, the serum bilirubin levels must be elevated in hepatic jaundice **(Moyer et al., 2000)**.The histopatholgical findings showed the presences of congested blood vessels of the liver and sinsuoids in TAA and paracetamol by several degrees, which consequently may block the normal excretory route and impair the clearance of bilirubin.

Paracetamol and thioacetamide showed a significant decreased in values of total protein, albumin and globulin. AAPP and TAA has been shown to reduce the number of viable hepatocytes and bile duct cells leading to accumulation of bile acids inside the hepatocyte, which promotes further liver damage that lead to decrease number of cells responsible for synthesis of albumin and proteins (**Rotruck et al., 1973)**.These findings may be due to the increment of injury in the hepatocytes, the reduction in SH-protein bond production, the disturbance in the endocrine system (T3, T4, cortisol and insulin), these data are in harmony with **Ruch et al., (1989)**.

Both treated group showed cause hypertriglyceridemia and hypercholesterolemia while significance decreased values of phospholipids showed in T group at the 90th day. This attributed to the excess level of the ROS formation by NAPQI produced by paracetamol which affect the functional mass of the liver and attack the biological molecules

such as DNA and Phospholipids (**Hinson et al., 2004**), also this in accordance to **(Ramachandran et al., 2010**) who observed that the plasma concentration of lipids is largely a balance of synthesis and degradation by the liver. The paracetamol cause significant increase in the level of triglyceride agreed with the data of **Kanchana and Sadiq, (2010**). These findings might be due to excessive release of triglycerides (**Iweala and Osundiya, 2010)** and/or decreased hepatic release of lipoprotein and increased esterification of free fatty acids **(Kanchana and Sadiq, 2010).** Elevated serum cholesterol values in paracetamol treated group are in harmony with **Raghavendran et al., (2005) and Setty et al., (2007).**

In thioacetamide group, **Bakhtiary et al., (2013)** said that TAA is a potent selective hepatotoxin due to oxidative injury, which has been recognized as the major mechanism in TAA-induced liver damage **(Bruck et al., 2004)** which leads to elevation of triglycerides, cholestrol level due to increase synthesis. This indicates disturbances in lipid metabolism induced by TAA intoxication as reported and agreed the results recorded by **Al-Attar, (2011)**. **Kabiri et al., (2013)** also showed reduce phospholipids levels. **Cooper et al. (1977)** reported that, alteration of bio-membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system. This suggested alteration in phospholipid and cholesterol ratio in cell membrane.

Histological findings of Liver showed that APAP administration to rats revealed a remarkable centrilobular necrosis, cytoplasmic changes, slight fibroblast formation and sinusoidal narrowings around the central vein, according to **Bauer et al., (2000)**. The liver of thioacetamide treated group showed marked congested central veins and sinusoids, presence of multifocal area of necrosis, fatty changes and inflammatory cell with granular swelling. The obtained histopathological change is in agreement with **Irani et al., (2001)**.

Conclusion

We can conclude that liver damage either acute or chronic has effect on hormonal level as it responsible for its degradation and metabolism.

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