THE VALUE OF OZONE IN THE MANAGEMENT OF ACUTE CARBON TETRACHLORIDE INTOXICATION IN ALBINO RATS

BY

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ABSTRACT

Ozone administration in acute toxicity with CCl4 affection is a new line of therapy, the value of which is not yet well estimated. Aim: The present work was an in vivo experimental study to investigate the toxic effects of CCl4 on certain serum enzymes levels and histopathological picture of the liver and kidney. Also the influence of repeated administration of ozone in atoxic doses on the progress of CCl4 toxicity was evaluated. After CCl4 oral intoxication of 45 rats for one cession, 15 animals were isolated to be considered as a toxic group (received CCl4 only). The remaining 30 animals were subdivided into two subgroups one subgroup was treated with atoxic dose of O_2-O_3 mixture while the other subgroup was given placebo (nitrogen) for 2 weeks. From these animals blood samples were taken for estimation of: Liver function tests: serum albumin, SGOT, SGPT, cholinesterase and γ-GT, beside serum creatinine. Thereafter, the rats were dissected and their livers and kidneys were taken for histopathological studies. Administration of oxygen-ozone mixture as intraperitoneal injection for 15 sessions at a daily dose of 1 ml into the CCl4 intoxicated rats showed that there was a significant improvement of the liver function tests in the form of a significant decrease of the elevated serum GOT, GPT and γ-GT and significant increase of the inhibited cholinesesterase. This feature was a trial to restore these enzymes to their normal limits. In addition, histopathological studies of the liver and kidney, showed marked improvement after ozone administration, if compared to the placebo. So, qualitative and quantitative changes of hepatocytes, in the form of mild macro and microvesicular steatosis were less noted whereas the central necrosis and hyperplasia of hepatocytes disappeared completely. At the same time, mild cloudy swelling in the renal proximal tubules occurred less frequent while no casts inside the tubules were detected. Ozone administration enabled the rats to maintain the hepatocellular integrity after CCl4 poisoning. Subsequently, by treatment with ozone significant reduction of transaminases and significant increase of cholinesterase together with improvement of the histopathological features in rats which previously received CCl4.

Key words: Carbon tetrachloride, Haematoxylin and Eosin, Fatty stains .

INTRODUCTION

Untoward chemicals (pesticides, toxins) may be found in drinking water, ingested food and the atmosphere beside some medical drugs (Hayes, 1993).

Carbon tetrachloride (CCl4) intake induced liver injury (Sugihara et al., 1992; Delrat et al., 1994; Muriel et al., 2001; Sheweita et al., 2001; Mohammad et al., 2007). However, little attention has been paid to the effect of atoxic doses of CCl4 on the kidney (Bareceloux, 2001; El-Azab, 2002; Mohammad et al., 2007).

Treatment of CCl4 toxicity using ozone had been tried before (El-Azab, 2002; Mohammad et al., 2007) and the obtained results were optimistic (Leon et al., 1998). In this respect, Candelario-Jalil et al. (2001) suggested that, ozone is able to promote oxidative tolerance preventing hepatocellular damage mediated by free radicals due to CCl4 toxicity. Recently, Mohammad and colleagues (2007) stated that ozone, contains a large excess of energy which makes it a treatment of choice in certain pathological conditions and an adjunct to treatment in others. On the other hand, Chen et al. (2007) found that chronic exposure to ozone was associated with elevated biomarkers of lipid peroxidation and oxidative damage while inhibition of antioxidant capacity was only induced by short term exposure to O_3.

In turn, the effect of ozone on acute toxicity with CCl4, is still in need of comprehensive study. Ozone administration in such disastrous affection is a new line of therapy, the value of which is not yet well estimated (Mohammad et al., 2007).

The purpose of the present study was to evaluate the effectiveness of treatment with ozone in protecting rats intoxicated with carbon tetrachloride, a strong hepatotoxic agent.

MATERIAL AND METHODS

Male albino rats weighing 250-300 g were included in this study. They were obtained from the animal house at Faculty of Medicine, Mansoura University and were fed on the ordinary food. The animals were housed under ordinary room temperature, which ranged in summer between 30-38°C, during which the study was performed.

The present study was conducted on 45 rats. Each of them was injected by 1 ul/g carbon tetrachloride intraperitoneally only once (Leon et al., 1998). Then 24 hours later, 13 rats of the 43 were separated and sacrificed (two animals died before sacrification). Their blood samples were obtained, left to clot and unhaemolysed sera were separated. These sera were analysed for liver and kidney functions. The results were statistically analysed and compared

to that of the normal rat controls.

The remaining 30 rats were subdivided into 2 equal subgroups. Each rat included in the 1st subgroup received oxygen-ozone mixture (Humazon-PM, Gml HD 76356 Weingarten-Karlsruhe) by intraperitoneal injection daily [dose : 1ml which contained 40 ul of ozone per one milliliter oxygen] for 15 sessions (Leon et al., 1998). The 2nd subgroup acted as a drug control receiving placebo (inert nitrogen: Masser Gases, Alex. Egypt) by intraperitoneal injection of 1.0 ml.

At the end of the experiment, animals in each subgroup were sacrificed and blood samples were obtained, left to clot and unheamolysed sera were separated. These sera were analysed for albumin, SGOT, SGPT, γ-GT and pseudo cholinestrase as well as creatinine. The results of the ozone group were statistically analysed and compared to the corresponding data of placebo group.

At the same time, 15 male albino rats served as a control group were managed without any chemical (CCl4, ozone or nitrogen) intake.

Methods: The following substances were estimated in each rat's serum:

1- Albumin (Drupt, 1974).
2- Aminotransaminases: SGOT and SGPT (Reitman and Frankel, 1957).
3- Gamma-glutamyl transferase (Szasz, 1969).
4- Cholinesterase (Ellman, 1961).
5- Creatinine (Henry, 1974).

After the end of each experiment, the rats the of respective subgroup were dissected, and their livers and kidneys were taken for histopathological studies.

Histopathological examination:

Livers and kidneys of sacrificed animals were fixed in 10% formaline, embedding in hard paraffin to form blocks, serial sections of 5 microns in thickness were cut for Haematoxylin and Eosin (Hx. & E.) stain according to Drury and Wallington (1967).

Statistical analysis:

The biochemical data were collected and statistically analysed using (statistical package for social sciences) SPSS program for windows release version 11.

Qualitative data were presented as %. Quantitative data were presented as mean ± SD. Then the difference between each two respective means was tested by student t-test. The P values was considered statistically significant if P value was <0.05.

RESULTS

Table (1) shows the liver and kidney

function tests of rats which received CCl4 course only versus the normal control rats: There was affection of the liver functions following CCl4 toxicity in the form of a significant acute toxic increase of the level of SGOT and SGPT. However, the level of serum albumin did not show significant change. At the same time, there was a significant toxic decrease of the level of serum cholinesterase and a significant toxic increase of the serum γ-GT levels following CCl4 toxicity.

On the other hand, there was no significant change of the level of serum creatinine following CCl4 acute toxicity.

Table (2) shows liver and kidney functional status of rats which received CCl4 together with oxygen-ozone mixture versus those which received placebo (nitrogen) together with CCl4 intoxication.

In ozone treated group, there was significant decrease of the level of SGOT and SGPT (P value 0.000). However, the level of serum albumin did not show significant change.

In rats which received placebo with CCl4 intoxication, there was a persistent disturbance of liver functions in the form of a toxic increase of the level of SGOT and SGPT. Moreover, there was a persistent toxic decrease of γ-GT level and a toxic increase of the level of serum cholinesterase in rats which received CCl4 together with placebo.

On the other hand, in rats treated with oxygen-ozone mixture, there was significant increase of the level of serum cholinesterase and significant decrease of the serum γ-GT levels (P value 0.000).

However, there was no significant changes of the level of serum creatinine following the treatment with oxygen-ozone mixtures or placebo.

Histopathological findings of the liver of rats which received carbon tetrachloride (CCl4) only (Fig. 1):

In the hepatocytes of rats which received CCl4 only, microscopical examination revealed the presence of degenerative changes in the form of foci of necrotic hepatocytes in the form of central necrosis was noticed in 76% of rats, whereas hyperplasia of hepatocytes appeared in 65% of rats.

Histopathological findings of the liver of rats which received carbon tetrachloride (CCl4) and placebo (inert nitrogen).

In the hepatocytes, foci of micro and macro vascular steatosis occurred in 100% of rats, central necrotic foci in 74% of rats, and hepatocyte hyperplasia in 66% of rats.

Histopathological findings of the liver of rats received carbon tetrachloride (CCl4) with oxygen-ozone mixture (Fig. 2):

Fatty changes of hepatocytes in the form of mild micro vesicular steatosis appeared in 70% of rats. Whereas the central necrosis and hyperplasia of hepatocytes disappeared completely.

Histopathological finding of the kidney of rats which received carbon tetrachloride (CCl4) only:

In the renal proximal tubules of the rats which received CCl4, microscopical examination revealed the presence of degenerative changes in the form of cloudy swelling occurred in 85% of rats, however casts inside the tubules appeared in 33% of rats (Fig. 3).

Histopathological finding of the kidney of rats received carbon tetrachloride (CCl4) and placebo (inert nitrogen):

In the proximal renal tubules, cloudy swelling appeared in 82% of rats, while casts inside the tubules in 30% of rats.

Hisopathological findings of the kidney of rats received carbon tetrachloride (CCl4) and treated with oxygen-ozone mixture:

Mild cloudy swelling of the proximal renal tubules occurred in only 52% of rats. No casts detected inside the tubules (Fig. 4).

DISCUSSION

Carbon tetrachloride is a well-known hepatotoxin. The exact chemical presentation depends on the dose, duration of exposure and individual susceptibility. Acute exposures to CCl4 are presented initially with CNS depression followed one week latter by the development of hepatic and renal dysfunction (El-Azab, 2002).

Leon et al. (1998) stated that administration of a single dose of CCl4 produced hepatocellular injury through generation of free radicals and subsequent induction of lipid peroxidation.

The present work was an in vivo experimental study to investigate the toxic effects of CCl4 on certain serum hepatic enzymes and the histopatho-logical changes in the livers or kidneys. Also the influence of repeated administration of ozone in atoxic doses on the progress of the CCl4 toxicity was evaluated.

In the present study, the results of intraperitoneal injection of a single acute dose (1 ml/kg) of CCl4 to male albino rats induced deterioration of liver functions compared to normal control (Table 1). Subsequently, there was a significant increase of serum GOT, GPT and γ-GT and decrease of serum pseudocholinesterase levels. At the same time, serum albumin did not show significant changes (Table 1).

It is known that the circulating albumin has a long half life and its plasma concentration is only depressed in chronic liver disease while hepatic enzyme alterations occurred immediately after acutely injured hepatic cells (Murray et al., 2003). The liver has a great reserve capacity. Moreover, some functions may be affected earlier and/or severer than others. So, a group of tests should be performed to detect liver dysfunction. By the liver, many injurious substances are detoxicated into less toxic or more readily excreted metabolites. In the course of a chemically toxic liver disease, the increase of SGPT and SGOT commonly return to normal in uncomplicated cases. Maintained rise or exacerbation of SGPT and SGOT indicates worse affection. Liver enzyme increase is a more prognostic rather than diagnostic tests. As regard the kidney function, there was no significant changes of the obtained level of creatinine. This indicates that the encroachment on the renal reserve capacity was not yet complete by CCl4 toxicity (Varley et al., 1976). However, serum creatinine level determines prognosis better than diagnosis in renal disease (Tietz, 1999) (Table 1).

In this respect, Sugihara et al. (1992) observed that the plasma albumin concentration showed insignificant changes by CCl4 induced liver injury. Also, Delrat et al. (1994) found that CCl4 given intraperitoneally to rabbits induced an increase in the plasma enzmatic activities (ALT, AST, gamma GT), while plasma proteins and creatinine levels remained unaltered. Also, animals treated with CCl4 and killed 24h after its administration showed significant elevations of serum γGT, ALT, AST, and alkaline phosphatase activities (Muriel et al., 2001; Sheweita et al., 2001). The results of the present study agree with the above findings.

In the same group, hisopathological examination of the liver and kidney of rats following CCl4 toxicity showed the some nonspecific changes compared to normal rat picture of the liver (Fig. 1 and 2). In the hepatocytes, there were degenerative changes in the form of foci of micro and macrovesicular steatosis which occurred in 100% of rats, necrotic hepatocytes in the form of central necrosis appeared in 76% of rats, whereas hyperplasia of hepatocytes appeared in 65% of rats (Fig. 1). In the kidney, the proximal renal tubules showed degenerative changes in the form of cloudy swelling which occurred in 85% of rats, however, casts inside the tubules appeared in only 33% of rats (Fig. 3). It should be declared that hepatorenal biopsy is the best diagnostic tool in hepatorenal disease.

These results are consistent with those obtained by Barceloux (2001), who stated that, histopathological changes of the liver due to CCl4 toxicity were characterized by

a classical yellow fatty liver picture with discrete damage concentrated in the midzonal and centrilobular regions (centrilobular necrosis). Whereas the renal lesion of the same toxin appeared in the form of acute tubular necrosis affecting primarily the proximal tubules and the loop of henle, and cloudy swelling of the tubular epithelium particularly the convoluted tubules. An earlier study (Leon et al., 1998) reported that rats received CCl4 (1 ml/kg), as a single toxic dose by intraperitoneal injection, develop hepatic damage in the form of hepatocellular necrosis, lipidosis and mesenchymal reaction.

Many authors explained how CCl4 exerts toxic effects. In this respect, Castro et al. (1997), proved the role of free radicals produced during CCl4 biotransformation. They promoted lipid peroxidation process of liver microsomal plus their ability to attack DNA bases (guanine, cytosine, and thymine) induced liver damage. At the same time, Sundari and Ramakrishna (1997) concluding, protein oxidation may play a role in the pathogenesis of CCl4 induced liver injury and that accumulation of oxidised proteins may be an early indication of CCl4 induced liver damage. Also, Campo et al. (2001) reported that CCl4-induced free radical which activate transcription factors regulating both the TNF-alpha gene and the early-immediate genes involved in tissue regeneration.

In the present study, administration of oxygen-ozone mixture by intraperitoneal injection into the CCl4 intoxicated rats for 15 sessions (at a dose of 1 ml. daily), produced a significant improvement of the liver function tests in the form of a significant decrease of the elevated serum GOT and GPT and gGT and a significant increase of the inhibited serum cholinesesterase (Table 2). Whereas, the kidney functions showed non significant changes after CCl4 intoxication or following treatment with either ozone or placebo (Table 2).

In the same group, histopathological studies of the liver and kidney, showed marked improvement, after ozone administration if compared to the placebo group. So, the fatty changes of hepatocytes, appeared in only 70% of rats in the form of mild microvesicular steatosis whereas the central necrosis and hyperplasia of hepatocytes disappeared completely (Fig. 2). At the same time, mild cloudy swelling in the renal proximal tubules occurred in only 52% of rats, with no casts inside the renal tubules (Fig. 4). While the group received placebo display no differences from the toxic group.

The treatment of CCl4 toxicity using ozone had been tried before (El-Azab, 2002) and the obtained results were optimistic and consistent with those in the present study. So, Leon et al. (1998) stated that repeated administration of ozone in

atoxic doses induced an adaptation to the oxidative stress. Thus ozone administration enabling the animals to maintain hepatocellular integrity after CCl4 poisoning. In this respect, Chen and colleagues (2007) found that chronic exposure to ozone was associated with elevated biomarkers of lipid peroxidation and oxidative damage. On the other hand, Candelario-Jalil et al. (2001) suggested that, ozone is able to promote oxidative tolerance preventing hepatocellular damage mediated by free radicals due to CCl4 toxicity. According to Mohammad et al. (2007) ozone improved hepato-renal dysfunctions in some other drug-induced hepato-renal affections.

CONCLUSION

Carbon tetrachloride (CCl4) overdose has a pronounced experimental toxic effects, producing necrotic injury in rats' livers and kidneys. These pathological changes were reflected on the biochemical liver and kidney function tests and histopathological examination of both organs.

Repeated administration of ozone in atoxic dose protected the rats from the CCl4 poison-induced damage. The effects on hepatorenal system can be attributed to an induction of tolerance to O3 and ROS generated by the toxic agent. Ozone therapy was able to preserve the organ integrity by either inducing enzymes or activation of pathways that maintain the equilibrated redox balance. In conclusion, appropriate ozone therapy can up regulate the antioxidant system, and so represents a complementary medical approach.

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Table (1): Liver and kidney function tests of the rats group receiving only CCl4 versus the normal rat control group.

Data	Normal Control group Mean ± SD (n=13)	CCl4 group Mean ± SD (n=13)	P value
Albumin (g/dl)	4.10 ± 0.32	3.71 ± 0.36	0.062
SGOT (u/l)	36.7 ± 9.15	181.90 ± 16.08	0.000*
SGPT (u/l)	20.3 ± 5.58	60.77 ± 7.41	0.000*
Cholinesterase (u/l)	2982.9±273.24	1441.05±224.61	0.000*
γ-GT (u/l)	1.0±0.03	3.51 ± 0.71	0.000*
Creatinine (mg/dl)	1.03 ± 0.48	1.06 ± 0.16	0.701

Table (2): Liver and kidney function tests of rats which received CCl4 and treated with oxygen-ozone mixture versus those which received placebo (nitrogen) beside CCl4 intoxication.

Data	CCl4+O_2-O_3 Mean ± SD (n=15)	CCl4+nitrogen Mean ± SD (n=15)	P value
Albumin (g/dl)	3.81 ± 0.38	3.73 ± 0.46	0.093
SGOT (u/l)	96.13 ± 16.28	183.80 ± 15.09	0.000*
SGPT (u/l)	29.13 ± 4.51	52.67 ± 4.32	0.000*
Cholinesterase (u/l)	2082.4±123.86	1446.06±235.62	0.000*
γ-GT (u/l)	1.20 ± 0.41	3.60±0.63	0.000*
Creatinine (mg/dl)	1.05±0.12	1.04 ± 0.11	0.765

Table (3): Histopathological findings of the liver of rats received carbon tetrachloride (CCl4) (Fig. 1).

Histopathological findings	Occurrence	%	Severity
Portal tract:			
• Thickening with hyperplastic lining in the blood vessels.	-		
• Lymphocytic infiltration.	-		
Liver cells:			
• Degeneration	+	100%	Severe
• Necrosis	+ central	76%	Severe
• Hyperplasia.	+	65%	Moderate
Sinusoids:			
• Kupffer cell hyperplasia	-		

Table (4): Histopathological findings of the liver of rats received carbon tetrachloride (CCl4) and placebo (inert nitrogen).

Histopathological findings	Occurrence	%	Severity
Portal tract:			
• Thickening with hyperplastic lining in the blood vessels.	-		
• Lymphocytic infiltration.	-		
Liver cells:			
• Degeneration	+	100%	Severe
• Necrosis	+ central	74%	Severe
• Hyperplasia.	+	66%	Moderate
Sinusoids:			
• Kupffer cell hyperplasia	-		

Table (5): Histopathological findings of the liver of rats received carbon tetrachloride (CCl4) and treated with oxygen-ozone mixture (Fig. 2).

Histopathological findings	Occurrence	%	Severity
Portal tract:			
• Thickening with hyperplastic lining in the blood vessels.	-		
• Lymphocytic infiltration.	-		
Liver cells:			
• Degeneration	+	70%	Mild
• Necrosis	-		
• Hyperplasia.	-		
Sinusoids:			
• Kupffer cell hyperplasia	-		

Table (6): Histopathological findings of the kidney of rats received carbon tetrachloride (CCl4) .

Histopathological findings	Occurrence	%	Severity
Proximal tubules:			
• Degeneration	+	85%	Moderate
• Necrosis	-		
• Lymphocytic infiltration	-		
• Casts inside the tubules.	+	33%	Moderate
Collecting tubules:			
• Thickening of its lining	-		

Table (7): Histopathological findings of the kidney of rats received carbon tetrachloride (CCl4) and placebo (inert nitrogen).

Histopathological findings	Occurrence	%	Severity
Proximal tubules:			
• Degeneration	+	82%	Moderate
• Necrosis	-		
• Lymphocytic infiltration	-		
• Casts inside the tubules.	+	30%	Moderate
Collecting tubules:			
• Thickening of its lining	-		

Table (8): Histopathological findings of the kidney of rats received carbon tetrachloride (CCl4) and treated with oxygen-ozone mixture.

Histopathological findings	Occurrence	%	Severity
Proximal tubules:			
• Degeneration	+	52%	Mild
• Necrosis	-		
• Lymphocytic infiltration	-		
• Casts inside the tubules.	-		
Collecting tubules:			
• Thickening of its lining	-		

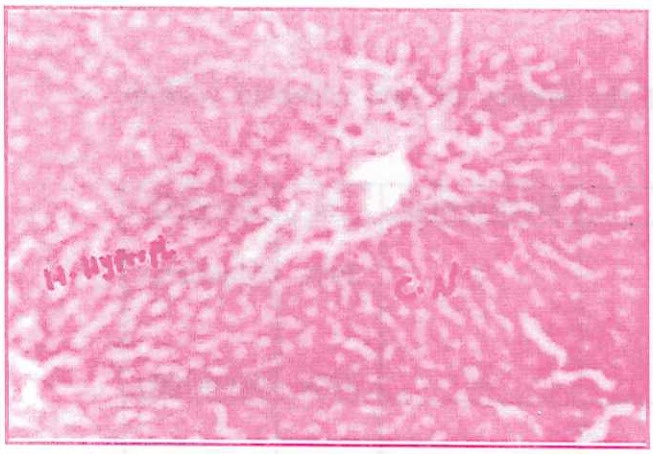

Fig. (1) : A photomicrograph of the liver of rats received CCI4 showing central necrosis (C.N), hepatocyte hyperplasia (H.S) (H&E stain)x200.

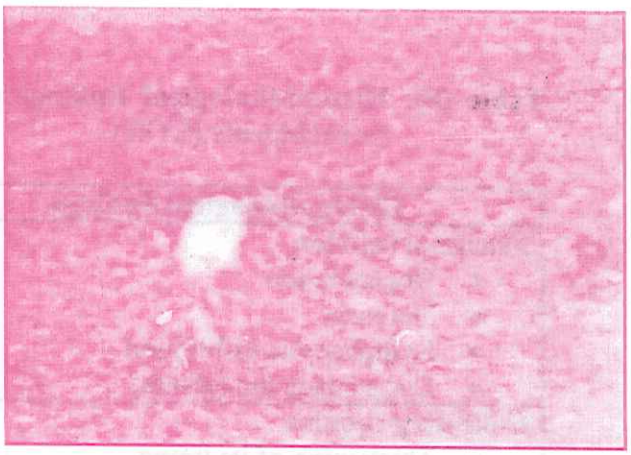

Fig. (2) : A photomicrograph of the liver of rats received CCl4 and treated with oxygen-ozonc mixture showing mild micro vesicular steatosis (H&E stain).x200.

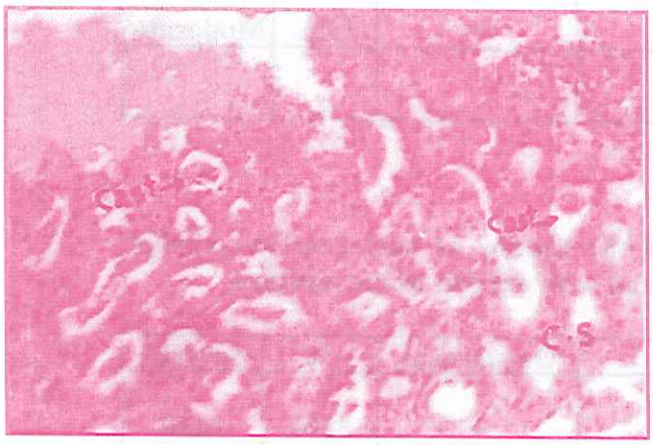

Fig. (3) : A photomicrograph of the kidney of rats received CCl4 showing cloudy swelling (C.S) and casts inside the tubules (H&E stain) x 200.

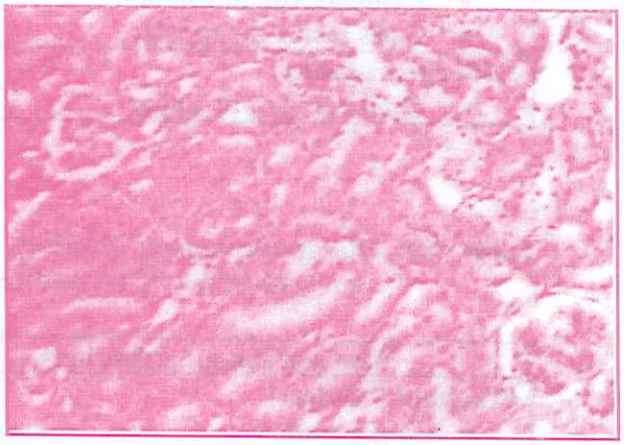

Fig. (4) : A photomicrograph of the kidney of rats received CCl4 and treated with oxygen-ozone mixture showing mild cloudy swelling (C.S) (H&E stain) x 200.

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دراسة عن التسمم الحاد برابع كلوريد الكربون فى الجرذان ودرجة إستجابته لتناول غاز الأوزون

المشتركون فى البحث

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كلية الطب - جامعة المنصورة

إستهدف هذا البحث دراسة تأثير رابع كلوريد الكربون فى الجرذ المعملى الأبيض منفرداً أو مع الأوزون على وظائف الكبد والكلى.

لقد أجرى هذا البحث على مجموعة تضم ٤٥ جرذاً تم إعطاؤها رابع كلوريد الكربون كجرعة واحدة عبارة عن ميكروليتر رابع كلوريد الكربون / جرام من وزن الجرذ وذلك عن طريق الحقن فى تجويف البريتون، من بين هذه المجموعة الكلية وأن ١٥ جرذ تركت بلا معالجة أخرى بينما تم إعطاء ١٥ جرذ أخرى مخلوط الأوزون - الأكسجين والـ ١٥ جرذاً الأخيرة غاز النيتروجين لمدة خمسة عشر يوماً بدءاً من تعاطى رابع كلوريد الكربون، ولقد تم أخذ عينة دم من كل جرذ وترك الدم للتجلط وفصل السيرم لإجراء تحاليل للألبيومين - وإنزيمات جلوتاميل - بيروفيك - ترانزأمينيز وجلوتاميل أوكساليك ترانزأمينيز وجاما جلوتاميل ترانزفيريز إلى جانب الكولين استريز.

وفى نهاية البحث تم ذبح الحيوانات وتشريحها وإستخراج الكبد والكلى لفحص التغيرات الهستوباثولوجية ثم بعد العلاج بالأوزون بالمقارنة مع النتروجين.

كانت نتائج الدراسة فى الجرذان المتعاطاه رابع كلوريد الكربون فقط هى :

١- زيادة ذات مغزى إحصائى فى معدلات إنزيمات جلوتاميل بيروفيك ترانز أمينيز وجلوتاميل أوكساليك ترانزأمينيز وجاما جلوتاميل ترانزفيريز ونقص ذو مغزى إحصائى فى معدل كولين إستريز بمصل الدم.

٢- بفحص خلايا الكبد ميكروسكوبيا وجد إعتلال فى الغدد الدهنية على هيئة تحوصل دهنى كبير وصغير فى الجرذان تنكرز مركزى فى الخلايا فى الجرذان كما وجد تكثر نسيجى وفرط نمو فى الخلايا فى الجرذان كذلك وجد تورم غيمى فى خلايا القنيات الملتفة العليا للكلى فى الجرذان بينما وجدت الكتل الليفية فى الجرذان.

وفى النهاية أثبت الفحص الهستوباثولوجى فاعلية غاز الأوزون فى تقليل الآثار الضارة الناجمة عن تعاطى رابع كلوريد الكربون على الكبد والكلى كما يلى :

- نقص ذو مغزى إحصائى عن معدلات الإنزيمات جلوتاميل - بيروفيك ترانز أمينيز وجلوتاميل أوكساليك ترانزأمينيز وجاما جلوتاميل

ترانزفيريز بمصل الدم بينما وجد زيادة ذات مغزى إحصائى فى مستوى كولين استيريز بمصل الدم عن مثيلاتها بدون تعاطى الأوزون.

- أثبت الفحص الهستوباثولوچى بعد العلاج بالأوزون أن فى خلايا الكبد ظهر التحوصل الدهنى الدقيق فى ٧٠٪ من الجرذان بينما لم يظهر التنكرز المركزى أو فرط النمو النسيجى للخلايا فى أى من الجرذان، بينما بفحص القنيات الملتفة العليا للكلى ظهر تورم غيمى بسيط فى ٥٢٪ من الجرذان بينما لم تظهر الكتل الليفية فى أى من الجرذان.

٣- بعد إعطاء النتروچين لم يظهر الفحص الكيميائى أو الهستوباثولوچى أى تغير يذكر.

على ضوء هذه النتائج نستنتج أن الأوزون له فاعلية فى حماية الأعضاء الداخلية (الكبد والكلى) بالجسم من التأثير الضار لرابع كلوريد الكربون.