CYTOTOXICITY INDUCED BY ALPRAZOLAM IN RAT VITAL ORGANS

Nadia A. Abdelmajeed and Amani M Manaa

Department of Biochemistry , Girls college of Education ,

king Abdulaziz University Jeddah , Kingdom of Saudi Arabia

P.O.Box 50098 Jeddah 21523

ABSTRACT

The current study demonstrated the cytotoxic effects of alprazolam on the vital organs of rats. Animals were divided into two groups; G1: normal control (not received any drug), G2: alprazolam treated group. Alprazolam was ingested orally using a single dose of 1 mg/ 100gm body weight. The effects of this drug toxicity on different tissue vital organs tissues (liver, kidney and heart) were studied after 10, 20 and 30 days from drug administration. The results revealed that administration of alprazolam induces oxidative tissue damage indicated by significant increase in the activity of xanthine oxidase (XO) with concomitant elevated level of nitric oxide (NO) in liver, kidney and heart of alprazolam -treated rats in comparison with normal animals. The increment of such oxidative tissue damage markers was accompanied by increased malondialdehyde (MDA, index of lipid peroxidation) in kidney ,and decrease in adenosine triphosphatase (ATPase) and lactate dehdrogenase (LDH) activities in cardiac tissue. The oxidative tissue damage induced in liver in response to alprazolam ingestion was supported by a depletion in the activity of hepatic sorbitol dehydrogenase (SD) coupled with elevation in serum enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) (indices of liver function). Also the obvious increase of serum markers of kidney, creatinine and uric acid levels as well as the increase in serum marker enzyme of heart, creatine phosphokinase (CPK) in alprazolam treated animals versus normal ones, indicating cardiorenopathy. Moreover, investigation also revealed that ingestion of alprazolam to rats led to decreased level of hemoglobin (Hb) compared with normal animals The deteriorations in the biochemical results were severe in 20 and 30 days after the drug administration and documented by the histopathological pictures of the studied organs.

Key words: *alprazolam, cytotoxicity, tissue damage, histopathological pictures, enzymes.*

INTRODUCTION

In the last few decades, the exponential increase in the use of powerful pharmacological agents has led to increased concerns about drug-induced adverse health events **(Skegg, 2001)**. Pre-marketing clinical trials provide conclusive information on drug effectiveness, but typically they are underpowered for de-

tecting adverse effects. Carefully designed and analyzed large-scale epidemiological post-marketing surveillance studies are therefore essential to establish the associations between drugs and adverse reactions **(Abrahamowicz et al., 2006)**.

Benzodiazepines (BDZs) are widely prescribed for the chronic treatment of epilepsy and panic disorders. Alprazolam is a member of BDZs group of drugs, widely used as antipsychotic and anxiolytic agent **(Isbister et al., 2004; Moroz 2004).** However, Patients using this drug report benzodiazepine related adverse events, such as drowsiness, dizziness, and reduced alertness **(Verster and Volkerts 2004)**. In addition, a vast amount of studies have shown that alprazolam in doses of 0.5 mg and higher induce changes in brain oxidative metabolism and impairs a variety of cognitive and psychomotor skills such as memory impairment, speed of responses, tracking performance and a considerable potential to induce dependence and abuse **(Verster and Volkerts 2004; Pardo et al., 2006; Leufkens et al., 2007)**. It also induces conformational changes in hemoglobin **(Maitra et al., 2007)** and intercalates into the DNA **(Saha et al., 2009a)**. In addition, it was found that alprazolam produced severe cytotoxicity in human cell lines as it induced elevated level of reactive oxygen species (ROS) and depletion of antioxidants **(Saha et al., 2009b)**. Generally, it was reported that all benzodiazepines drugs can cause respiratory depression **(Woodcock et al., 1981)**, hepatic dysfunction **(Ananth et al., 1994)**, Hypotension and thrombophlebitis **(Donaldson and Gibson, 1980; Glaser et al., 1982)**, brain damage **(Meador, 1994)**.

None of the previous studies investigated the pathological effects of these drugs on different organs in experimental animals, so the objective of this investigation is to study the effects of alprazolam in inducing oxidative tissue injury on rat liver, kidney and heart.

MATERIALS AND METHODS

Chemicals:

All chemical reagents were of analytical grades purchased from Sigma Chemical Co. (St. Louis, Mo, USA), Merk (Germany) and BDH (England). Diazepam drug was obtained from Swiss Hofman Laroch Limited Company.

Animals :

60 adult male albino rats (100-120gm) were obtained from animal house, King Fahed Center for Medicinal Research, King Abdul-Aziz University, Jeddah. The animals were housed in cages under standard hygienic condition and were fed with rat chow and water ad libitum. In order to optimize drug absorption. All animals were fasted for 1 hour prior to drug administration.

Experimental Design :

Rats were divided into Two groups normal healthy group (group 1) and drug treated group (group 2), each of 30 rats. Alprazolam drug was administered orally using a single dose of 1mg/100gm body weight **(Leufkens et al., 2007)**. The effect of this drug toxicity on different tissue organs (liver, kidney and heart) was studied after 10, 20 and 30 days from drug ingestion. After each studied period the blood samples were collected from animals into sterilized tubes for serum separation and into tubes containing heparin for hemoglobin determination. Serum was

separated by centrifugation at 3000_ g for 10 minutes and used for biochemical analysis. At the end of each experiment, rats of each experimental period were sacrificed under ether anesthesia and the liver, kidney and heart samples were collected, minced and homogenized in either ice cold bidistilled water or 10% to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 minutes at 10000g. at 4ºC and the supernatants and used for different biochemical tissue analysis.

Biochemical analysis :

All the following biochemical parameters were measured spectrophotometrically .

Tissue analysis :

Nitrite concentration (an indirect measurement of NO synthesis) was assayed using Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) in acidic medium **(Moshage et al., 1995)**. Lipid peroxidation was determined by measuring the formed MDA **(Buege and Aust. 1978)**. XO activity was determined by the reduction of nitroblue tetrazolium (NBT) **(Fried and Fried, 1974)**. ATPase was determined using the method of Tsakiris and Deliconstantinos (1984). LDH activity was evaluated according to **(Bergmeyer, 1975)**. Sorbitol dehydrogenase was measured by the method of **Bergmeyer (1974)**.

Serum analysis:

ALT and AST activities were determined according to the method described by **(Bergmeyer et al., 1986)**. Gamma Glutamyl Transferees (GGT) was measured by the method described by **Shaw et al. (1983)**, uric Acid (UA) by the method described by **Bulgar and Johns (1941)**, creatinine (Crea) by the method of **Larsen (1972)** and creatine phosphokinase (CPK) by the method described by **Rosalki (1967)**. GGT was assayed by the method of **Schmidt and Schmidt (1981)**.

Blood analysis :

Hb was estimated in the whole heparinized blood by cyanmethaemoglobin method **(Drabkin and Austin, 1932)**.

Histological evaluation :

Representative slices from liver, kidney and heart tissues were taken from the eviscerated animals and fixed in 10% formalin. For light microscopy examination, the tissues were embedded in paraffin, sectioned at 5 _m and stained with hematoxylin and eosin (H&E).

Statistical analyses:

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.D. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) coupled with post-hoc (LSD) and followed by Bonferoni as a post ANOVA test **(Evans and O'Connor, 2007)**.

RESULTS

Oxidative tissue injury markers in different tissue organs in normal and alprazolam treated groups after the three studied different periods are shown in table (1). Alprazolam administration to rats led to marked elevated activity of xanthin oxidase (XO) with concomitant increase in nitric oxide (NO) level in liver, kidney and heart of alprazolam-treated rats compared with normal one. The

elevation of such markers was accompanied by reduction in the activity of sorbitol dehydrogenase (SD) in liver, elevated level of malondialdehyde (MDA) in kidney ,and depletion in adenosine triphosphatase (ATPase) and lactate dehdrogenase (LDH) activities in heart tissue. Table (2) shows the levels of blood functional markers in normal and alprazolam treated rats after three studied different periods. The results revealed increased in liver function marker enzymes, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT). Marked increment of serum markers of kidney function, creatinine and uric acid levels and increase in serum marker enzyme of heart, creatine phosphokinase (CPK) in alprazolam treated animals versus normal ones were also observed. The table also shows that administration of alprazolam to rats led to decreased level of hemoglobin (Hb) in alprazolam treated animals compared with normal animals. The histopathological pictures of liver, kidney and heart of alprazolam treated animals were observed in figures 1,2 and 3 respectively. The pictures showed severe necrotic degenerative changes in the liver and kidney in rats treated with alprazolam. Inflammation and necrotic changes in the muscle fibres of hearts of animals under the effect of the used drug was also observed. These changes were severe in 20 and 30 days after the drug ingestion.

DISCUSSION

The benzodiazepines (BZs) are still the most widely used anxiolytic compounds. However, recent study demonstrated that these drugs have serious toxic effects **(Saha et al., 2009b)**. The current investigation was designed to evaluate the undesirable toxic effects of alprazolam which is a member of BDZs group of drugs.

Administration of alprazolam drug to rats induced oxidative tissue damage in different organs of rats which was proved by obvious increment in the activity of free radical producing enzyme, XO accompanied by elevated NO level in livers, kidneys and hearts of alprazolam treated rats in compared with normal animals. The increment of such biomarkers was accompanied by increased MDA in kidney, and decreased in ATPase and LDH activities in heart tissue suggesting that liver, kidney and heart are target organs for alprazolam toxicity. Our results agree with previous study stated that this drug produced severe cytotoxicity in human cell lines as it induced elevated level of reactive oxygen species (ROS) and depletion of antioxidants **(Saha et al., 2009b)**.

Chronic and acute overproduction of ROS under pathophysiologic conditions forms an integral part of the development of liver, kidney as well as cardiovascular diseases **(Victor et al., 2009; Hinson et al., 2010; Zhang et al., 2010)**. XO is one of the oxidases enzymes which have the major role in the production of ROS and reactive nitrogen species (RNS) that can produce oxidative tissue injury **(Harrison 2002; Fadillioglu et al., 2003; Victor et al., 2009)** . It catalyzes the reduction of nitrite to NO which has various pathological dangerous effects on different tissues **(Mohamed et al., 2001; Gao, 2009)**.

Nitric oxide (NO) exerts a broad range of effects on bodily functions including muscle

contractility, platelet aggregation, metabolism, neuronal activity, and immune responses. The underlying mechanisms rely primarily on elevating guanosine 3',5'-cyclic monophosphate due to the stimulation of soluble guanylyl cyclase, inhibiting mitochondria respiration by the action on cytochrome C oxidase, and nitrosylating proteins and enzymes. Under pathophysiological conditions, an increased production of NO concurrently with an enhanced generation of superoxide leads to the formation of peroxynitrite, a potent oxidative agent, and thus tissue injuries **(Gao, 2009)**. Production of peroxynitrite was found coupled with oxidizing cellular structure and causes lipid peroxidation **(Weinstein et al., 2000; Sayed Ahmed et al., 2001)**. Lipid peroxidation and lipid-radical cycles are two alternative processes. These cycles activate membrane proteins, protect membrane lipids from oxidation and prevent from formation of endogenous aldehydes. Experimental and clinical evidence suggests that production of endogenous toxic aldehyde, such as MDA is the major initiators of the metabolic alterations **(Dmitriev and Titov, 2009)**.

ATPase is an important membrane bound enzyme that is directly involved in energy conversion and has an important role in maintaining the integrity of the myocardial membrane **(Devika and Mainzen Prince 2008)**. The reduction in such enzyme may attributed to the ability of alprazolam to induce lipid peroxidation which has deleterious effects on the cell membrane and coupled with inactivation of membrane-bound enzymes **(Hazarika and Sarkar 2001)**. A significant fall in ATPase activity of rat hearts in response to alprazolam treatment may lead to a decrease in metabolic energy turnover due to toxic effects of alprazolam.

The present work revealed that the toxic dangerous effects of alprazolam induced in livers of alprazolam treated rats was documented by a decrease in the activity of hepatic SD coupled with increase in liver serum marker enzymes, AST, ALT and GGT. This result was supported by previous clinical studies stated the complex interaction between hepatic dysfunction and benzodiazepines treatment which may directly contribute to hepatic encephalopathy, **(Ananth et al., 1994)**. The abnormalities in such marker enzymes activities may be due to the release of these enzymes from liver cytosol into the blood stream as a result of tissue damage caused by alprazolam toxic effect. This result was also documented by liver histopathological picture which showed severe damage ensured by severe degenerative necrotic changes observed after the three different experimental periods. The significant elevated levels of serum creatinine and uric acid in animals treated with alprazolam were coupled with deteriorative changes in both glomeruli and tubules of kidney tissue observed in histopathological pictures of animals treated with the used antipsychotic drug and these abnormal changes are well indicators of renopathy.

The cardiac tissue damage induced by toxic effect of alprazolam in rats was ensured by pronounced increased in the activity of diagnostic serum marker enzyme, CPK and a decrease in LDH of heart tissue in alprazolam treated rats compared to normal ones. This

was supported by the hitopathological picture of heart tissue which demonstrated inflammation and myonecrotic lesion of heart muscle fibres. These findings confirm the onset of myocardial lesion and leaking out of the marker enzymes from heart to blood **(Ganesan et al., 2009)**.

Ingestion of alprazolam to rats led to decreased level of Hb in alprazolam treated rats compared with normal animals. This result is coped with previous published data stated that Hb deficiency in response to alprazolam may be its ability to induce conformational changes in hemoglobin **(Maitra et al., 2007)**. Decreased of Hb level induced a state of anemia which may lead to thrombocytosis which might also related to cardiovascular disease, biochemical abnormalities and impaired cell-mediated immunity with increased susceptibility to infection **(Farthing, 1989; Silverberg et al., 2009)**.

It could be concluded that, the changes in both biochemical markers and in histological pictures of liver, kidney and heart of alprazolam treated animals leads to suggestion that alprazolam, the antipsychotic drug, might have serious toxic effects on different body organs.

Table 1 : Oxidative stress markers in different organs of normal and alprazolam treated rats.

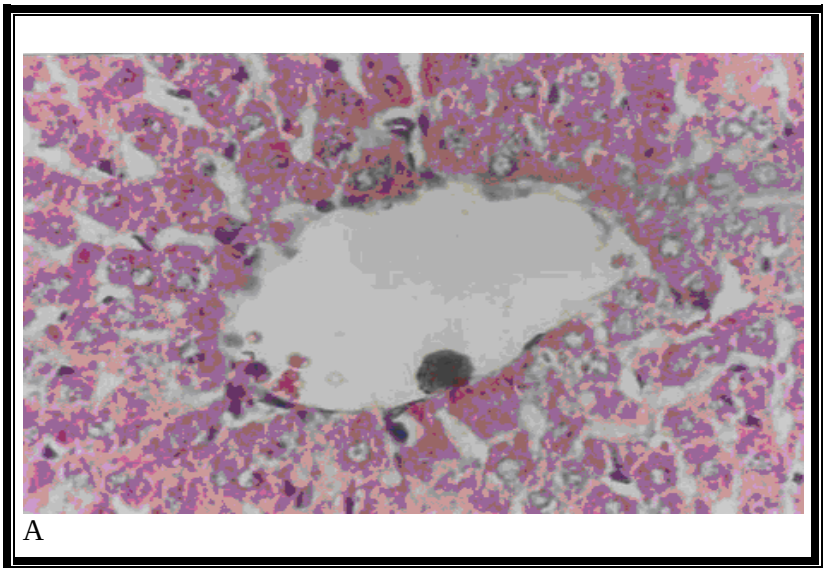
Parameters	Normal	10 days	20 days	30 days
Liver				
XO activity	3.15 ± 0.26	25.39 ± 2.40[a]	33.09 ± 2.29[a]	41.15 ± 2.44[a]
NO activity	17.30 ± 1.28	28.09 ± 2.9[b]	37.38 ± 1.88[a]	41.11 ± 3.34[a]
SD activity	32.5 ± 1.60	3.87 ± 0. 45[a]	2.82 ± 0.20[a]	2.08 ± 0.20[a]
Kidney				
XO activity	1.97 ± 0.26	10.65± 1.48[a]	21.73 ± 1.90[a]	28.42 ± 2.62[a]
NO activity	5.82 ± 0.90	28.58 ± 2.13[a]	37.23 ± 1.43[a]	44.12 ± 3.18[a]
MDA level	10.17 ± 1.78	37.41 ± 2.08[b]	54.05 ± 3.64[a]	87.56± 3.24[a]
Heart				
XO activity	2.12 ± 0.23	12.05 ± 0.84[a]	16.62 ± 0.52[a]	21.95 ± 1.88[a]
NO activity	12.40 ± 2.1	59.16 ± 2.30[a]	70.24 ± 2.55[a]	77.96 ± 3.16[a]
LDH activity	5.18 ± 0.12	2.87 ± 0.32[a]	2.50 ± 0.22[a]	1.28 ± 0.10[a]
ATPase	5.06 ± 0.25	1.56 ± 0.62[a]	2.87 ± 0.53[a]	1.17 ± 0.22[a]

Data are expressed as mean± SD of 8 rats in each group, XO and SD are expressed in **n** mol/ min/mg protrein, NO is expressed in umol/g tissue, LDH and ATPase are expressed in u mol/ min/mg, MDA is expressed in nmol/g tissue. [a]P< 0.001, [b]P < 0.01, when compared with normal group.

Table 2 : Blood functional markers of different organs in normal and alprazolam treated rats after three different periods.

Parameters	Normal	10 days	20 days	30 days
AST (µl /l)	19.57± 2.3	292.66 ± 4.32***	321.16 ± 6.63***	375.83 ±5.67***
ALT(µl /l)	17.70 ± 0.76	70.15 ± 6.46***	82.13 ± 3.84***	91.86 ± 1.49***
GGT (µl /l)	15.56 ± 0.69	22. 06 ± 1.78*	24.33 ± 1.50**	25.66 ± 1.75**
Uric acid (mg/dl	2.60 ± 0.057	3.47 ± 0.36*	5.41 ± 0.29**	7.81 ± 0.72***
Creatinine (mg/dl)	0.59± 0.004	4.00± 0.64***	6.45 ± 0.33***	9.20± 0.32***
CPK (µl/l)	30.34 ± 8.46	105.00 ± 4.85***	110.5 ± 4.72***	145.66 ± 11.5***
Hb (g/dl)	16.38 ± 0.61	12.11 ± 0.82*	10.88 ± 0.37**	10.18 ± 0.56**

Data are expressed as mean± SD of 8 rats in each group . *P< 0.05, **P < 0.01 , ***P < 0.001 when compared with normal group.



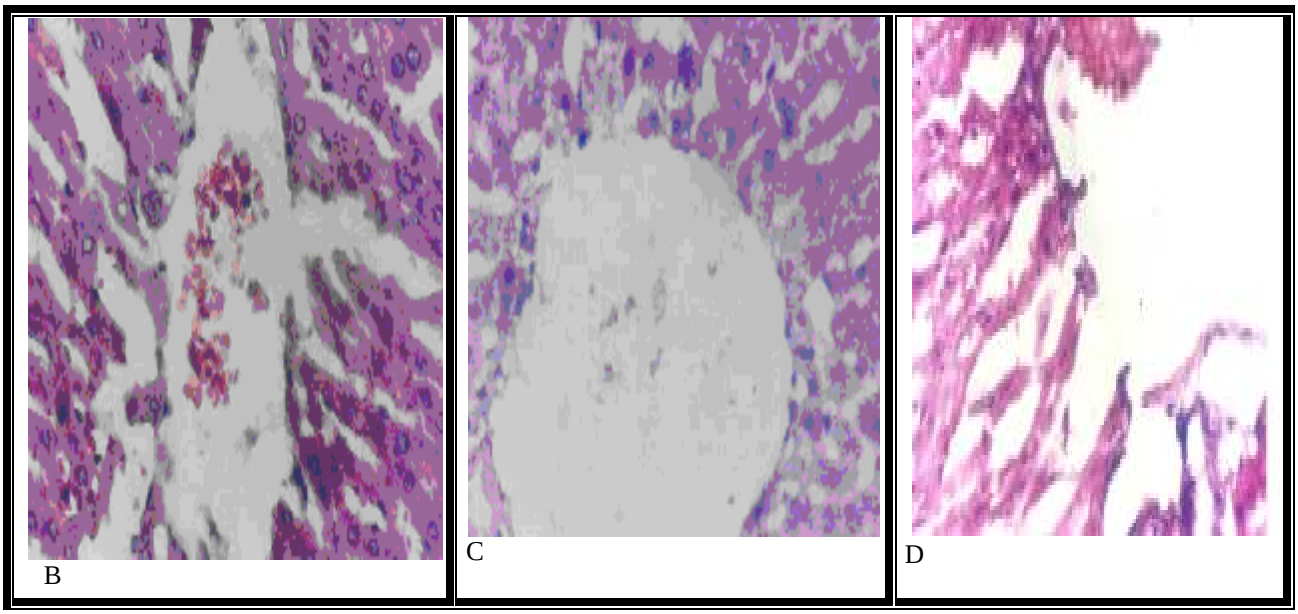


Figure (1) : Effect of alprazolam on liver tissue. A-light microscopic picture of normal liver showing normal hepatocytes B - liver of rats treated with alprazolam after 10 days commenced just after of drug administration, showing loss of liver normal architecture observed by degenerative changes in hepatocytes. (C and D) - Liver of rats treated with alprazolam after 20 days and 30 days respectively commenced just after of drug administration, showing severe degenerative change in hepatocytes (H & E ×400).

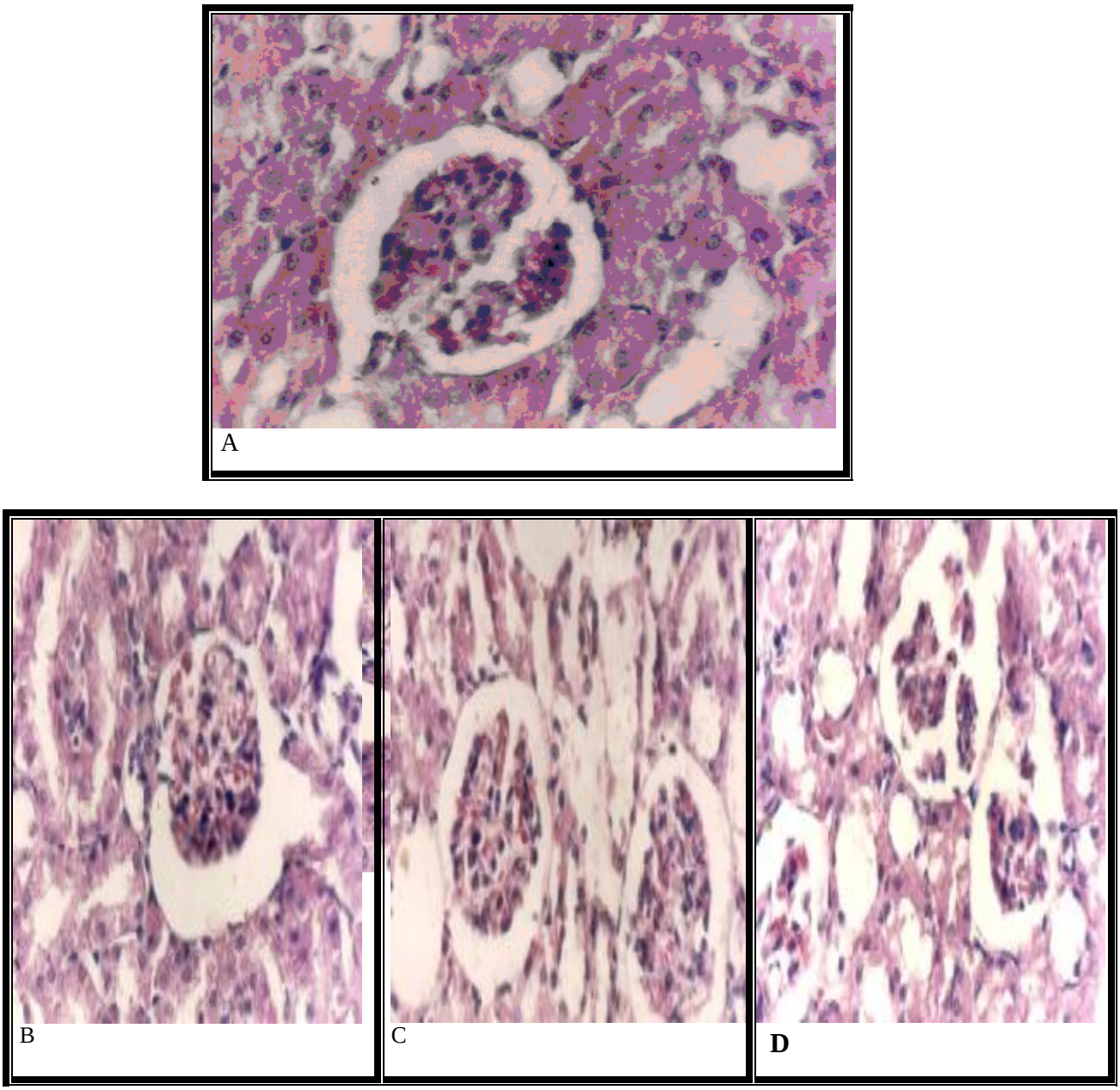


Figure (2): Effect of alprazolam on Kidney Tissues. A-light microscopic picture of normal kidney showing normal tubules and glomeruli B - kidney of rats treated with alprazolam after 10 days, commenced just after of drug administration, showing shrinkage of glomeruli, shrinkage of epithelial lining of some tubules and dilation of the lumen of the tubules. (C and D) - kidney of rat treated with alprazolam after 20 days and 30 days respectively, commenced just after of drug administration, showing severe degenerative change in glomeruli and tubules (H&E ×400).

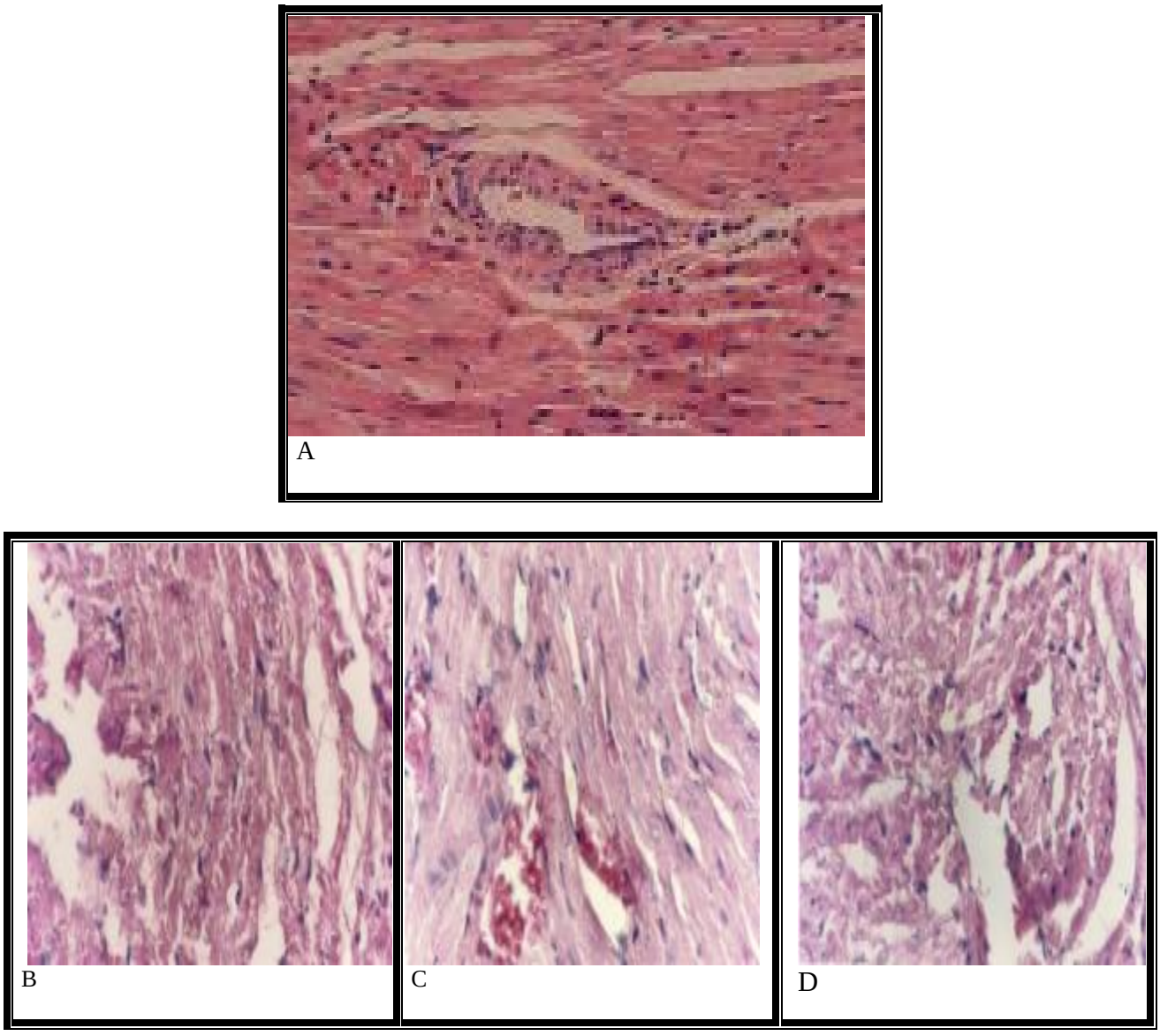


Figure (3): Effect of alprazolam on heart Tissues. A-light microscopic picture of normal heart showing normal muscle fibers B - Heart of rat treated with alprazolam 10 days after drug administration showing necrotic degenerative change of muscle fibres in some areas (C and D) - Hearts of rats treated with alprazolam 20 days and 30 days after drug administration respectively showing inflammatory cells (C and D) and severe necrotic changes in heart muscle fibers (D) (H&E ×400).

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الملخص العربى

السمية الخلوية التى يسببها ألبرازولام فى الأجهزة للجرذان

د/ ناديه أمين عبدالمجيد د/ أمـــانى مـنـــاع

قسم الكيمياء الحيوية – كلية التربية للبنات – جامعة الملك عبدالعزيز – جدة – المملكة العربية السعودية

أظهرت الدراسة الحالية التأثير السام للألبرازولام على خلايا الأجهزة الحيوية للجرذان، وتم تقسيم الحيوانات إلى مجموعتين؛ المجموعة الأولى : المجموعة العادية الحاكمة، المجموعة الثانية : المجموعة المعالجة بالبرازولام، تم تناول ألبرازولام عن طريق الفم باستخدام جرعة واحدة (١ملجرام / ١٠٠ جم من وزن الجسم)، وتم دراسة التأثيرات السمية لهذا العقار على الكبد والكلى والقلب بعد ١٠ و ٢٠ و ٣٠ يوم من تناول هذا العقار.

وأظهرت النتائج بأن إعطاء هذا العقار أدى إلى إحداث تلف مؤكسد للأنسجة وتبين هذا من زيادة ملحوظة فى نشاط إنزيم الزانسين أوكسيديز ويصاحب ذلك إرتفاع مستوى أكسيد النيتريك فى الكبد والكلى والقلب فى الجرذان المعالجة بالبرازولام بالمقارنة مع الحيوانات العادية، وتبين أيضاً فى هذا البحث أن الزيادة فى هذه المؤشرات الدالة على تلف الأنسجة يصاحبه زيادة فى المالونالديهيد (المؤشر الدال على أكسدة الدهون وتلف جدار الخلايا) فى الكلى وانخفاض فى نشاط إنزيم الأدينوساين ترای فوسفتيز واللاكتات ديهدروچينيز فى أنسجة القلب.

وقد أوضحت النتائج أن التأكسد الناجم عنه تلف الأنسجة فى الكبد نتيجة لتناول البرازولام تم تأكيده بانخفاض فى نشاط إنزيم السوربيتول ديهيدروچينيز فى الكبد مقروناً بارتفاع فى إنزيمات الدم، الاسبارت أمينو ترانسفيراز والألانين أمينوترانسفيراز والجاما جلوتاميل ترانسفيراز (GGT) (مؤشرات وظائف الكبد)، وأصبحت الدراسة أيضاً زيادة ملحوظة فى المؤشرات الدالة على وظائف الكلى ووظائف القلب فى الدم وهى الكرياتينين وحمض اليوريك، (مؤشرات وظائف الكلى) وإنزيم الكرياتين فوسفوكيناز (مؤشر وظائف القلب) وذلك فى الحيوانات المعالجة بالبرازولام مقارنة بالمجموعة العادية الحاكمة وهذه المؤشرات تشير بالحالة المرضية للكلى والقلب.

وعلاوة على ذلك، كشف هذا البحث أن إعطاء ألبرازولام للجرذان أدى إلى إنخفاض فى مستوى الهيموجلوبين فى الدم بالمقارنة مع الحيوانات الطبيعية، ووجد فى هذه الدراسة أن التدهور فى النتائج البيوكيميائية كانت شديدة بعد ٢٠ و ٣٠ يوماً من تناول هذا العقار والتى أكدتها الصور التشريحية المرضية لكلاً من الكبد والكلى والقلب.

الكلمات المفتاحية : ألبرازولام – السمية الخلوية – أضرار بالأنسجة – الصور التشريحية المرضية – والإنزيمات.