TOXICOLOGICAL EVALUATION OF THE NEUROENDOCRINOLOGIC, PANCREATIC AND HEPATIC EFFECTS OF BACLOFEN IN ALCOHOL DEPENDENT ALBINO RATS

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ABSTRACT

Alcohol is a toxic substance to cells and tissues. Chronic alcohol consumption damages the brain, liver and many other organs like pancreas. Recently, new lines of treatment that maintain abstinence and preventing relapse to alcohol have been escalated like baclofen and acamprosate. Baclofen is a $GABA_B$ receptor analog recently used as an effective substitution drug. The aim of the current work was to evaluate the neuroendocrinologic, pancreatic and hepatic effects of baclofen treatment in alcohol dependent albino rats from the toxicological point of view. Thirty adult male albino rats were divided into 4 groups, Group 1: control rats: 12 rats equally subdivided into 1-a: negative control and 1-b: positive control (n=6), daily received distilled water by gavage. Group 2: Baclofen group: Six rats gavaged baclofen 7.5mg/kg/d for 3 days then, 15 mg/kg for the remaining of 4 weeks, Group 3: Ethanol group: Six rats gavaged ethanol 2.5 g/kg/d for 4 weeks, Group 4: Ethanol and baclofen group: Six rats gavaged ethanol 2.5 g/kg/d for 4 weeks, then received baclofen (7.5 mg/kg/d in the first 3 days and 15 mg/kg/d), for the remaining of 4 weeks. All rats were investigated by assessment of prolactin, leptin, ALP, ALT, AST, bilirubin, GGT, amylase and lipase serum levels, with histopathological examination of the brain, pancreas and liver. It was found that baclofen induced a significant decrease in the mean values of prolactin and leptin ($P<0.05$), with a significant increase in amylase ($P<0.05$), lipase ($P<0.001$), ALP, ($P<0.01$), bilirubin ($P<0.001$), ALT ($P<0.001$) and GGT ($P<0.05$), without any hisolopathological changes, as compared with +ve control group. Ethanol dependent rats showed a significant increase in the mean values of prolactin and leptin hormones levels, amylase, lipase, ALP, bilirubin, AST, ALT, and GGT ($P<0.001$) with severe alteration of the normal architecture of the brain, pancreas and liver. The alcohol dependent rats treated with baclofen showed a significant increase in the mean values of serum amylase, lipase, ALP, bilirubin, AST, ALT, and GGT ($P<0.01$), with a non significant decrease in the prolactin and leptin levels ($P>0.05$). However the biochemical changes were significantly less than those of alcohol, the hisopathological changes in both groups were similar. It was concluded that baclofen induced functional pancreatic and hepatic toxic changes in normal rats. Moreover, the functional and structural toxic effects of chronic alcohol dependence on the brain tissues, pancreas and liver were almost similar, after 4 weeks of the baclofen administration therapy. Further studies were recommended regarding the toxic effects of baclofen with a special concern to the molecular mechanisms underlying such effects.

Keywords: Ethanol toxicity, alcohol dependence, baclofen, prolactin, leptin, liver function tests, amylase.

INTRODUCTION

Alcohol (ethanol) is a CNS depressant. Large amounts consumed rapidly can cause respiratory depression, coma, and death. Large amounts chronically consumed damage the liver and many other organs. Alcohol exerts its toxic effects by several mechanisms. It binds directly to γ-aminobutyric acid (GABA) receptors in the CNS, causing sedation. Tolerance to alcohol develops rapidly due to its adaptational changes of CNS cells. The physical dependence accompanying tolerance is profound, and withdrawal has potentially fatal toxic effects [(1)].

Disulfiram is the first drug used to prevent relapse in alcohol dependence through the interference with the metabolism of acetaldehyde. Naltrexone, one of the opioid antagonists, has been evaluated in preclinical animal models of ethanol consumption and found to be effective in most reports. In clinical use, naltrexone has not proved to be as efficacious in preventing relapse. While naltrexone targets opioid receptors, many other neurotransmitter systems are targeted by ethanol and, to a greater or lesser extent, contribute to modulating ethanol's reinforcing effects [(2)]. Furthermore, it has been found that drugs active at the gamma amino butyric acid B ($GABA_B$) receptors can affect the self-administration of many drugs with abuse potential [(3)].

Recently, new lines of treatment that maintain abstinence and preventing relapse to alcohol, through their action on GABA receptors, have been escalated like baclofen and acamprosate [(4)].

Knapp et al., (2007) stated that baclofen (the prototypic γ-aminobutyric acid B receptor agonist), is a promising pharmacological compound for treatment of alcohol dependence. In particular, it has been found to suppress symptoms of alcohol withdrawal syndrome. It is also effective in the prevention of relapse due to its ability to reduce alcohol intake and craving in alcoholic patients. Moreover, it produces no significant side effects and displaying no addictive properties [(5)].

Despite of the extensive research studies on the effectiveness of baclofen as a substitution drug used in cases of alcohol dependence, when retrieving the published data concerned with the

safety profile of baclofen and its toxic effects, it was limited.

AIM OF THE WORK

The aim of this work was to evaluate the neuroendocrinologic, pancreatic and hepatic effects of baclofen treatment in alcohol dependent albino rats from the toxicological point of view.

Through the assessment of prolactin, leptin, ALP, ALT, AST, bilirubin, GGT, amylase and lipase serum levels, with histopathological examination of the brain, pancreas and liver.

MATERIAL AND METHODS

* **Chemicals, Drugs and Kits:**

1. Absolute ethyl alcohol-201302 was obtained from ADWIC, El-Nasr Pharmaceutical Co., Egypt, diluted with water to get 50% v/v solution (for each 53 ml ethanol, water was added to reach 100 ml).
2. Baclofen was obtained from Misr Co. For Pharmaceuticals and Chemical Industries.
3. Eli Tech-Diagnostic kits were used for estimation of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Bilirubin.
4. Gamma–glutamyl transpeptidase (GGT), Amylase and Lipase assays kits were purchased from Sigma-Aldrich Co.
5. Alkaline phosphatase (ALP) diagnostic kit was obtained from Bio-Systems Co.
6. ELISA Kits for prolactin and leptin assays were obtained from SCETI Bioscience, Japan.

* **Animal Groups:** Thirty adult male albino rats weighing 150-200g were obtained from the animal house of the Faculty of Veterinary Medicine, Zagazig University, divided into four groups, housed in stainless steel cages with standardization of the environmental conditions for minimization of fallacies.

Group 1: Control rats: 12 rats equally subdivided into **1-a:** negative control and **1-b:** positive control (n=6), received distilled water (1 ml/d) by gavage.

Group 2: Baclofen group: Six rats gavaged baclofen 7.5mg/kg/d for 3 days then, 15 mg/kg (1/10 of LD_{50}) for the remaining of 4 weeks [6].

Group 3: Ethanol group: Six rats gavaged ethanol 2.5 g/kg/d 50% (v/v) solution for 4 weeks [7].

Group 4: Ethanol and baclofen group: Six rats gavaged ethanol 2.5 g/kg/d (50% (v/v) solution) for 4 weeks, then received baclofen (7.5 mg/kg/d in the first 3 days) and 15 mg/kg/d (1/10 of LD_{50}), for the remaining of 4 weeks.

After 4 weeks of the study, all rats were investigated by measuring the levels of prolactin and leptin hormones, ALT, AST, bilirubin, GGT, ALP, amylase, and lipase enzymes. After scarification, the brain, pancreas and liver were submitted to histopathological examination by using H&E stain. Masson Trichrome special stain was also used for pancreas and liver tissues to detect formation of collagen fibers in these tissues.

Methods:

* Blood Sampling: Blood samples (3 ml) were collected from the retro orbital plexus by using capillary tubes, centrifuged to separate sera for the assessment of AST, ALT, bilirubin, GGT, ALP, amylase, lipase, prolactin and leptin levels, using sterile plain tubes, as following:

1. Serum levels of aspartate transaminase, alanine transaminase (AST and ALT) and bilirubin were measured spectro photometically at a wave length 546 nm using the methods described by **Bergmeyer et al., (1978), Price and Alberti (1979) and Jendrassik and Grof (1983)** [8, 9, 10].
2. Serum levels of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) were assessed by a colorimetric method according to **Belfield and Coldberg (1971)** and **Reitman and Frankle (1957)** [11, 12].
3. Lipase and amylase serum levels were measured by enzymatic colorimetric test by following the method of **Lott et al. (1986) and Kurahashi and Inomata (1988)** [13, 14].
4. Prolactin and leptin levels were assayed through Enzyme Linked Immuno-Assay method (ELISA) as described by **Uotilaet al., (1976) and Maffei et al., (1985)** [15,16].

***Histopathological Examination:** For light microscopic study, brain, liver and pancreas specimens were fixed in 10% formalin saline. Paraffin sections 5 um were prepared, stained with H&E stain by following the method described by **Wilson and Gamble (2002)** [17]. Another paraffin sections (5 um) of pancreas and liver stained with special stain, Masson Trichrome by following the method of **Masson et al., (1929)** were examined [18].

* **Statistical Analysis:** Data were represented as mean±SD. The student's t-test and ANOVA test were performed to compare between the studied groups. Difference was considered significant at $p<0.05$. The statistical analysis was performed using Epi-Info version 6.1.

RESULTS

The presented work showed a non significant difference between negative control and positive control rats in the tested parameters ($P>0.05$). When comparing the baclofen-treated group (group 2) with the +ve control group, a significant decrease in the mean values of prolactin and leptin hormones was found ($P<0.05$), with a significant increase in the mean values of amylase ($P<0.05$), lipase ($P<0.001$), alkaline phosphatase (ALP),

(P<0.01), bilirubin (P<0.001), alanine transeferase (ALT) (P<0.001) and gamma glutamyl transpeptidase (GGT) (P<0.05) serum levels. However, there was a non significant increase in the mean value of aspartate transaminase (AST) (P>0.05) as compared to the control group (P>0.05) **Table (1).**

Comparison between the ethanol dependent rats (group 3) and the +ve control rats showed that chronic administration of alcohol induced a significant increase in the mean values of prolactin and leptin hormones levels, amylase, lipase, ALP, bilirubin, AST, ALT, and GGT enzyme activities (P<0.001) as shown in **Table (1).**

This study also showed a significant increase in the mean values of serum amylase (P<0.01), lipase, ALP, bilirubin, AST, ALT, and GGT levels (P<0.001) in the baclofen-treated alcohol-dependent rats (group 4) as compared to the +ve control group. However, the mean values of serum prolactin and leptin levels of this group showed a non significant increase, when compared to the control group (P>0.05) **Table (1)**.

When comparing the ethanol dependent group (group 3) with the baclofen-treated group (group 2), there was a significant increase in the mean values of all parameters (prolactin, leptin, amylase, lipase, ALP, bilirubin, AST, ALT, and GGT) (P<0.001) **Table (2)**.

The alcohol dependent rats treated with balcofen (group 4) showed a significant increase in the mean values of serum prolactin, leptin, amylase, lipase, ALP, bilirubin, AST and GGT (P<0.001) with a significant decrease in the mean value of ALT (P<0.001) as compared to the balcofen group (group2) **Table (2).**

When comparing the same group (group 4) with the ethanol dependent rats (group 3), it showed a significant decrease in the mean values of prolactin, leptin, amylase, lipase, ALP, AST, ALT and GGT (P<0.001) with a significant increase in the mean value of serum bilirubin (P<0.001) as shown in **Table (2).**

Histopathological examination of the brain tissues of the control group showed normal structures of the brain, pancreas and liver specimens **Figs. (1A, 2A, 3A)**. The baclofen-treated group (group 2) showed normal structures of the brain including the polymorphic, pyramidal, and molecular layers. The pyramidal nerve cells appeared as large triangular cells as shown in **Fig. 1B.** The pancreatic and the liver tissues were also more or less similar to the control rats represented by normal closely packed acini with basal basophilia, apical acidophilia and basal rounded nuclei. The acini were separated by scanty connective tissue contain blood vessels **Fig. 2B**. The hepatocytes showed acidophilic cytoplasm of with central rounded vesicular nuclei and prominent nucleoli **Fig. 3B.**

When examining the brain tissue of the ethanol dependent rats (group3), it showed decreased thickness of the pyramidal layer, irregular faintly stained cells with few shrunken pyramidal nerve cells and a darkly stained cytoplasm with lost nuclear details **Fig. 1C**. It was also observed that the pancreatic acini were disorganized with loss of apical acidophilia and dilated congested blood vessel. Islets of langerhans showed many cells with dark small /pyknotic nuclei and loss of some islet cells leaving empty spaces with decreased basal basophilia and inflammatory infiltrate around the ducts **Figs. 2C, 2D.** The liver specimens showed loss of normal hepatic architecture with vacuolated hepatocytes with a signet ring nucleus, indicating the microvesicular steatosis, accompanied by congested blood vessel and lymphocytic infiltration as shown in **Figs. 3C, 3D.**

When the alcohol-dependent rats with baclofen (group 4), it was noticed that the brain tissue had the same changes induced by ethanol administration represented by the decreased thickness of the pyramidal layer and the presence of irregular faintly stained cells. However, other nerve cells had vesicular nuclei with prominent nucleoli **Fig. 1D.** The pancreatic tissues of the same group (group 4) showed wide spaces between the acini. Some acini were destroyed leaving empty spaces, whereas others showed vacuolation with dilated congested blood vessels **Figs. 2E, 2F.** The liver specimens showed acidophilic finely vacuolated hepatocytic cytoplasm. Some hepatocytes were binucleated with congested portal vein and few bile ductules **Figs. 3E, 3F.**

Masson trichrome-stained liver and pancreatic specimens showed that mild collagen fibers were noticed especially around the central vein and pancreatic blood vessels in the baclofen treated group (group 2). The ethanol dependent rats (group 3) showed massive collagen formation in the hepatic and pancreatic specimens. When examining the pancreases and livers of the alcohol-dependent rat treated with baclofen, they also showed marked collagen fibers deposition especially around the vessels **Figs. (4 , 5)**.

Table (1): Student's t test statistical analysis for comparison between the control group and each administrated substance in adult albino rats.

Parameter		Control	Baclofen	Ethanol	Ethanol+ Baclofen
Prolactin (ng/ml)	**Mean±SD**	**12.35±6.53**	**5.63±3.41**	**41.35±13.48**	**21.24±7.6**
	t		2.23	4.74	2.17
	P		**0.049***	**0.001***	**0.054#**
Leptin (ng/mL)	**Mean±SD**	**5.9 ± 3.7**	**2.1±7.6**	**19.1± 8.1**	**7.1±5.6**
	t		2.30	3.71	0.437
	P		**0.043***	**0.001***	**0.67 #**
Amylase (U/L)	**Mean±SD**	**72.4 ± 12.9**	**98.3±21.2**	**206.7±19.3**	**118.6±24.1**
	t		2.55	14.17	4.13
	P		**0.028***	**0.001***	**0.002***
Lipase (U/L)	**Mean±SD**	**29.2±0.07**	**11.4±0.9**	**66.3±0.12**	**42.1±0.11**
	t		48.29	654.14	242.3
	P		**0.001***	**0.001***	**0.001***
ALP (U/L)	**Mean±SD**	**85.34±6.15**	**95.21±2.13**	**221.1±14.7**	**164.50±11.97**
	t		3.71	20.86	14.408
	P		**0.004***	**0.001***	**0.001***
Bilirubin (mg/dl)	**Mean±SD**	**1.1 ± 0.03**	**1.87± 0.01**	**2.2± 0.04**	**2.6 ± 0.01**
	t		59.64	53.88	116.18
	P		**0.001***	**0.001***	**0.001***
AST (U/L)	**Mean±SD**	**57.3±8.7**	**65.15±2.32**	**146.68±11.6**	**119.76±9.02**
	t		0.58	15.09	12.20
	P		**0.058#**	**0.001***	**0.001***
ALT (U/L)	**Mean±SD**	**22.2±2.6**	**54.21±3.1**	**77.3±8.7**	**45.15±2.32**
	t		19.37	14.86	16.13
	P		**0.001***	**0.001***	**0.001***
GGT (U/L)	**Mean±SD**	**27.33±3.05**	**31.13±0.17**	**79.32±4.37**	**45.67±1.86**
	t		3.047	23.90	12.58
	P		**0.012***	**0.001***	**0.001***

*n = 6 , * P<0.05 Significant , # P>0.05 non Significant*

Table (2): Anova one way statistical analysis for comparison of biochemical parameters in the different studied groups of adult albino rats.

Parameter	Control	Baclofen	Ethanol	Ethanol+ Baclofen	F	P
Prolactin (ng/ml)	**12.35±6.53**	**5.63±3.41 AB**	**41.35±13.48 AD**	**21.24±7.6 ABD**	**19.672**	**0.001***
Leptin (ng/mL)	**5.9 ± 3.7**	**2.1±7.6 AB**	**19.1± 8.1 AD**	**7.1±5.6 ABD**	**7.696**	**0.001**
Amylase (U/L)	**72.4 ± 12.9**	**98.3±21.2 AB**	**206.7±19.3 AD**	**118.6±24.1 ABD**	**51.95**	**0.001***
Lipase (U/L)	**29.2±0.07**	**11.4±0.9 AB**	**66.3±0.12 AD**	**42.1±0.11 ABD**	**1521**	**0.001***
ALP (U/L)	**85.34±6.15**	**95.21±2.13 AB**	**221.1±14.7 AD**	**164.50±11.97 ABD**	**242.1**	**0.001***
Bilirubin (mg/dl)	**1.1 ± 0.03**	**1.87± 0.01 AB**	**2.2± 0.04 AD**	**2.6 ± 0.01 ABD**	**359**	**0.001***
AST (U/L)	**57.3±8.7**	**65.15±2.32 AB**	**146.68±11.6 AD**	**119.76±9.02 ABD**	**150.2**	**0.001***
ALT (U/L)	**22.2±2.6**	**54.21±3.1 AB**	**77.3±8.7 AD**	**45.15±2.32 ABD**	**127.9**	**0.001***
GGT (U/L)	**27.33±3.05**	**31.13±0.17 AB**	**79.32±4.37 AD**	**45.67±1.86 ABD**	**421.4**	**0.001***

*n = 6 , * P<0.05 Significant , P>0.05 non Significant , A: significant difference between baclofen group and ethanol group, B: significant difference between baclofen group and ethanol+baclofen group, D: significant difference between ethanol group and ethanol+baclofen group (P<0.001).*

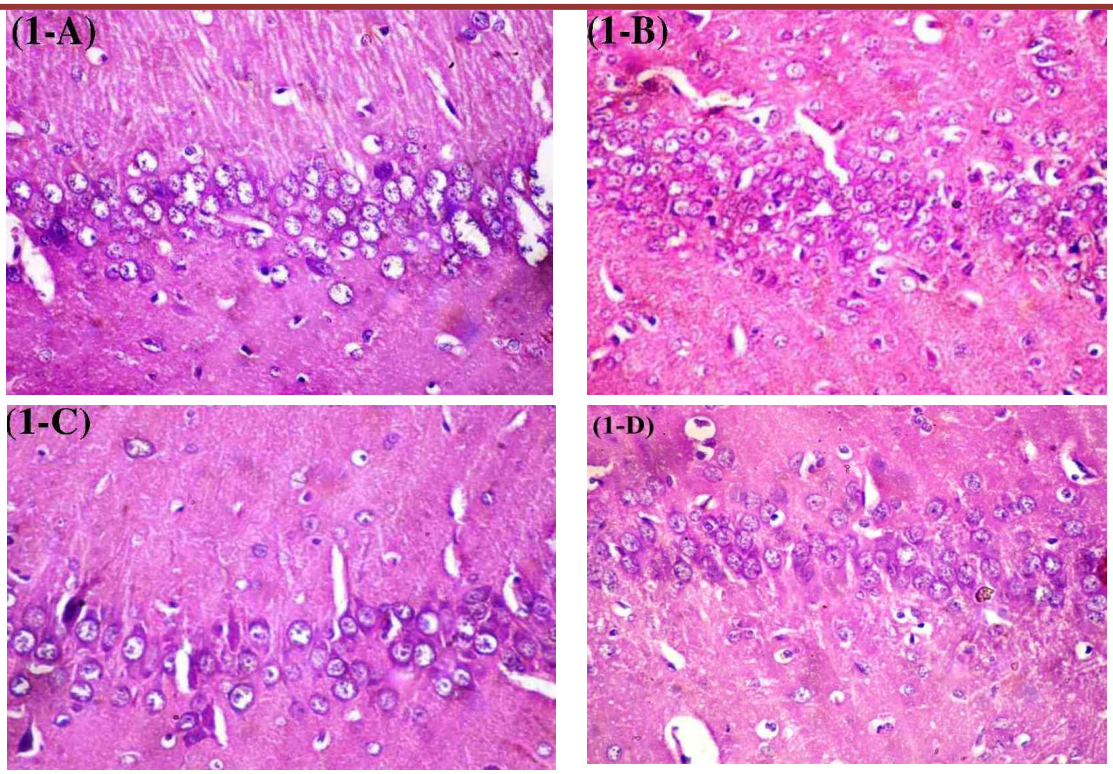
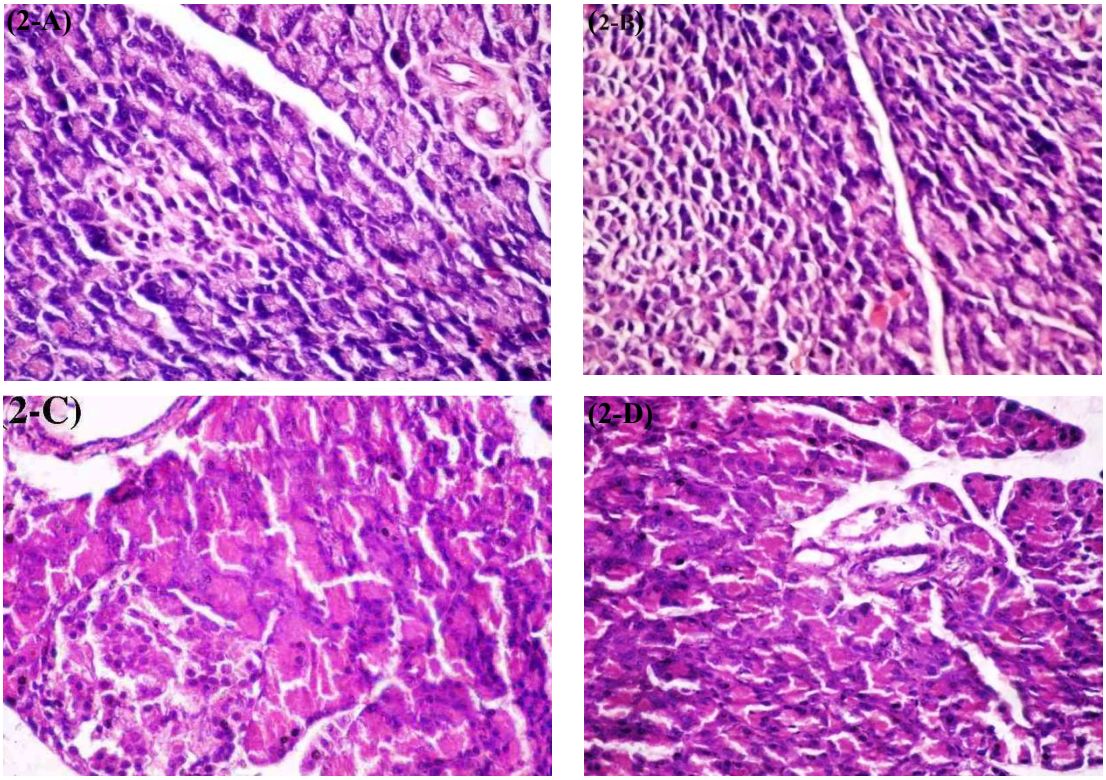


Fig. (1): Photomicrographs of brain sections of :**A**) a control rat showing normal structure, **B**): baclofen-treated rat showing polymorphic (PP), pyramidal (P), and molecular layers (M). The pyramidal nerve cells appear as large triangular, **C)** ethanol dependent rat showing decreased thickness of the pyramidal layer, irregular faintly stained cells (arrow) and other nerve cells have vesicular nuclei with prominent nucleoli (arrow head) and few shrunken pyramidal nerve cells with a darkly stained cytoplasm and lost nuclear details, **D)** alcohol-dependent rat treated with baclofen showing decreased thickness of the pyramidal layer *H&E, × 400.*



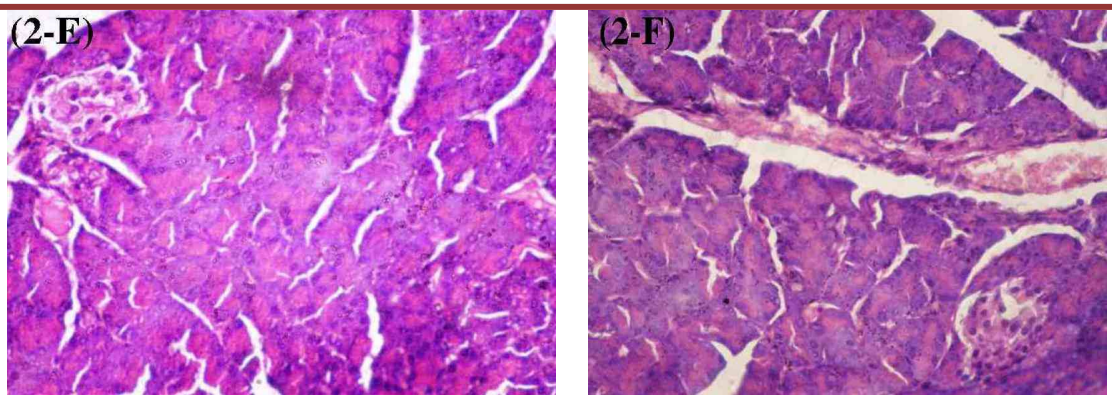
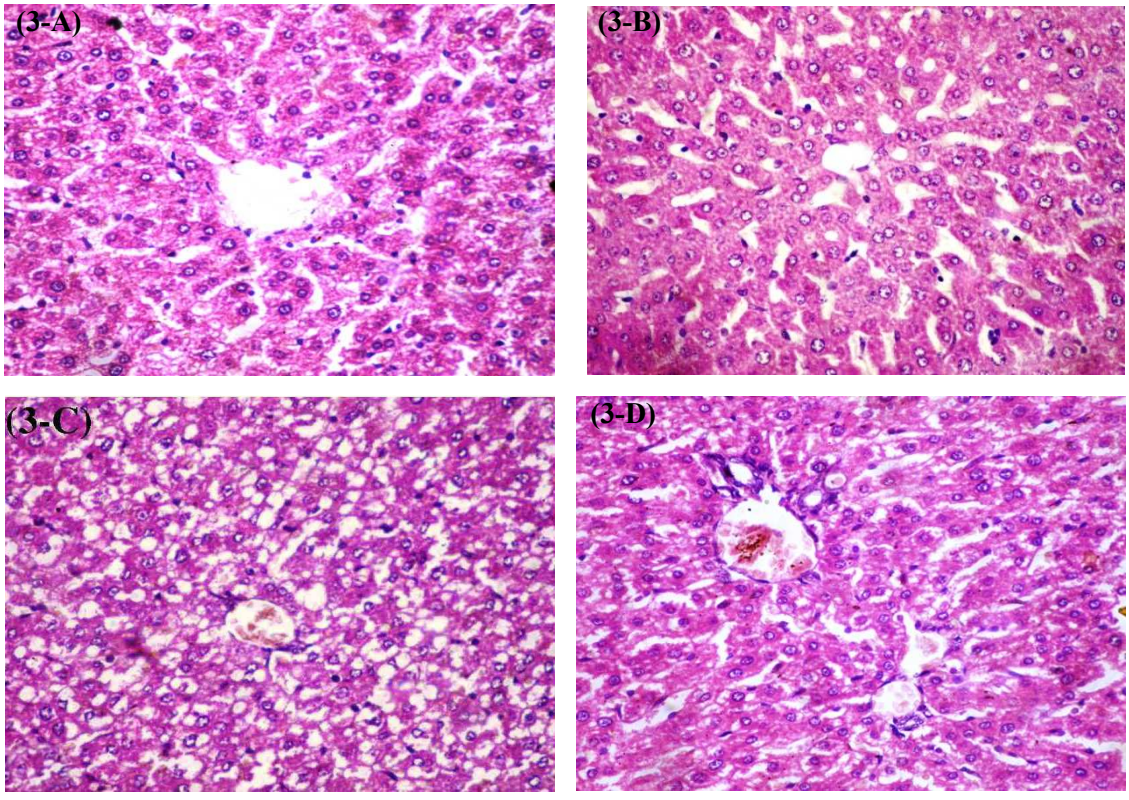
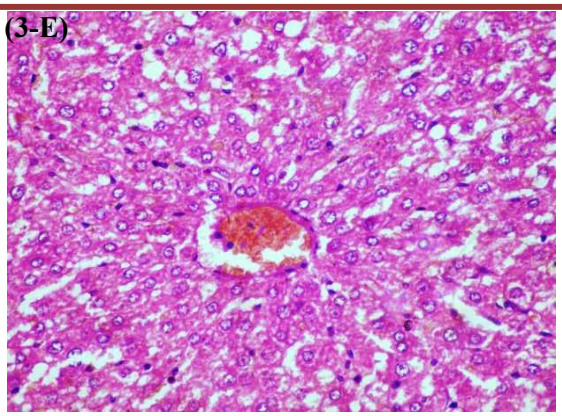


Fig (2): Photomicrographs of pancreatic sections of: **A**) a control rat showing normal structure, **B**): baclofen-treated rat showing more or less normal structure, **C)** ethanol dependent rat showing some disorganized acini with loss of apical acidophilia (*), Islet of langerhans shows many cells with dark small /pyknotic nuclei and loss of some islet cells leaving empty spaces. **D)** ethanol dependent rat showing decrease in basal basophilia (-) and inflammatory infiltrate around the ducts, **E)** alcohol-dependent rat treated with baclofen showing wide spaces between the acini. Some acini were destroyed leaving empty spaces, whereas others showed vacuolation with dilated congested blood vessels *H&E, × 400.*





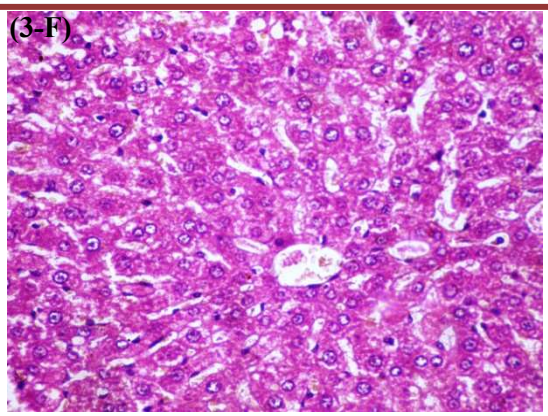
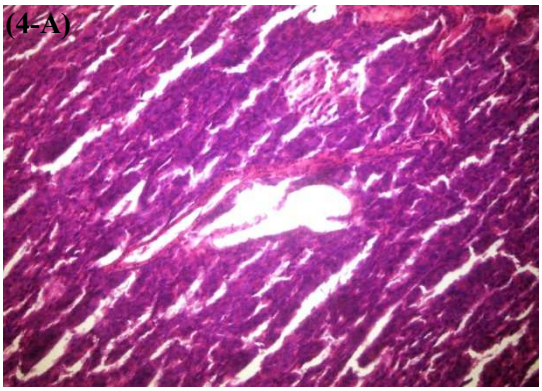
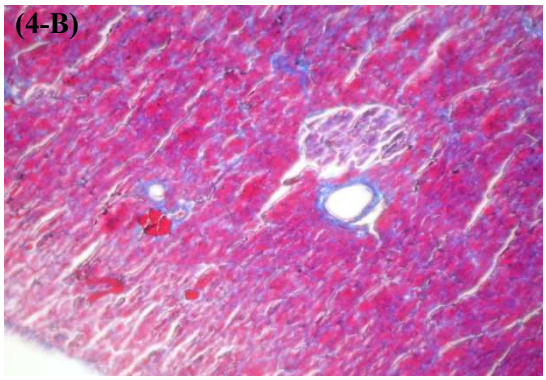
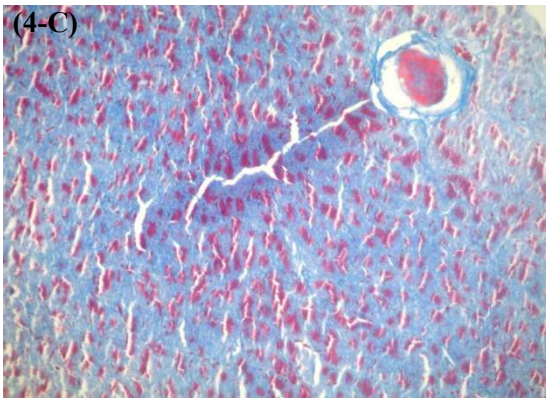


Fig (3): Photomicrographs of liver sections of: **A**) a control rat showing normal structure, **B**): baclofen-treated rat showing more or less normal structure, the hepatocytes have acidophilic cytoplasm and central rounded vesicular nuclei with one or two prominent nucleoli (thick arrow), **C)** ethanol dependent rat showing loss of normal hepatic architecture with vacuolated hepatocytes with a signet ring nucleus, indicating the microvesicular steatosis, **D)** ethanol dependent rat showing congested blood vessel and marked lymphocytic infiltration, **E)** alcohol-dependent rat treated with baclofen showing that most of the hepatocytes with a highly vacuolated cytoplasm (arrow), **F)** alcohol-dependent rat treated with baclofen showing an ill-defined borders of the hepatocytes with irregular nuclei (arrow head) *H&E, × 400*.







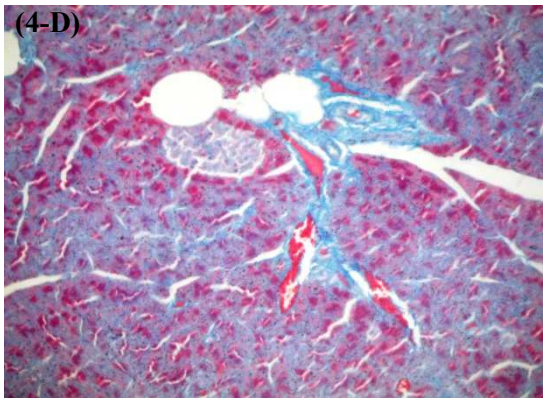


Fig. (4): photomicrographs of sections from pancreases stained with Masson Trichrome showing: **A)** normal collagen in control rat**, B)** mild collagen formation especially around the pancreatic vessels in Baclofen-treated rat, **C)** marked collagen formation in ethanol dependent rat, **D)** marked collagen formation in ethanol dependent rat treated with baclofen Masson Trichrome x 400

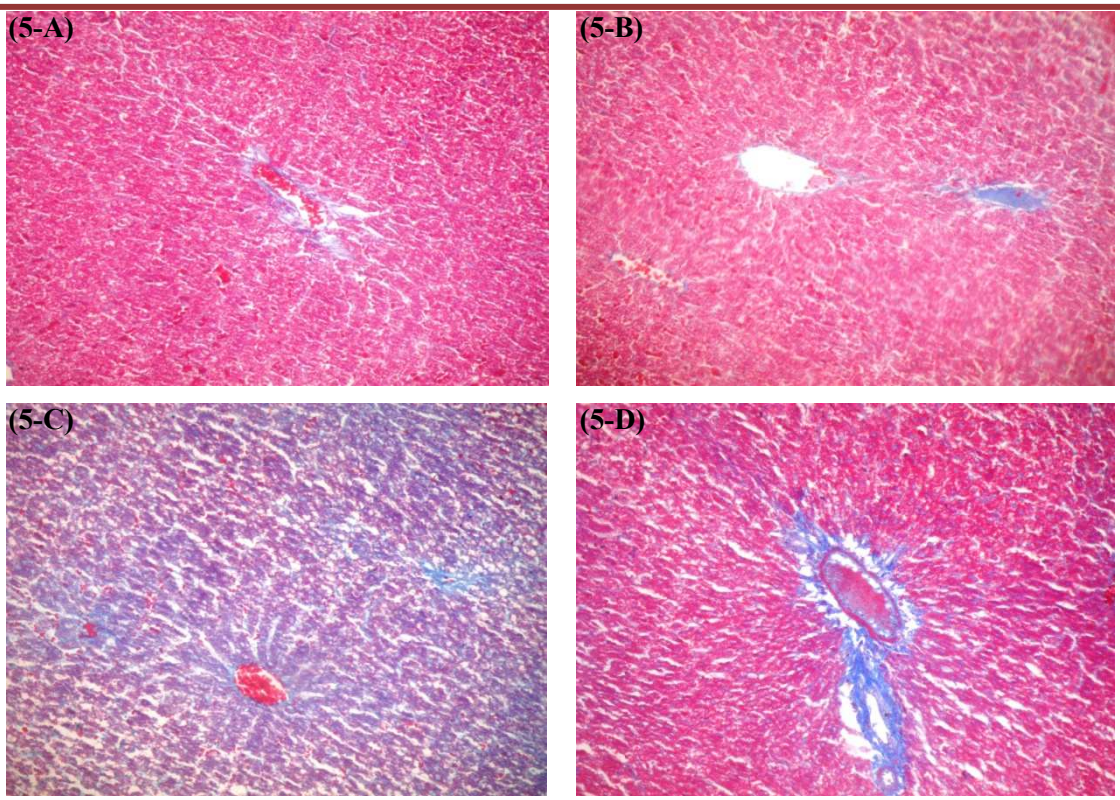


Fig. (5): photomicrographs of sections from livers stained with Masson Trichrome showing: **A)** normal collagen in control rat, **B)** mild collagen formation in Baclofen-treated rat, **C)** marked collagen formation in ethanol dependent rat, **D)** marked collagen formation especially around the central vein in ethanol dependent rat treated with baclofen *Masson Trichrome x 400*

DISCUSSION

Alcohol is a toxic compound on cells or tissues. It is readily metabolized by alcohol dehydrogenase to acetaldehyde, and then this primary metabolite is catabolized to CO_2 and H_2O. Acetaldehyde has a cytotoxic effect within the cells or tissues and also remains capable of reacting covalently with nucleophiles including nucleic acids, proteins, peptides, amino acids, lipids, and carbohydrates, especially in high chronic alcohol consumption [(7)].

The use of pharmacotherapy together with psychosocial interventions has enhanced the percentage of success in maintaining alcoholic patients in remission and assisting the development of a lifestyle compatible with long-term alcohol abstinence. However, drugs with proven efficacy are very few. The discovery of new medications capable of positively affecting the components of alcohol dependence syndrome, such as craving and protracted abstinence symptoms, became mandatory [(19)].

Baclofen is a potent and stereo selective γ-aminobutyric acid ($GABA_B$) receptor agonist used clinically to control spasticity. Recent preclinical experiments have demonstrated the efficacy of baclofen in suppressing both alcohol withdrawal signs in rats made physically dependent on alcohol and voluntary alcohol intake in alcohol-preferring rats [(20)]. Moreover, preliminary clinical open studies have confirmed the ability of baclofen to reduce alcohol craving and intake and alcohol withdrawal symptoms in alcohol-dependent patients [(4, 21)].

It was noticed that, there is limited data about the effects of baclofen (when used as a substitution therapy) on the target organs of chronic alcohol consumption like the brain, pancreas and liver. The aim of the current work was to evaluate the neuroendocrinologic, pancreatic and hepatic effects of baclofen in alcohol dependent rat model. To achieve this goal, serum levels of prolactin, leptin, amylase and lipase, ALP, AST, ALT, bilirubin and GGT, were assessed and the brain, pancreas and liver were submitted to histopathological examination.

The current study showed that baclofen administration to normal rats induced a significant decrease in the prolactin serum level. In **1985, Morosini et al.,** studied the effects of baclofen on the prolactin level. Baclofen was unable to significantly raise serum prolactin levels in healthy subjects and in patients affected by prolactinoma. On the contrary baclofen decreased

prolactin rise induced by cimetidine. It was concluded that in basal conditions, $GABA_B$ receptor doesn't play an obvious role in modulation of prolactin secretion and suggested that $GABA_B$ modulation of prolactin secretion doesn't obtain itself by dopaminergic pathways.[22] On the other hand, **D'Eramo et al. (1986)** found that baclofen blocks prolactin release when release of the hormone is dynamically stimulated by stress [23]. **Rigamonti and Müller, (2000)** also found that baclofen failed to increase the level of prolactin and growth hormone in normal rats [24].

It was also found that baclofen significantly decreased the serum leptin level. Leptin is a 16-kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite/hunger and metabolism. It is secreted from adipocytes, and leptin receptors, expressed in the hypothalamus and fat tissue to regulate energy balance and adiposity. Leptin is considered one of the most important adipose-derived hormones [25]. The finding of the present work was in a harmony with the study of **Amira and Oiso, (2010)** who found that serum leptin levels, which possibly reflect the amount of adipose stores, were decreased significantly by baclofen [26].

In this study, baclofen induced a significant alteration of the pancreatic and hepatic functions, represented as increased serum levels of all studied biochemical markers. However, AST level showed a non significant rise. On contrary, the histopathological findings showed that baclofen didn't induce significant changes in the examined organs of the normal rats. **Braun et al., (2004)** studied the role of $GABA_B$ receptor activation in pancreatic changes. They found that pancreatic islets contain high levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). It has recently been reported that baclofen reduces synaptic transmission by inhibition of vesicle priming. By contrast, the physiological roles of GABA in the pancreatic islets are not fully understood. GABA is stored in the β-cell in synaptic-like microvesicles, which accumulate GABA by active transport. Finally, they suggested that the ability of baclofen to inhibit Ca^{2+}-dependent exocytosis involves activation of the protein phosphatase calcineurin [27].

Biju et al., (2002) studied the relation between $GABA_B$ receptor enhancement and the hepatic neoplasia. They found that $GABA_B$ receptor enhancement induces hepatic neoplasia. Also, baclofen is seen to act as a potent co-mitogen, triggering DNA synthesis in primary cultures of rat hepatocytes, mediated through the G (i) protein coupled $GABA_B$ receptors [28].

Nevertheless, **Garbutt et al., (2010)** stated that limited data are available on the hepatotoxicity of baclofen. Among the many clinical trials evaluating the safety and efficacy of baclofen none mention hepatic toxicity or rates of serum ALT elevations occurring during chronic therapy. Although the product insert mentions that 5% of patients develop mild serum aminotransferase elevations, but little documentation is available on the significance, severity or duration of these abnormalities [29].

The current study showed that alcohol induced a significant increase in the mean values of prolactin. This result was compatible with the previous studies. They stated that alcohol induces hyper prolactinemia in both alcoholic men and women, but the mechanism is not fully established. They suggested that alcohol resulted in elevated serum prolactin level in normal probably through enhancing pituitary gland cell proliferation combined with altered hypothalamic neurotransmitters that regulate prolactin level [30].

Wilhelm et al., (2011) also found that prolactin is significantly elevated in alcohol-dependent patients during alcohol withdrawal and early abstinence, not showing a rapid decline after cessation of drinking. Moreover, prolactin, serum levels during alcohol withdrawal are associated with the severity of alcohol dependence and withdrawal symptoms [31].

In the present study, it was also found that alcohol induced a significant increase in the mean value of serum leptin. Previous studies demonstrated that chronic ethanol consumption increases body adiposity and circulating leptin levels. Additionally, it was suggested that chronic ethanol intake affects metabolism of the body by altering the leptin system that regulates energy balance. It was also found that chronic ethanol consumption not only increases leptin levels, but also alters leptin receptors in the hypothalamus and the perigonadal fat of mice [32].

Moreover, in this study it was observed that chronic ethanol administration induced significant functional and structural toxic changes, as seen from the statistical analysis of the biochemical results and the histopathological examination of the studied organs. These findings were consistent with all previous data concerning alcohol toxicity. It has long been accepted that excessive alcohol use can cause structural and functional abnormalities of the brain and other organs. Even heavy social drinkers who have no specific neurological or hepatic problems show signs of regional brain damage and cognitive dysfunction. Changes are more severe and other brain regions

are damaged in patients who have additional vitamin B1 (thiamine) deficiency [33].

Kirpich et al., (2008) stated that γ-glutmyl transferase (GGT) levels in the blood are very sensitive to changes in liver function and increased with consumption of even small amounts of alcohol. Higher levels are found in chronic heavy drinkers accompanied with high levels of ALP due to altered liver metabolism and damage [34].

The findings of this study were also in accordance with **Apte and Wilson (2003) and Oruc and Whitcomb (2004)** [35, 36]. They reported that chronic pancreatitis is one of the consequences of alcohol abuse and characterized by progressive pancreatic exocrine and endocrine insufficiency. Chronic pancreatitis is characterized by irregular sclerosis with destruction, loss of exocrine parenchyma and complete replacement of acinar, ductal and endocrine tissue by fibrotic tissue. It has been also reported that acute alcoholic pancreatitis develop on a pancreas already affected by chronic pancreatitis.

In recent years, **Clemens and Mahan (2010)** explained that these toxic changes progresses to irreversible pancreatic damage. They also showed that alcohol may promote chronic pancreatitis changes through toxic effect of its metabolites on acinar cells, oxidant stress or by facilitating activation of pancreatic stellate cells and key fibrogenic cells in the pancreas [37].

To clarify the mechanisms of hepatotoxic effects induced by chronic ethanol consumption, **Bruckner and Warren, (2001) ; Plaa and Carbonneau, (2001) and Jaeschke et al., (2002)** found that ethanol induced liver injury produced via several ways. Acetaldehyde and lipid peroxidation-derived adducts are generated in the early phase of alcohol-induced liver disease. Chronic ethanol consumption can alter metabolic activity of hepatocytes which causes an increase in acetaldehyde production. This event may result in morphologic changes in hepatocytes including cellular hypertrophy, micro and macro vesicular structure, fatty changes, liver necrosis and hemorrhages [38,39,40].

Regarding the results of baclofen-treated alcohol-dependent rats in the current study, significant functional toxic changes were observed as compared to the control group. However, these changes were significantly less than that those induced by ethanol administration except for the bilirubin level. The histoapathological findings of the current results supported the biochemical findings. The encountered changes by ethanol administration were more or less noticed through the examination of brain and pancreas.

It was reported that baclofen in subjects with alcoholic liver cirrhosis could significantly reduce the risk of relapse into alcohol-dependence, with a very good safety level [41]. However, the exact efficacy of low doses of baclofen on alcohol abuse is under debate, even if it appears that this molecule is quite safe at low doses [42]. In parallel, the use of high-dose baclofen to treat heavy drinking has recently increased in the medical community [43, 44, 45]. However, they stated that the precise safety level of this therapeutic practice is uncertain.

CONCLUSION

From the previously mentioned results it was concluded that baclofen induced functional pancreatic and hepatic toxic changes in normal rats. Moreover, the functional and structural toxic effects of chronic alcohol dependence on the brain, pancreas and liver were almost similar, after 4 weeks of the baclofen administration therapy.

RECOMMENDATIONS

Further studies were recommended regarding the toxic effects of baclofen with focusing on the molecular mechanisms which underlying such effects.

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التقييم السمى للتأثيرات الهرمونية العصبية والبنكرياسية والكبدية لعقار الباكلوفين
فى الجرذان البيضاء المعتمدة على الكحول

المشتركون فى البحث
غادة السيد المسلمى[1] ونرمين رأفت عبد الفتاح[2]
قسمى الطب الشرعى والسموم الاكلينيكية[1] والكيمياء الحيوية[2] ، كلية الطب البشرى – جامعة الزقازيق

الكحول هو مادة سامة للخلايا والأنسجة. الاستهلاك المزمن للكحول يتلف المخ والكبد وأعضاء أخرى كثيرة مثل البنكرياس. مؤخرا تم تصعيد طرق جديدة من العلاج التي تحافظ على الامتناع عن الكحول ومنع الانتكاس مثل باكلوفين واكامبروسيت. باكلوفين هو مثيل لمستقبلات الجابا استخدم مؤخرا كعقار بديل فعال. استهدف العمل الحالي تقييم الآثار العصبية الهرمونية، البنكرياسية والكبدية للعلاج بالباكلوفين في الجرذان البيضاء المعتمدة على الكحول من وجهة النظر السمية. تم تقسيم ثلاثين من ذكور الجرذان البيضاء البالغة إلى ٤ مجموعات، المجموعة ١: الجرذان الضابطة: ١٢ جرذ قسمت بالتساوي إلى ا-أ :ضابطة سلبية ، ١-ب ضابطة إيجابية (ع = ٦)، اعطيت الماء المقطر يوميا بالفم. المجموعة ٢ : مجموعة الباكلوفين: ستة جرذان اعطيت عن طريق الفم ٧.٥مجم /كجم/اليوم لمدة ٣ أيام، ثم ١٥ مجم / كجم/اليوم فيما بقى من ال ٤ أسابيع، المجموعة ٣: مجموعة الإيثانول: ستة جرذان اعطيت عن طريق الفم الإيثانول ٢.٥ جم / كجم / اليوم لمدة ٤ أسابيع، المجموعة ٤: مجموعة الإيثانول والباكلوفين: ستة جرذان اعطيت عن طريق الفم الإيثانول ٢.٥ جم / كجم / اليوم لمدة ٤ أسابيع ، ثم اعطيت الباكلوفين (٧.٥مجم /كجم/اليوم لمدة ٣ أيام، ثم ١٥ مجم / كجم/اليوم فيما بقى من ال ٤ أسابيع)، جميع الجرذان تم فحصهم عن طريق تقييم مستوى البرولاكتين، هرمون الليبتين، الكالين فوسفاتيز، الانين ترانسفيراز، اسبارتات ترانسبيبتيداز، البيليروبين، الأميليز والليباز والجاما جلوتاميل ترانسفيراز، مع فحص أنسجة المخ، البنكرياس والكبد. وقد جد أن باكلوفين أحدث انخفاض ذو دلالة احصائية في متوسط قيم البرولاكتين وهرمون الليبتين، مع زيادة كبيرة في الأميليز، الليباز، الكالين فوسفاتيز، والجاما جلوتاميل ترانسفيراز والبيليروبين، دون أي تغيير هستوباثولوجى .وأظهرت الجرذان المعتمدة على الإيثانول زيادة كبيرة في متوسط قيم البرولاكتين والليبتين، والأميليز والليباز، الكالين فوسفاتيز ، البيليروبين، الانين ترانسفيراز، اسبارتات ترانسبيبتيداز ، والجاما جلوتاميل ترانسفيراز مع تغيير شديد في التركيب الطبيعى للبنكرياس والمخ والكبد. كما أظهرت الجرذان المعتمدة على الكحول التى اعطيت الباكلوفين زيادة كبيرة في متوسط قيم الأميليز ،الليباز، الكالين فوسفاتيز والبيليروبين، الانين ترانسفيراز، اسبارتات ترانسبيبتيداز ,والجاما جلوتاميل ترانسفيراز مع انخفاض كبير في مستويات البرولاكتين والليبتين. كما كانت التغييرات الهستوباثولوجية في كل من المجموعتين متماثلة ، ولكن التغييرات الكيميائية الحيوية كانت أقل من تلك التي أحدثها الكحول. وتم استخلاص أن الباكلوفين أحدث تغيرات وظيفية سمية فى البنكرياس والكبد في الجرذان العادية. وعلاوة على ذلك، فإن التأثيرات الوظيفية والتركيبية السمية على أنسجة المخ والبنكرياس والكبد، الناتجة من إدمان الكحول المزمن، كانت متماثلة تقريبا بعد ٤ أسابيع من إعطاء عقار الباكلوفين . تمت التوصية بمزيد من الدراسات حول الآثار السمية للباكلوفين مع الاهتمام الخاص بفحص الآليات الجزيئية الكامنة وراءها.