COMPARATIVE EFFICACY OF ZINC AND VITAMIN A ON ALLOXAN AND N-ALKYL ALLOXAN INDUCED TOXICITY IN ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: Diabetes has been associated with several complications occasioned by oxidative stress. High levels of free radicals lead to damage of cellular organelles and enzymes. Aim of the study: The aim of this work was to assess the beneficial effect of zinc or vitamin A (vit. A) on alloxan or N-alkyl (N-A.) alloxan induced toxicity. Materials and Methods: 110 male adult albino rats were used in this study and were divided into 11 equal groups, 10 rats each. GI: The negative control group, GII: Saline group, GIII: Corn oil group, GIV: Zinc chloride group, GV: Vit. A group, GVI: Alloxan group, GVII: N-A. alloxan group, GVIII: Alloxan-zinc group, GIX: Alloxan-vit. A group while groups X and XI were N-A. alloxan-zinc and N.A. alloxan-vit. A groups respectively. Oxidative stress markers [superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA)] concentrations were measured in addition to blood glucose and glycosylated hemoglobin (Hb) levels as well as liver enzymes as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Lipid profile [total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDLP) and low density lipoproteins (LDLP)] was also assessed. Semen analysis including sperm motility and count was evaluated. Furthermore, histopathological examination of the liver and pancreas was carried out. Results: Alloxan or N-alkyl alloxan led to diabetes (hyperglycaemia and increased glycosylated Hb levels). There was also decrease in the levels of SOD and GSH with increase of MDA level. Considering ALT and AST concentrations, there was significant increase of both enzymes. Hyperlipidaemia was also evident in the form of increased total cholesterol, TG and LDLP levels with reduction of HDLP level. Nevertheless, semen analysis showed significant reduction both in number and motility of sperms. Histopathological examination of both the liver and the pancreas showed severe changes in both hepatic and pancreatic architecture after alloxan or N-A. alloxan (being more toxic) intake. Zinc supplementation caused a favorable protective effect with normalization of almost all the biochemical parameters as well as improvement of the histopathological changes. Vit. A also protected against alloxan or N-A. alloxan induced toxicity but was less effective than zinc. Conclusion: Zinc is more effective than vit. A. in protecting against alloxan or N-A. alloxan induced toxicity not only on the pancreatic tissue but also on other tissues as the liver.

Keywords: Diabetes, Alloxan, N-A. alloxan, Zinc, Vit. A.
INTRODUCTION

Recently alarming reports warned that traces of the dangerous chemical alloxan and its analogue N-alkyl alloxan are present in white bread and other commercial baked goods (cookies, crackers and donuts) which are made with bleached wheat flour (Luise, 2006). Bleaching toughens the protein molecules in flour and yields chlorine gas and various oxides of chloride which are believed to combine with proteins in flour, producing either alloxan or its analogue N-alkyl alloxan as an unintended byproduct which directly interacts with the protein in wheat flour and it is postulated that they are not safe to eat (Bullock, 2009).

Alloxan is used to induce experimental diabetes by destroying pancreatic β-cells (Lankin et al., 2004) and subsequently generates reactive oxygen species (ROS) (Ramakrishna and Rama, 2007) which is a direct consequence of hyperglycaemia (Brownlee, 2001) and this leads to lipid peroxidation of the cellular organelles and enzymes (Demozay et al., 2008). Other studies have suggested that increased free fatty acids may also result in ROS formation (Bakker et al., 2000). Because of their ability to directly oxidize DNA, proteins and lipids, ROS are believed to play a direct role in the pathogenesis of many diabetic complications (Nishikawa et al., 2001). Furthermore, Yao et al. (2009) postulated that chronic plasma glucose elevation did caused many of the complications of diabetes including nephropathy, neuropathy, vascular damage and abnormalities in male reproductive functions. Increased oxidative stress in diabetes recommends the use of antioxidants which may reduce such stress (Shi et al., 2003). The best treatment of diabetes and its complications remains the prevention of ROS generation through strict glycaemic control (Anuradha, 2009).

Rudolf et al. (2001) stated that zinc is a fundamental element for maintenance of the structural and functional integrity of cells and tissues because of its ability as a strong free radical scavenger and a biological membrane stabilizer. Moreover, Evans et al. (2003) has reported the beneficial effect of zinc supplementation in restraining the progression of tissue degeneration and even in the protection against cancer.

Vit. A is a lipid soluble antioxidant which acts by protecting the unsaturated fatty acids, a main compartment of the cell membrane, and hence could prevent complications of diabetes (Ottaviano et al., 2008).

AIM OF THE STUDY

This study has been designed to throw light on the toxicity of alloxan and its analogue N-alkyl alloxan and the possible...
protective role of either zinc or vit. A. in adult male albino rats.

**MATERIALS AND METHODS**

**Chemicals:**
Alloxan and its analogue N-alkyl alloxan were purchased from Sigma Chemical Company in the form of white powder freely soluble in saline.

Zinc chloride was obtained from El-Gomhoreya company for chemical and pharmaceutical preparations (ARE) in the form of white powder freely soluble in saline.

Vitamin A was obtained from Kahira Pharmaceutical Company (ARE) 5000 IU/capsule, which is freely soluble in corn oil.

**Animals:**
One hundred and ten male adult albino rats, purchased from medical research centre, Faculty of Medicine, Ain Shams University, weighing 180-200 gm were used in this study.

They were housed in cages and maintained on a 12 h, light 12 h dark cycle at room temperature and relative humidity of 60%. They were fed ad lib and offered free access to water. All animals were kept for 7 days before the start of the experiment for acclimatization. All ethically approved conditions for animal housing and handling were considered.

**Animal groups:**
The animals were divided into 11 equal groups, 10 rats each as follows:

**Group I:** (Negative control)
The animals in this group were kept in the same environment without handling to demonstrate the normal basic parameters.

**Group II:** (Saline group)
The animals were injected with 0.25 ml of sterile saline I.P once per week for 3 weeks.

**Group III:** (Corn oil group)
The animals were given corn oil in a dose of 1 ml / day orally for 2 weeks.

**Group IV:** (Zinc chloride group)
The animals received zinc chloride (Zn Cl₂) at a dose of 5 mg/kg I.P. as a single dose according to Moustafa (2001).

**Group V:** (Vitamin A group)
The animals were given 100 IU/day orally of vit. A for 2 weeks according to Olson (1987).

**Group VI:** (Alloxan group)
The rats were injected I.P. with 100 mg/kg alloxan once per week for three weeks according to Gwarzo et al. (2010).

**Group VII:** (N-alkyl alloxan group)
The animals were given N-alkyl alloxan in a dose of 75 mg/kg I.P once per week
for three weeks after Munday et al. (1997).

**Group VIII:** (Alloxan-zinc group)
After the last dose of alloxan the animals received a single dose of ZnCl₂ at a dose of 5 mg/kg IP.

**Group IX:** (Alloxan-vit. A group)
In this group the rats were given vit. A at a dose of 100 IU/day orally for 7 days then alloxan with the same regimen as before followed by vitamin A for another 7 days as above.

**Group X:** (N-alkyl alloxan-zinc group)
The rats were given 5 mg/kg I.P. Zn Cl₂ as a single dose after the last dose of N-alkyl alloxan.

**Group XI:** (N-alkyl alloxan-vit. A group)
The rats were given 100 IU of Vit. A daily orally for 7 days before N-A. alloxan which was given with the same regimen as before then for another 7 days after N-A. alloxan with the same mentioned dose and route as above.

**Experimental parameters:**
**A- Biochemical tests:**
At the end of the experimental period the animals were sacrificed after being anaesthetized with pentothal sodium, then the intra-abdominal organs were exposed and the abdominal aorta was cannulated to collect blood samples. A portion of the blood was collected in a heparinized tube for spectrophotometric determination of:

**I. Oxidative stress markers:** Superoxide dismutase (SOD) according to Nishikimi et al. (1972), reduced glutathione (GSH) after Beutler (1982) and malondialdehyde (MDA) according to Yoshida et al. (1980).

The rest of the blood sample was left to coagulate and centrifuged at 3000 r.p.m. for determination of:

**II. Blood glucose level** after the method of Barham and Trinder (1972) and glycosylated Hb level was assessed according to the method of Nayak and Pattabiraman (1981).

**III. Aspartate aminotransferase (AST)**
and alanine aminotransferase (ALT) were also assayed according to Henry et al. (1974).

**IV. Lipid profile:** Total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDLp) were determined by the methods of Allain et al. (1974), Fossati and Prencipe (1982) and Lopes-Virella et al. (1977) respectively while low density lipoproteins (LDLP) was calculated by the ratio of Friedewald et al. (1972) as follows:

\[ \text{LDLP} = \left[ (\text{TC} - \text{HDLp}) - \text{TG} / 5 \right]. \]

**B. Semen analysis:**
* Motility:
A drop of semen was collected from the epididymis, put on a glass slide then
sodium citrate buffer (2.9%) was added and mixed gently according to the method of Zemjanis (1970). The percentage of motility was evaluated microscopically within 2-4 minutes of semen collection.

* Spermatic count:
It was performed using special haemocytometer slide after (Oyeyemi and Fayomi, 2011).

C. Histopathological examination:
The liver and the pancreas were excised, fixed in 10% formalin and then embedded in paraffin and processed. Serial sections of 5 µm thickness were cut, and stained by Hx & Eosin (Drury and Wallington, 1980).

Statistical analysis:
The data were collected then tabulated and presented to the statistical package for social science (SPSS) version 17. Mean±SD were used to describe variables. One way analysis of variance (ANOVA test) with post hoc tests and least significant difference (LSD) were used for multiple comparisons between the different studied groups. The level of significance was at P < 0.05.

RESULTS

I. Biochemical results:
On comparing the different biochemical parameters between group I (negative control group) and groups II, III, IV and V, no significant changes were found between these different groups (table 1).

Considering semen analysis, table (1) also showed non significant difference regarding the sperm count and motility between group I and groups II, III, IV and V.

On the other hand, on comparing the negative control group (GI) with either G VI (alloxan group) or G VII (N-A. alloxan group) there was significant increase as regards blood glucose, glycosylated Hb, MDA, total cholesterol, triglycerides as well as LDLP levels. There were also significant decrease between these different groups as regards SOD, GSH and HDLP (table 2).

Table (2) also showed a significant decrease as regards sperm count and motility between GVI and GVII when compared with GI.

When comparing both groups together (GVI & GVII) significant difference was noticed regarding all the tested biochemical parameters as well as regards sperm count and motility and revealed that N-A. alloxan is more toxic than alloxan (table 2).

Table (3) showed marked improvement among all the tested biochemical parameters but still significant differences were
Present when comparing GI with either group VIII (Alloxan-zinc group) or group IX (Alloxan-vit. A group).

Although, table (3) showed that administration of either zinc or vit. A. with alloxan offered beneficial effects on alloxan induced testicular toxicity, yet there is a significant difference found between GI and groups VIII and IX.

Table (4) showed restoration of the mean values of all the tested parameters, nevertheless significant differences were noticed between GI and groups X and XI.

Furthermore, tables (3 and 4) showed that zinc is more beneficial than vit. A in mitigating alloxan or N-A. alloxan induced toxicity.

II. Histopathological results:

a) Liver: Examination by light microscopy showed that the liver of the negative control group had a lobular architecture. The hepatocytes appeared polyhydral with rounded central nuclei and abundant cytoplasm. They were oriented in cords composed of single row of cells separated by sinusoids lined by endothelial and Von Kupffer’s cells. In one of the corners of the hepatic lobule there was a portal tract (Fig. 1).

Alloxan group (GVI) showed congestion of the central vein, mononuclear cellular infiltration in the area of the portal tracts and the hepatocytes showed vacuolations (Fig. 2).

Focal poliosis, fibrous tissue expansion of the portal tracts with inflammatory cell infiltration were noticed in N-alkyl alloxan group (GVII) when compared with the negative control group (GI), (Fig. 3). Also figure (4) showed that N-alkyl alloxan did caused changes in the hepatocytes which appeared vacuolated and showed pyknotic nuclei with cellular infiltration.

On administration of zinc together with alloxan (GVIII) the picture started to approach the control in some areas (Figs. 5 & 6), while on administration of vit. A together with alloxan (GIX) the liver appeared nearly normal with some congestion in the central veins (Figs. 7 & 8). N-A. alloxan-zinc group (GX), and N-A. alloxan - vit. A. A group (GXI) showed nearly normal appearance of the hepatic tissue but still some congestion and cellular infiltration were present (Figs. 9 & 10)

b) Pancreas: Figure (11) shows the pancreatic tissue of a control rat showing normal acini and cellular population in islets of Langerhans. In alloxan group (GVI) the pancreatic tissue showed damaged islets of Langerhans and reduced islet’s size (Fig. 12). In N-alkyl alloxan group (GVII) extensive degeneration of islets of Langerhans with loss of plasma membrane and condensed nuclei were noticed (Fig. 13).
arise due to oxidative stress (Palsamy and Subramanian, 2008) and increased lipid peroxides formation and this was seen in animal models of diabetes mellitus (Anjaneylu and Chopra, 2004 and Szkudelska, 2010).

Persistent hyperglycaemia results in glycation of hemoglobin that leads to the formation of glycosylated hemoglobin (Yabe-Nishimura, 1998). This was evident in the current study by the significant elevation of glycosylated Hb level after alloxan or N-alkyl alloxan administration. Exposure to alloxan or N-A. alloxan in this study caused lipid peroxidation proved by the reduction in SOD and GSH levels, together with elevation in MDA concentrations. This was also reported by Sailaja-Devi and Das (2006) and Marjani (2010) who observed a significant decrease in SOD and GSH together with increase in MDA level after alloxan or N-A. alloxan intake.

Tas et al. (2007) also recorded reduction of SOD and GSH with elevated MDA levels in diabetic rats. Hyperglycaemia in diabetic rats resulted in generation of free radicals and consumption of antioxidants and hence disruption of the cellular functions and oxidative damage of the membrane with enhanced susceptibility to lipid peroxidation (Andallu and Varadacharjulu, 2003) which led to increased activity of MDA and decreased activity of SOD and
GSH (Bhatia et al., 2003) and that is why many studies had addressed the importance of anti-oxidants for the control of abnormalities in diabetes (Agardh et al., 2002).

In the present work, diabetic rats (after either alloxan or N-A. alloxan exposure) showed a significant increase in AST and ALT activities. These results were also noticed by Khalil (2009); Silmara et al. (2008) and Butterfield and Poon (2005).

The hepatic insult which was reflected by the increase in AST and ALT activities after alloxan or N-A. alloxan exposure may be due to either direct toxic damage to the liver or due to increase in oxidative damage and decrease in the antioxidant capacity of the liver leading to increase in the membrane permeability with elevation of the hepatic transaminases (Khalil, 2009 and Kechrid et al., 2007).

The hyperlipidemia caused by alloxan or N-A alloxan had been demonstrated in the current work by significant increase in total cholesterol, TG and LDLP levels and decreased HDLP level.

This goes hand in hand with the work done by Kakadiya et al. (2010) who showed that hyperlipidaemia is an associated complication of alloxan exposure. Hanhinveva et al. (2010) reported that elevation of all plasma lipid fractions often coexist with hyperglycaemia. Insulin inhibit lipolysis and promotes storage of TG in adipocytes. Therefore, insulin lack in alloxan induced diabetes led to hydrolysis of TG and re-esterification into TG for the storage or secretion of LDLP (Zang et al., 2006). Consequently, the clearance of LDLP can be delayed resulting in hyperlipidaemia (Lui et al., 2008). Hassan (2007) also recorded the same changes in the lipid profile after either alloxan or N-A. alloxan intake as there were significant increase in total cholesterol, TG and LDLP and significant decrease in HDLP concentrations.

Regarding the sperm count and motility following induction of diabetes with alloxan or N-A. alloxan, there was a significant reduction in the number and the percentage of sperm motility compared with the control non-diabetic group.

This can be explained by Azeez et al. (2010) who attributed this toxic effect to the damage of the secretory cells of the semineferous tubules probably due to oxidative damage from glucose auto-oxidation and excessive production of superoxide radicals (Kim and Moley, 2008), in addition to formation of advanced glycation end products associated with diabetes (Agbaje et al., 2007).

Histopathologically, alloxan caused congestion of the central vein and mononuclear cellular infiltration of some of the
portals, in addition to vacuolation of the hepatocytes. N-A. alloxan led to polio- sis and fibrosis of the portal tracts.

The same picture was described by Jörns et al. (1997) who found the same histopathological changes in the liver of rats intoxicated by alloxan or N-A. alloxan and postulated that N.A. alloxan is more toxic than alloxan which was also clarified in this study.

Histopathological examination of the pancreatic tissue showed cell necrosis in some of β-cells of the pancreas after alloxan administration, while N-A. alloxan caused much more necrosis of the β cells. Meanwhile, non of both agents affected the exocrine parenchymal cells.

In a study by Gwarzo et al. (2010) they reported that in diabetes induced rats the pancreatic β-cells appeared necrotic and little in number. Furthermore, Jörns et al. (1997) stated that N-A. alloxan is much more toxic to the pancreatic β cells than alloxan.

Alloxan or N-A. alloxan induced pancreatic toxicity appeared to be multifactorial including not only the impaired capacity of insulin synthesizing cells of the pancreas but also to the insulin resistance in other cells that are targets to insulin (Gwarzo et al., 2010).

Additionally, it had been reported that in alloxan induced diabetes there is a poor response of the pancreatic cells to the glycaemic effect of insulin (Altomare et al., 1997). Accordingly, alloxan or its analogue N-A. alloxan induced changes in the redox state of the pancreatic cells account for the impaired insulin synthesis by the pancreatic cells and also for its destruction (Sempoux et al., 2001).

Zinc supplementation in this study concomitantly with either alloxan or N-alkyl alloxan improved all the biochemical changes that had been altered and exhibited a good degree of improvement of the histopathological picture of the liver and the pancreas. Similar results were observed by Channon and Guzik (2002) who reported that zinc may protect the microsomal membrane against lipid peroxidation and hence cellular protection.

This can be explained by the protective effect of zinc against lipid peroxidation of cellular and subcellular membranes which may reverse the impairment in glucose transport and metabolism in cells exposed to alloxan and its derivatives N-A. alloxan (Kocic et al., 2002). Zinc was also found to induce metallothionein (MT) synthesis (Hanna et al., 1993). The high sulphydryl content in zinc enables MT efficiently to scavenge oxyradicals (Suntres and Lui, 1990). Another possible protective mecha-
nism of MT is its ability to release zinc for binding at sites on membrane surfaces, thereby, inhibiting lipid peroxidation (Chevion, 1991).

Moreover, the suggested effect of zinc in inducing SH-rich MT synthesis, may preserve the SH-residue in many functional proteins. Therefore, zinc preserve the structural and functional integrity of SH-dependent enzymes including those regulating glucose metabolism (St Croix et al., 2002).

Gwarzo et al. (2010) recorded that vitamin A intake protected against alloxan or N-A. alloxan induced diabetes in rats.

Administration of vitamin A with alloxan or N-A. alloxan did offered some sort of protection with slight amelioration of the altered biochemical parameters in this study. It also caused a little improvement of the histopathological changes of the liver and the pancreas.

Demozay et al. (2008) stated that vit. A supplementation with alloxan or N-A. alloxan had shown protection against chemically induced destruction of B-cells via free radical scavenging mechanism. The histological architecture of the liver and pancreas was also regained by vit. A (Lankin et al., 2004).

Ramakrishna and Rama (2007) suggested that vit A may prevent the occurrence of diabetes complication induced chemically by alloxan or N-A alloxan. On the contrary, Sharma and Kumar (2011) stated that the use of vit. A appeared to be difficult and costy as it needs the use of concentrations that may be toxic.

**CONCLUSION**

From the above mentioned results it can be concluded that alloxan or N-A. alloxan has a toxic effect on the pancreas, liver and male reproductive system and provided the possibility of amelioration of this toxicity by the use of either zinc or vitamin A. Zinc supplementation proved to be a potent agent in protecting against the changes associated with increased free radical activity induced by alloxan or N-A. alloxan and is more beneficial than vit. A which caused mild protection.

Additional studies are required to determine the benefits of the use of combination of zinc and vitamin A in oxidant stress induced diabetes at the lower safe effective doses and for a longer period of time. This may open prospects for the use of zinc or vitamin A in therapeutic strategies that aim to interrupt the stress sensitive pathway mediating diabetes complications like nephropathy,
retinopathy and CVS damage. Furthermore, the use of alloxan or its analogue N-A. alloxan in bread bleaching and in other commercial backed goods must be prohibited completely and the use of other safe chemicals is advised.
### Table (1): Comparison of different measured parameters in the studied groups I, II, III, IV and V.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (n=10) (mean±SD)</th>
<th>Group II (n=10) (mean±SD)</th>
<th>Group III (n=10) (mean±SD)</th>
<th>Group IV (n=10) (mean±SD)</th>
<th>Group V (n=10) (mean±SD)</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg%)</td>
<td>85.6±4.06</td>
<td>87.2±4.8</td>
<td>88.2±0.305</td>
<td>88.25±5.6</td>
<td>86.8±4.1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>g. Hb (%Hb)</td>
<td>6.8±0.68</td>
<td>6.7±0.60</td>
<td>6.55±0.59</td>
<td>6.59±0.61</td>
<td>6.61±0.65</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>SOD (µ/mgHb)</td>
<td>339.66±27.46</td>
<td>340.7±29.7</td>
<td>336.9±25.1</td>
<td>341.0±29</td>
<td>338.9±27</td>
<td>0.04</td>
<td>0.9</td>
</tr>
<tr>
<td>GSH (mg/g Hb)</td>
<td>46.6±3.8</td>
<td>47.1±2.7</td>
<td>48.5±2.8</td>
<td>48.0±3.7</td>
<td>49.0±2.6</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>2.09±0.09</td>
<td>2.08±0.11</td>
<td>2.07±0.15</td>
<td>2.1±0.1</td>
<td>2.06±0.12</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>6.5±0.92</td>
<td>6.75±1.0</td>
<td>6.8±1.2</td>
<td>6.3±1.06</td>
<td>6.6±0.7</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>16.28±1.6</td>
<td>16.8±1.2</td>
<td>16.8±1.8</td>
<td>16.9±1.7</td>
<td>16.9±1.6</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Total cholesterol (mg%)</td>
<td>77.8±2.6</td>
<td>78.25±2.4</td>
<td>76.8±2.3</td>
<td>77.3±2.7</td>
<td>77.5±2.3</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>TG (mg%)</td>
<td>89.5±3.5</td>
<td>86.5±2.5</td>
<td>86.7±4.2</td>
<td>88.3±3.3</td>
<td>87.5±3.0</td>
<td>1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>H.D.L.P (mg%)</td>
<td>37.6±1.6</td>
<td>36.6±1.0</td>
<td>37.7±2.8</td>
<td>36.7±1.4</td>
<td>37.3±1.5</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>L.D.L.P (mg%)</td>
<td>32.3±4.1</td>
<td>30.6±5.7</td>
<td>30.4±3.7</td>
<td>31.7±2.8</td>
<td>31.2±2.5</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Sperm count (million/ml)</td>
<td>7.23±1.1</td>
<td>7.20±1.0</td>
<td>7.22±0.9</td>
<td>7.21±0.9</td>
<td>7.24±1.1</td>
<td>0.001</td>
<td>1.0</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>77.9±6.0</td>
<td>78.0±6.1</td>
<td>78.2±6.0</td>
<td>77.7±6.3</td>
<td>78.5±6.0</td>
<td>0.02</td>
<td>0.9</td>
</tr>
</tbody>
</table>

N.B. glycosylated haemoglobin (g.Hb), superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL).

N = Number  
SD: Standard deviation  
P: < 0.05 Significant difference
Table (2): Comparison of different measured parameters in the studied groups I, VI and VII.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (n=10) (mean±SD)</th>
<th>Group VI (n=10) (mean±SD)</th>
<th>Group VII (n=10) (mean±SD)</th>
<th>F</th>
<th>P-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg%)</td>
<td>85.6±4.06</td>
<td>265.7±10.4</td>
<td>280.9±10.5</td>
<td>150.5</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>g. Hb (%Hb)</td>
<td>6.84±0.68</td>
<td>13.37±1.1</td>
<td>15.8±1.2</td>
<td>172.4</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>SOD (µ/mgHb)</td>
<td>339.66±27.46</td>
<td>177.1±15.2</td>
<td>199.1±13.9</td>
<td>252.5</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>GSH (mg/g Hb)</td>
<td>46.6±3.8</td>
<td>9.60±0.43</td>
<td>6.3±0.3</td>
<td>107.1</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>2.09±0.09</td>
<td>4.0±0.1</td>
<td>5.7±0.12</td>
<td>299.2</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>6.5±0.92</td>
<td>20.75±1.8</td>
<td>45.9±2.1</td>
<td>141.1</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>16.28±1.6</td>
<td>39.5±2.1</td>
<td>60.1±3.0</td>
<td>90.6</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>Total cholesterol (mg%)</td>
<td>77.8±2.6</td>
<td>124.8±6.01</td>
<td>155.0±6.2</td>
<td>55.0</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>TG (mg%)</td>
<td>89.5±3.5</td>
<td>159.25±8.6</td>
<td>175.0±9.0</td>
<td>37.1</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>H.D.L.P (mg%)</td>
<td>37.6±1.6</td>
<td>28.2±2.1</td>
<td>23.8±1.9</td>
<td>14.0</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>L.D.L.P (mg%)</td>
<td>32.3±4.1</td>
<td>75.02±4.82</td>
<td>98.2±5.0</td>
<td>51.6</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>Sperm count (million/ml)</td>
<td>7.23±1.1</td>
<td>5.51±1.2</td>
<td>3.78±1.2</td>
<td>21.5</td>
<td>0.000</td>
<td>I vs VI = 0.021</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>77.7±6.0</td>
<td>45.5±5.4</td>
<td>35.3±3.4</td>
<td>191.0</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
</tbody>
</table>

N.B. glycosylated haemoglobin (g.Hb), superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), high density lipoproteins (HDL.P) and low density lipoproteins (LDL.P).

n: number  SD: Standard deviation  P: < 0.05 significant difference  LSD: Least significant difference
Table (3): Comparison of different parameters measured in the studied groups I, VIII and IX.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (n=10) (mean±SD)</th>
<th>Group VIII (n=10) (mean±SD)</th>
<th>Group IX (n=10) (mean±SD)</th>
<th>F</th>
<th>P-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg%)</td>
<td>85.6±4.6</td>
<td>130±2.2</td>
<td>150.0±3.5</td>
<td>65.2</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>g. Hb (%Hb)</td>
<td>6.8±0.68</td>
<td>8.3±0.86</td>
<td>10.77±0.82</td>
<td>34.3</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>SOD (µ/mg Hb)</td>
<td>339.9±27.5</td>
<td>329.9±10.8</td>
<td>310.1±22.6</td>
<td>9.8</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>GSH (mg/g Hb)</td>
<td>46.6±3.8</td>
<td>43.9±3.6</td>
<td>40.7±0.72</td>
<td>9.3</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>2.09±0.09</td>
<td>3.1±0.13</td>
<td>4.2±0.11</td>
<td>72.8</td>
<td>0.000</td>
<td>I vs VI = 0.020</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>6.5±0.92</td>
<td>8.5±0.7</td>
<td>10.2±1.2</td>
<td>20.1</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>16.28±1.6</td>
<td>18.13±2.7</td>
<td>19.9±2.7</td>
<td>4.62</td>
<td>0.003</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>Total cholesterol (mg%)</td>
<td>77.8±2.6</td>
<td>79.8±2.2</td>
<td>82.8±4.1</td>
<td>4.5</td>
<td>0.010</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>TG (mg%)</td>
<td>89.5±3.5</td>
<td>92.12±5.6</td>
<td>94.7±3.6</td>
<td>8.7</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>H.D.L.P (mg%)</td>
<td>37.6±1.6</td>
<td>35.12±2.03</td>
<td>33.9±2.7</td>
<td>7.5</td>
<td>0.000</td>
<td>I vs VI = 0.021</td>
</tr>
<tr>
<td>L.D.L.P (mg%)</td>
<td>32.3±4.1</td>
<td>33.9±2.8</td>
<td>35.9±2.4</td>
<td>6.3</td>
<td>0.000</td>
<td>I vs VI = 0.040</td>
</tr>
<tr>
<td>Sperm count (million/ml)</td>
<td>7.23±1.1</td>
<td>5.9±1.0</td>
<td>4.8±0.8</td>
<td>4.8</td>
<td>0.002</td>
<td>I vs VI = 0.023</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>77.7±6</td>
<td>75.7±5.8</td>
<td>69.1±3.9</td>
<td>5.2</td>
<td>0.001</td>
<td>I vs VI = 0.010</td>
</tr>
</tbody>
</table>

N.B. glycosylated haemoglobin (g.Hb), superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), high density lipoproteins (HDL-P), low density lipoproteins (LDL-P).

n= number  SD: Standard deviation  P: < 0.05 significant difference
LSD: Least significant difference
Table (4): One way analysis of variance (ANOVA test) and post hoc tests (LSD) comparing between group I and groups X and XI.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (n=10)</th>
<th>Group X (n=10)</th>
<th>Group XI (n=10)</th>
<th>F</th>
<th>P-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg%)</td>
<td>85.6±4.6</td>
<td>135.0±4.5</td>
<td>160.0±6.5</td>
<td>12.0</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>g. Hb (%Hb)</td>
<td>6.84±0.68</td>
<td>8.9±1.0</td>
<td>11.2±1.1</td>
<td>13.0</td>
<td>0.000</td>
<td>I vs VI = 0.010</td>
</tr>
<tr>
<td>SOD (μ/mgHb)</td>
<td>339.66±27.46</td>
<td>319.3±21.8</td>
<td>305±20.9</td>
<td>6.0</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>GSH (mg/g Hb)</td>
<td>46.6±3.8</td>
<td>40.9±3.7</td>
<td>34.97±3.5</td>
<td>5.4</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>MDA (mmol/ml)</td>
<td>2.09±0.09</td>
<td>3.4±0.10</td>
<td>4.6±0.9</td>
<td>36.9</td>
<td>0.000</td>
<td>I vs VI = 0.020</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>6.5±0.92</td>
<td>9.0±1.6</td>
<td>10.6±1.8</td>
<td>20.9</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>16.28±1.6</td>
<td>19.0±1.7</td>
<td>21.1±1.8</td>
<td>10.0</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>Total cholesterol (mg%)</td>
<td>77.8±2.6</td>
<td>82.0±2.0</td>
<td>84.2±4.3</td>
<td>12.5</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>TG (mg%)</td>
<td>89.5±3.5</td>
<td>94.3±4.6</td>
<td>96.9±6.01</td>
<td>6.1</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>H.D.L.P (mg%)</td>
<td>37.6±1.6</td>
<td>29.4±1.5</td>
<td>26.3±1.3</td>
<td>20.2</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>L.D.L.P (mg%)</td>
<td>32.3±4.1</td>
<td>45.5±6.4</td>
<td>48.6±7.6</td>
<td>19.4</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>Sperm count (million/ml)</td>
<td>7.2±1.1</td>
<td>5.2±1.0</td>
<td>4.0±1.0</td>
<td>6.7</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>77.7±6</td>
<td>70.9±8.5</td>
<td>65.1±8.12</td>
<td>7.8</td>
<td>0.000</td>
<td>I vs VI = 0.000</td>
</tr>
</tbody>
</table>

N.B. glycosylated haemoglobin (g.Hb), superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), high density lipoproteins (HDLp) and low density lipoproteins (LDLP).

n= number                SD: Standard deviation       P: < 0.05 significant difference
LSD: Least significant difference
Figure (1): A photomicrograph of a section of the liver of the negative control group showing radiating cords of hepatocytes surrounding a central vein (Hx & E x 100).

Figure (2): A photomicrograph of a section of the liver of alloxan toxic group showing congestion of the central vein (*), mononuclear cellular infiltration of the portal tracts (†) and the hepatocytes appear vacuolated (Hx & E x 100).

Figure (3): A photomicrograph of a section of the liver of N-alkyl alloxan toxic group showing portal tract with fibrous tissue expansion and inflammatory cell infiltrate. Focal poliosis was also noticed (†) (Hx & E x 100).

Figure (4): A photomicrograph of a section of the liver of N-alkyl alloxan toxic group showing most of the hepatocytes are vacuolated, others showed pyknotic nuclei (†) (Hx & E x 250).
Figure (5): A photomicrograph of a section of the liver of alloxan-zinc group showing radiating cords of hepatocytes surrounding central veins (CV) (Hx & E x 100).

Figure (6): A higher magnification of the previous figure showing a central vein surrounded by hepatocytes having a nearly normal appearance, some blood sinuoids show few blood cells (↑) (Hx & E x 400).

Figure (7): A photomicrograph of a section of the liver of alloxan-vit. A group showing nearly normal hepatocytes surrounding congested central veins and portal tracts (Hx & E x 100).

Figure (8): A photomicrograph of a section of the liver of alloxan-vit. A group showing mild congestion in the central veins (↑) and sporadic areas of mild mononuclear cellular infiltration (*) (Hx & E x 100).
Figure (9): A photomicrograph of a section of the liver of N-alkyl alloxan-zinc group showing normal radiating liver cords surrounding a central vein and blood sinusoids show congestion (Hx & E x 250).

Figure (10): A photomicrograph of a section of the liver of N-alkyl alloxan-vitamin A group showing nearly normal appearance of the hepatocytes. Note the congestion and cellular infiltrate (↑) (Hx & E x 250).

Figure (11): A photomicrograph of a section of the pancreas of the negative control group showing islets of Langerhans surrounded by pancreatic acini (Hx & E x 250).

Figure (12): A photomicrograph of a section of the pancreas of alloxan group showing damaged islets of Langerhans and reduced islet's size (Hx & E x 250).
Figure (13): Photomicrograph of a section of the pancreas of N-alkyl alloxan group showing condensed nuclei (↑) with islets cell degeneration (Hx & E x 640).

Figure (14): Photomicrograph of a section of the pancreas of alloxan-zinc group showing normal appearance of the islets of Langerhans and pancreatic acini (Hx & E x 640).

Figure (15): Photomicrograph of a section of the pancreas of alloxan-vitamin A group showing nearly normal appearance of the islets of Langerhans and pancreatic acini. Note mild congestion (Hx & E x 250).

Figure (16): Photomicrograph of a section of the pancreas of N-alkyl alloxan-zinc group showing normal islets of Langerhans and pancreatic acini. (↑) (Hx & E x 640).

Figure (17): Photomicrograph of a section of the pancreas of N-alkyl alloxan-vitamin A group showing nearly normal islets of Langerhans and pancreatic acini. Note minimal congestion (↑) (Hx & E x 640).
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مقارنة لكفاءة كل من الزنك وفيتامين أ ضد سمية الألوكسان والنون الكبالي ألوكسان في ذكور الفئران البيضاء البالغة

المشتركون في البحث

RSA الحسيني أبو عززة
من صيد حمس

أقسام الطب الشرعي والسموم الأكلينيكية* و الكيماوية الحيوية**
كلية الطب - جامعة عين شمس

إن مرض السكري يصاحبه العديد من المضاعفات بسبب الأكسيدة الضاغطة. تؤدي النسب المرتفعة من الشوارد المرمرة إلى إتلاف العضيات الخلوية والأنزيمات. ومنذ ذلك، فقد تؤثر النسبة المرتفعة من فيتامين أ على النوبات النارية من الألوكسان أو النون الكبالي ألوكسان.

الطريقة البحث: تم إجراء هذا البحث على 110 من ذكور الفئران البيضاء البالغة وتم تقسيمهم إلى 11 مجموعة متساوية عشر فئات لكل مجموعة كما يلي، المجموعة الأولي: وهي المجموعة الضاغطة السلبية، المجموعة الثانية: مجموعة محلول الملح، المجموعة الثالثة: مجموعة مزمنة زيت النبات، المجموعة الرابعة: مجموعة كلوريد الزنك، المجموعة الخامسة: مجموعة فيتامين أ، المجموعة السادسة: مجموعة ألوكسان، المجموعة السابعة: مجموعة النون الكبالي ألوكسان، المجموعة الثامنة: مجموعة الألوكسان مع الزنك، المجموعة التاسعة: مجموعة الألوكسان مع فيتامين أ، أما المجموعة العاشرة والحادية عشر فكانا مجموعات النون الكبالي ألوكسان مع الزنك ومجموعة النون الكبالي ألوكسان مع فيتامين أ على التوالي.

وتم قياس العلامات التي تشير إلى الأكسيدة الضاغطة (السوبر أكسيد ديمينوتاز، الجلوتاتيون المتخلل، والمالان تنسيل دهيلان). بالإضافة إلى سمية السكر بالدم، والهنسولين حامل السكر والأنزيمات الكبدية مثل الأالان أكسيد أسيتيراتسيز، والأنسيرين أسيتيراتسيز. تم تقسيم الدهن بالجملسترون، الدهن الثلاثي، الدهن الثلاثي، الدهن عالية الكفاءة. ودمن مخفضة الكفاءة) ثم تحليل السائل المرن في ذلك. حركة وعدد الهياكل المتاحة على ذلك أخرى لأشعة الكبد والبنكرياس.

النتائج: سبب كل من الألوكسان أو النون الكبالي ألوكسان مرض السكري (ارتفاع نسبة السكر بالدم والهنسولين حامل السكر) وكان هناك اختلاف في نسبة السكر أو كود مرنوتاز والخلطات المتخلل مع ارتفاع في نسبة المالان تنسيل دهيلان. بالنظر في نسبة الأالان أكسيد أسيتيراتسيز، الأسيتيرين أسيتيراتسيز كانت هناك زيادة ملحومة في كلا الألرين. ونتج ارتفاع نسبة الدهن في صورة زيادة نسبة الكلسترون. الدهن الثلاثي، الدهن مخفضة الكفاءة مع ارتفاع في الدهن مرتفعة الكفاءة، علامة على ذلك أظهر تحمل السائل المرن انخفاضاً ملحوظاً على حد سواء في عدد حركة الهياكل المتاحة. كما أظهر الفحص الهستوبيوتيولوجي لكل من الكبد والبنكرياس تشذبات
شديدة في كل منها بعد أخذ الألوكسان أو ألوكسان (الأكثر سمية). أدى تناول الزنك إلى تأثير وقائي مفضل مع تراجع كل القياسات الكيميائية الحيوية إلى معدلها الطبيعي تقريباً وتحسين في صورة التغييرات الهيستوتأثريولوجية. أدى أيضاً تناول فيتامين A إلى تأثير وقائي ضد سمية الألوكسان أو ألوكسان ولكن كان أقل من تأثير الزنك. وقد استنتج من ذلك أن الزنك له تأثير وقائي أفضل من تأثير فيتامين A ضد سمية كل من الألوكسان أو ألوكسان ليس فقط على أنسجة البنكرياس ولكن على أنسجة أخرى مثل الكبد.