Hepato- Toxicity Caused by Ecstasy

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Ecstasy (3,4-Methylenedioxymethamphetamine, MDMA) is abused mainly by young population. The liver is a vulnerable target for amphetamine toxicity, but the mechanisms involved in the hepatotoxicity remain poorly understood . For this reason, it is especially relevant to take into consideration the effects on the liver tissue. The influence of MDMA on the liver tissue of the male rat was assessed in this study. The present study was aimed at clarifying if daily oral administration of ecstasy at low doses(10mg kg body weight of ecstasy) for 45 days has any deleterious effects on liver functions of male rats. **Methods**: 100 male white albino rats of Sprague Dawley weighed about (100-150 g) were divided into three groups : one group served as control (20 rats) and the other two groups (40 rats in each group) were kept for treatment and administrated 10, 30 mg/kg body weight of ecstasy (chronic dose) by oral gavage for 15, 30 and 45 days. The 45-day of treatment was followed by 15 days of withdrawal (w15). All animals were sacrificed after 30 minutes from the last treatment and biochemical parameters in serum and homogenized liver were tested.

Results: A significant increase in transglutaminase activity and collagen content were recorded in the liver of treated rats. Significant increases in lipid peroxides, lipid hydroperoxides and conjugated dienes associated to significant decreases of reduced glutathione content, superoxide dismutase and catalase activities were also recorded. Significant increases ($P < 0.0001$) of serum ALT, AST, ALP and GGT activities were observed in rats that received 30 mg/Kg MDMA . Also, significant increases ($P < 0.0001$) in the content of total, direct and indirect bilirubin were observed in the rats that received 30 mg/kg MDMA. Withdrawal group ameliorated these increases. The results exhibited major changes in the protein pattern which included changes in the molecular weight of the treated bands and the relative percentage of protein fractions as well as the total number of

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bands compared with control ones, as a result of disappearance of some original bands and appearance of other new ones. The obtained results revealed that ecstasy10 mg has no significant effect on DNA damage, while ecstasy (30 mg) exerts DNA damage in different groups (fig. 2) and table (12) which represented DNA damage pattern obtained by DNA comet assay of rat hepatocytes. **In conclusion:** 10 mg/kg MDMA had a little significant effect on liver tissue, while 30 mg/kg MDMA significantly affected the liver tissue of male rats by induction of oxidative stress.

Introduction

The recreational use of the ring-substituted amphetamine derivative 3,4 methylenedioxymethamphetamine (MDMA, ecstasy) has been recognized as one of the most significant trends in drug abuse over the past decade. Acting as a potent releaser and/or reuptake inhibitor of presynaptic serotonin, dopamine and noradrenalin [1] .It has attracted a great deal of media attention in recent years due to its widespread abuse as a recreational drug by the youth[2] . Clinical evidence has shown that the liver is a great target for MDMA toxicity. In fact, MDMA is metabolized by cytochromes P_{450} 2D, 2B and 3A and reactive metabolites are readily oxidized to the corresponding O-qiuinones and in the long run formation of reactive oxygen species (ROS)[3,4].

Oxidative stress characterized by the overproduction of reactive oxygen species (ROS), which overwhelm the levels of antioxidants, was suggested as the pathogenic factor of a number of human diseases and was reported to cause tissue damage [5]. Acute exposure to MDMA alone or in combination with other substances, can damage several organs such as the heart, liver, kidney, and brain and can be fatal. The life threatening clinical manifestations of MDMA toxicity also include acute hepatic damage[6]. Despite the well-established toxicities associated with its abuse, MDMA is the second most common cause of liver injury in people under the age of 25 and has been shown to produce cell necrosis and fibrosis in the liver [7].

ROS can react with cellular macromolecules such as nucleic acids, polyunsaturated fatty acids in cellular membranes and sulfhydryl bonds in proteins to cause mutagenesis, carcinogenesis and

cell death. The reactive intermediates in this pathway covalently bind to hepatic macromolecules and eventually cause liver injury, whereby free radical-mediated lipid peroxidation contributes to the development of liver damage[8]. Prolonged administration of ecstacy causes hyperplastic liver nodules, liver cell adenomas and hepatocarcinomas. The free radicals produced during ecstasy metabolism interfere with ribosomal activity, thereby hindering protein synthesis[9].

Materials and Methods

All chemicals and reagents used in the experiments were of analytical grade and purchased from either Merck (Germany) or Sigma Aldrich Chemie (Germany). Assay kits for testing alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransaminase (AST), gamma glutamyltransferase (GGT), bilirubin and total protein were supplied by Diamond Diagnostics (Egypt).

Animal Groups

100 male white albino rats of Sprague Dawley weighed about (100-150g) were obtained from experimental animal house, Helwan, Egypt. Animals were maintained on stock diet in the form of pellets having the following composition: protein (18.8 w/w), barley (37% w/w), corn (15% w/w), salt and vitamins mixture (29.2% w/w)[10]. All animals were normal and healthy. Animals were divided into three groups: one group served as control (20 rats) and the other two groups (40 rats in each group) were kept for treatment and administered 10, 30 mg/kg body weight of ecstasy by oral gavage for 15, 30 and 45 days. The 45-day of treatment was followed by 15 days of withdrawal (w15)[11]. These daily doses in relation to their respective effective doses were calculated according to Paget and Barnes (1964)[12] for species interconversion of dosage. All animals were sacrificed after 30 minutes from the last treatment and biochemical parameters in serum and homogenized liver were tested.

Biochemical Assays:

Tissue transglutaminase (tTG) activity in liver homogenate was determined according to the direct spectrophotometric method of De Macedo *et al.*, 2000[13]. Total protein content in liver tissue was determined according to the method of Henry,1974[14]. Liver collagen content was determined according to Woessner, 1961[15].

Reduced glutathione concentration (GSH) in liver was determined according to Beutler *et al.*, 1963[16]. Superoxide dismutase (SOD) activity in liver was measured according to Masayasu & Hiroshi, 1979[17]. The colorimetric assay for liver catalase activity (Cat) was carried out according to Sinha,1972[18]. Lipid peroxides (LP) indicated by the formation of malondialdehyde (MDA) was assessed in liver homogenates according to Yoshioka *et al.*,1979[19]. Lipid hydroperoxides (LHP) in liver was determined according to the Fox method described by Jiang *et al.*,1992[20]. The levels of conjugated dienes (CD) in liver were measured according to Rechnagel and Gglende,1984 and Nowak *et al.*,1995[21, 22]. Serum ALP activity was determined according toTeitz,1976[23].

Activities of ALT and AST were determined colorimetrically according to Reitman and Frankel ,1957[24]. Serum gamma GGT activity was measured kinetically according to Szasz,1976[25]. Serum bilirubin (total, direct and indirect) contents were determined according to Perry *et al.*,1983[26].

Electrophoretic separation of serum proteins:

SDS-PAGE electrophoresis was carried out according to the method of Laemmli, 1970[27], the wide range SDS-PPAGE molecular weight standard mixture (Biorad) was applied to the first well. Scanning was applied using gel pro software (ver.3, USA, 1998), for Media Sci Image densitometry 700 Biorad.

DNA damage evaluated by Comet Assay:

Comet assay was performed by the method of Speit and Hartmann, 2005[28]. The comet assay is a simple method for measuring deoxyribonucleic acid (DNA) strand breaks in liver cells. Cells

embedded in agarose on a microscope slide are lysed with detergent and high salt to form nucleotides containing super coiled loops of DNA linked to the nuclear matrix. Electrophoresis at high pH results in structures resembling comets, observed by fluorescence microscopy; the intensity of the comet tail relative to the head reflects the number of DNA breaks[29].

Statistical analysis

The SPSS (version 23) was used in data analysis. Data were analyzed with one-way analysis of variance (ANOVA). The data were expressed as mean ± standard deviation (SD). P values < 0.05 were considered to be statistically significant.

Results

Results of tables (1-2): Tissue transglutaminase (tTG) activity and collagen content in liver tissue homogenates of rats under different treatment conditions.

As shown in table (1), 10 mg/kg MDMA had no significant effect on liver collagen content and tTG activity. On the other hand, chronic administration of 30 mg/kg MDMA for 15, 30 and 45 days showed significantly increased liver collagen content and tTG activity ($P < 0.0001$). Withdrawal group for 15 days significantly ameliorated the increase of collagen content and tTG activities in the rats (Table 2).

Table (1): Effect of Chronic Administration of 10 mg/kg MDMA on Transglutaminase (tTG) Activity and Collagen Content in Liver Tissue Homogenates of Rats.

Groups \ Parameter		tTG (anilide/umol/mg protein/min)	Collagen (mg/g wet tissue)
Control	Mean ± SD	1.26 ± 0.182	3.96 ± 0.346
D_{15}	Mean ± SD P<	1.37 ± 0.082 N.S	4.20 ± 0.499 N.S
D_{30}	Mean ± SD P<	1.28 ± 0.085 N.S.	4.11 ± 0.163 N.S.
D_{45}	Mean ± SD P<	1.3 ± 0.065 N.S.	3.99 ± 0.125 N.S.
W_{15}	Mean ± SD P<	1.24 ± 0.111 N.S	4.03 ± 0.221 N.S

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Table (2): Effect of Chronic Administration of 30 mg/Kg MDMA on Transglutaminase (tTG) Activity and Collagen Content in Liver Tissue Homogenates of Rats.

Groups \ Parameter		tTG (anilide/umol/mg protein/min)	Collagen (mg/g wet tissue)
Control	Mean ± SD	1.26 ± 0.182	3.96 ± 0.346
D_{15}	Mean ± SD P<	2.87 ± 0.166 0.0001	5.14 ± 0.318 0.0001
D_{30}	Mean ± SD P<	3.49 ± 0.185 0.0001	6.77 ± 0.075 0.0001
D_{45}	Mean ± SD P<	3.76 ± 0.162 0.0001	7.15 ± 0.155 0.0001
W_{15}	Mean ± SD P<	1.72 ± 0.061 0.0001	5.13 ± 0.221 0.0001

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Results of tables (3-6) can be summarized as follow:

Administration of10 mg/kg MDMA for 45 days to rats induced significant decreases ($P < 0.0001$) in liver GSH content, SOD and Cat activities (Table 3), which were parallel to significant increases in LP ($P < 0.01$) content (Table 5). 30 mg/kg MDMA for 15,30,45 and withdrawal period showed significant decrease in liver GSH content, SOD and Cat activities in association with significant increase in LP, LHP and CD content (Tables 4 and 6).

Table 3: Effect of Chronic Administration of 10 mg/kg MDMA on Superoxide Dismutase(SOD), Catalase (Cat) Activities and Redused Glutathione(GSH) Content in Liver Tissue Homogenate of Rats.

Groups \ Parameter		SOD (µg/g wet tissue)	Cat (µmol/g wet tissue)	GSH (mg/g wet tissue)
Control	Mean ± SD	12.06 ± 0.701	119.36 ± 9.799	24.75 ± 1.035
D_{15}	Mean ± SD P<	12.38 ± 0.499 N.S.	119.37 ± 5.334 N.S.	25.06 ± 0.752 N.S
D_{30}	Mean ± SD P<	11.84 ± 0.398 N.S	118.89 ± 4.522 N.S	23.26 ± 0.586 N.S
D_{45}	Mean ± SD P<	10.79 ± 0.054 0.0001	101.40 ± 3.561 0.0001	21.02 ± 0.699 0.0001
W_{15}	Mean ± SD P<	11.88 ± 0.031 N.S	117.32 ± 9.122 N.S	23.11 ± 0.867 N.S

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Table (4): Effect of Chronic Administration of 30 mg/Kg MDMA on Superoxide Dismutase(SOD), Catalase (Cat) Activities and Redused Glutathione (GSH) Content in Liver Tissue Homogenate of Rats.

Groups \ Parameter		SOD (µg/g wet tissue)	Cat (µmol/g wet tissue)	GSH (mg/g wet tissue)
Control	Mean ± SD	12.06 ± 0.701	119.36 ± 9.799	24.75 ± 1.035
D_{15}	Mean ± SD P<	10.64 ± 0.316 0.0001	91.01 ± 6.793 0.01	19.86 ± 0.263 0.0001
D_{30}	Mean ± SD P<	9.14 ± 0.408 0.0001	80.45±4.231 0.001	17.24 ± 0.816 0.0001
D_{45}	Mean ± SD P<	8.71 ± 0.464 0.0001	64.10 ± 5.511 0.001	15.14 ± 0.827 0.0001
W_{15}	Mean ± SD P<	10.81 ± 0.031 0.0001	97.32 ± 6.11 0.001	17.01 ± 0.644 0.0001

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Table (5): Effect of Chronic Administration of 10 mg/kg MDMA on Lipid Peroxides (LP), Lipid Hydroperoxide (LHP) and Conjugated Diene (CD) in Liver Tissue Homogenates of Rats .

Groups \ Parameter		LP (µg/g tissue)	LHP (µM)	CD (Abs_{234}/g tissue)
Control	Mean ± SD	108.27 ± 7.246	17.81 ± 0.841	2.20 ± 0.091
D_{15}	Mean ± SD P<	100.99 ± 4.910 N.S.	17.50 ± 0.919 N.S.	2.20 ± 0.091 N.S.
D_{30}	Mean ± SD P<	114.72 ± 5.170 0.05	17.90 ± 0.759 N.S.	2.24 ± 0.068 N.S.
D_{45}	Mean ± SD P<	127.47 ± 2.480 0.01	17.79 ± 2.949 N.S.	2.30 ± 0.200 N.S.
W_{15}	Mean ± SD P<	110.11 ± 6.133 N.S.	17.69 ± 0.911 N.S.	2.29 ± 0.088 N.S.

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Table (6): Effect of Chronic Administration of 30 mg/kg MDMA on Lipid Peroxides (LP), Lipid Hydroperoxide (LHP) and Conjugated Diene (CD) in Liver Tissue Homogenates of Rats.

Groups \ Parameter		LP (µg/g tissue)	LHP (µM)	CD (Abs_{234}/g tissue)
Control	Mean ± SD	108.27 ± 7.246	17.81 ± 0.841	2.20 ± 0.091
D_{15}	Mean ± SD P<	142.04 ± 14.09 0.01	17.88 ± 2.247 N.S.	2.47 ± 0.116 N.S.
D_{30}	Mean ± SD P<	169.04 ± 16.91 0.001	21.64 ± 1.99 0.0001	3.14 ± 0.042 0.0001
D_{45}	Mean ± SD P<	182.93 ± 9.560 0.0001	21.92 ± 1.958 0.0001	3.20 ± 0.015 0.0001
W_{15}	Mean ± SD P<	165.54 ± 13.19 0.001	20.91 ± 0.833 0.0001	2.54 ± 0.191 0.0001

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Results of tables (7-10):

Table (7, 8): ALT, AST, ALP and GGT activities in serum of rats under different treatment conditions. Significant increases ($P < 0.0001$) of serum ALT, AST, ALP and GGT activities were observed in rats that received 30 mg/Kg MDMA in comparison with control . Withdrawal group ameliorated these increases (Table 8).

Table (9,10): Bilirubin total, direct and indirect concentration in serum of rats under different treatment conditions.

Significant increases ($P < 0.0001$) in the content of total, direct and indirect bilirubin were observed in the rats that received 30 mg/Kg MDMA when comparison with control .withdrawal group ameliorated these increases (Table 10).

Table (7): Effect of Chronic Administration of 10 mg/kg MDMA on Alanine aminotransferase (ALT), Aspartate aminotransaminase (AST), Alkaline phosphatase (ALP) and Gamma-glutamyltransferase (GGT) Activities in Serum of Rats.

Groups \ Parameter		ALT (U/l)	AST (U/l)	ALP (U/l)	GGT (U/l)
Control	Mean ± SD	15.54 ± 1.987	8.00 ± 0.503	50.11 ± 1.24	1.00 ± 0.01
D_{15}	Mean ± SD P<	15.44 ± 2.001 N.S.	8.18 ± 0.804 N.S.	49.36 ± 1.01 N.S.	1.08 ± 0.14 N.S.
D_{30}	Mean ± SD P<	17.17 ± 1.89 N.S.	9.26 ± 0.585 N.S.	51.98 ± 1.59 N.S.	1.43 ± 0.09 N.S.
D_{45}	Mean ± SD P<	19.91 ± 1.371 0.0001	14.37 ± 0.567 0.0001	88.00 ± 1.76 0.0001	2.62 ± 0.24 0.0001
W_{15}	Mean ± SD P<	14.98 ± 1.28 N.S.	7.84 ± 3.380 N.S.	54.35 ± 2.43 N.S.	1.06 ± 0.21 N.S.

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Table (8): Effect of Chronic Administration of 30 mg/kg MDMA on Alanine aminotransferase (ALT), Aspartateaminotransaminase (AST), Alkaline phosphatase(ALP) and Gamma-glutamyltransferase (GGT) Activities in Serum of Rats.

Groups \ Parameter		ALT (U/l)	AST (U/l)	ALP (U/l)	GGT (U/l)
Control	Mean ± SD	15.54 ± 1.987	8.00 ± 0.503	50.11 ± 1.24	1.00 ± 0.01
D_{15}	Mean ± SD P<	23.58 ± 1.282 0.0001	16.04 ± 3.380 0.0001	94.35 ± 2.43 0.0001	4.66 ± 0.21 0.0001
D_{30}	Mean ± SD P<	24.60 ± 1.268 0.0001	21.20 ± 0.560 0.0001	104.40 ± 3.24 0.0001	5.78 ± 0.36 0.0001
D_{45}	Mean ± SD P<	30.11 ± 1. 71 0.0001	25.17 ± 1.61 0.0001	120.00 ± 2.16 0.0001	6.12 ± 0.64 0.0001
W_{15}	Mean ± SD P<	19.85 ± 0.813 0.0001	12.20 ± 1.344 0.0001	55.99 ± 2.79 0.0001	2.71 ± 0.28 0.0001

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Table(9): Effect of chronic administration of 10 mg/Kg MDMA on Bilirubin Total, Direct and Indirect Concentration in Serum of Rats.

Groups \ Parameter		Bilirubin total (mg/ml)	Bilirubin direct (mg/ml)	Bilirubin indirect (mg/ml)
Control	Mean ± SD	0.923 ± 0.022	0.146 ± 0.006	0.777 ± 0.026
D_{15}	Mean ± SD P<	0.917 ± 0.060	0.145 ± 0.007	0.772 ± 0. 061
D_{30}	Mean ± SD P<	0.936 ± 0.063 N.S.	0.144 ± 0.006 N.S.	0.792 ± 0.028 N.S.
D_{45}	Mean ± SD P<	0.923 ± 0.033 N.S.	0.162 ± 0.009 0.025	0.761 ± 0.030 N.S.
W_{15}	Mean ± SD P<	0.896 ± 0.053 N.S.	0.137 ± 0.080 N.S.	0.759 ± 0.040 N.S.

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Table (10): Effect of chronic administration of 30 mg/Kg MDMA on Bilirubin Total, Direct and Indirect Concentration in Serum of Rats.

Groups \ Parameter		Bilirubin total (mg/ml)	Bilirubin direct (mg/ml)	Bilirubin indirect (mg/ml)
Control	Mean ± SD	0.923 ± 0.022	0.146 ± 0.006	0.777 ± 0.026
D_{15}	Mean ± SD P<	1.302 ± 0.071 0.0001	0.199 ± 0.014 0.0001	1.103 ± 0.074 0.0001
D_{30}	Mean ± SD P<	2.281 ± 0.169 0.0001	0.250 ± 0.024 0.0001	2.031 ± 0.166 0.0001
D_{45}	Mean ± SD P<	3.137 ± 0.076 0.0001	0.281 ± 0.008 0.0001	2.856 ± 0.079 0.0001
W_{15}	Mean ± SD P<	1.310 ± 0.063 0.0001	0.226 ± 0.005 0.0001	1.084 ± 0.062 0.0001

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

Results of table (11) can be summarized as follows:

In the present study the changes induced in electrophoretic serum protein pattern of male rats by daily administration of 30mg/kg of MDMA for 15, 30 and 45 days. The results of electrophoretic

separation of serum proteins by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) are shown in table(11)and figure (1). The results exhibited major changes in the protein pattern which included changes in the molecular weight of the treated bands and the relative percentage of protein fraction as well as the total number of bands, as a result of disappearance of some original bands and appearance of other new ones.

The electrophoretic separation of serum protein showed a wide molecular weight range in the different groups with a maximum of 188.98 KDa and a minimum of 30.373 KDa. The banding pattern showed 14 distinct bands in control group ,16 distinct pattern in ES15 group ,14 for ES 30, 13 for ES 45. The 65..347 KDa band was similarly expressed in control group and ES 30group (around 13.6%). The 107.05 KDa band was similarly expressed in ES15 group((around 6.06%) and ES 45group (around 4.05%). The 29.816 KDa band was more expressed in ES15 group((around 8.45%) and less expressed in ES 45group (around 4.35%). The 81.93 KDa band similarly expressed in ES30 group((around 6.95%) and ES 45group (around 5.1%).The band of 188.98 KDa was expressed in control group only, while that of 123.8KDa was expressed in ES 45 group only.

There were 14 missing bands in ES15 treated group. These were (188.98, 115.73 ,112.76, 106.29, 101.39, 97.63, 79.315, 65.347, 58.865, 46.133, 39.534, 37.573, 33.938 and 30.373) versus the control group. On the other hand , 13 missing bands in ES 30(188.98, 115.73 ,112.76, 106.29, 101.39, 97.63, 79.315, 58.865, 46.133, 39.534, 37.573, 33.938 and 30.373) versus the control group and 14 missing bands in ES45(188.98, 115.73 ,112.76, 106.29, 101.39, 97.63, 79.315, 65.347, 58.865, 46.133, 39.534, 37.573, 33.938 and 30.373) versus the control group.

Table (11):Effect of Chronic Administration of 30 mg/Kg MDMA on Serum Protein Electrophoretic Patterns in Adult Male Rats.

Lanes Bands	Lane 1		Lane 2		Lane 3		Lane 4		Lane 5	
	KD	%	KD	%	KD	%	KD	%	KD	%
1	205	16.9	188.98	2.44	116	5.17	121.8	4.78	123.8	6.43
2	116	11.3	115.73	4.54	114.1	2.71	113.56	3.42	107.05	4.05
3	97.4	16.8	112.76	2.79	107.05	6.06	108.58	4.77	101.63	3.55
4	66	23.4	106.29	8.22	100.91	5.15	101.87	3.72	86.014	8.54
5	45	21.3	101.39	4.12	98.093	3.88	98.324	3.22	81.93	5.1
6	29	10.3	97.63	5.59	94.292	2.47	87.42	7.21	75.549	3.89
7			79.315	22.2	83.72	5.89	81.93	6.95	66.717	10.4
8			65.347	12.5	81.049	6.75	65.347	13.6	60.048	2.48
9			58.865	4.55	73.138	4.73	56.851	7.08	51.465	15.7
10			46.133	16.3	66	11.1	47.531	18.1	43.768	29.8
11			39.534	2.7	59.454	1.88	44.175	11.8	39.902	2.15
12			37.573	2.05	57.42	2.91	39.17	4.26	33.009	3.63
13			33.938	6.36	47.295	22.1	34.732	5.95	29.816	4.35
14			30.373	5.67	39.352	4.15	31.81	4.89		
15					35.71	6.64				
16					29.816	8.45				
Sum		100		100		100		100		100
In lane		100		100		100		100		100

Lane 1=Marker , Lane 2 =Control , Lane 3=ES15 , Lane 4ES30 ,Lane 5=ES45

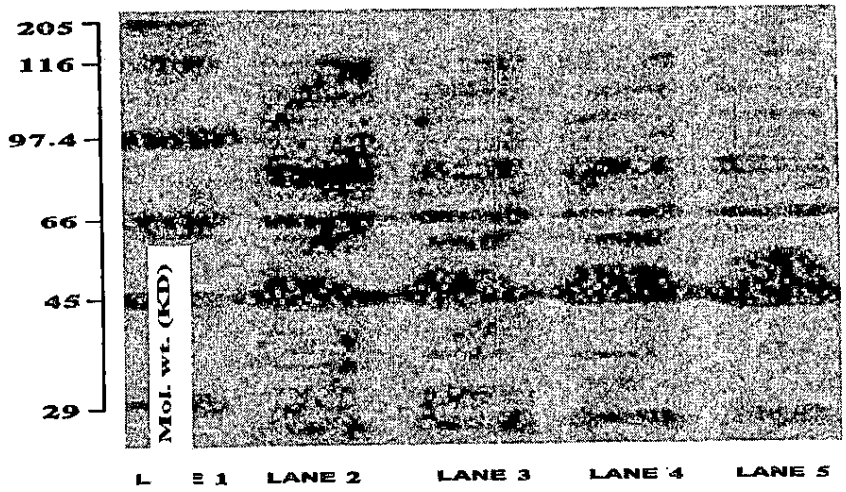

Fig1:Electrophoretic pattern of serum protein in adult male rat affected by daily administration of ecstasy for15,30 and45 days. Individual lanes represent individual samples per group. Lane1= marker,Lane2= Control group ;Lane3= (ES15)ecstasy treated group for 15 days;Lane4=(ES30) Ecstasy treated group for 30 days andLane5=(ES45) Ecstasy treated.

Results of table(12) and(fig. 2) can be explained as follows:
The obtained results revealed that ecstasy10 mg has no significant effect on DNA damage,while ecstasy (30mg) exerts DNA damage in different groups (fig. 2) and table (12) which represented DNA damage pattern obtained by DNA comet assay of rat hepatocytes. The intensity of the comet tail relative to the head reflects the number of DNA breaks. The comet head containing the high molecular weight DNA and the comet tail containing the leading ends of migrating fragments. The results of ecstasy (30 mg), revealed that 1 stgroup(control group), DNA% was found to be 1.15% and DNA tail was 1.77, while the 2 nd group after 15 days of ES treatment recorded that DNA tail was4.39and DNA% was 4.07, while the 3rd group of ES treatment increases the DNA tail up to 6.14 and DNA% up to 5.83, Meanwhile, the 4th group of ES treatment increases the intensity of the tail up to 6.82 and DNA% up to 6.55. The DNA damage was found in withdrawal group also with DNA tail of 6.08 and DNA% to 5 .29.

Table (12): Effect of Chronic Administration of 30 mg/kg MDMA on DNA Damage in Liver of Rats Using the Comet Assay.

Parameter / groups	Tail (um)	DNA%
Control Mean ±S.D	1.77±0.05	1.15±0.04
ES15 Mean ±S.D	4.39±0.01	4.07±0.014
%change	148.023	253.91
P<	0.0001	0.0001
ES 30 Mean ±S.D	6.14±0.06	5.83±0.04
%change	246.89	406.956
P<	0.0001	0.0001
ES45 Mean ±S.D	6.82±0.07	6.55±0.07
%change	285.311	469.565
P<	0.0001	0.0001
ESW Mean ±S.D	6.08±0.04	5.29±0.05
%change	243.5	360
P<	0.0001	0.0001

Each value represents mean ± SD of 10 determinations. P-value <0.05 statistically significant, D_{15}, D_{30}, D_{45} and W_{15} = duration of time taken 15, 30, 45 and withdrawal 15 days.

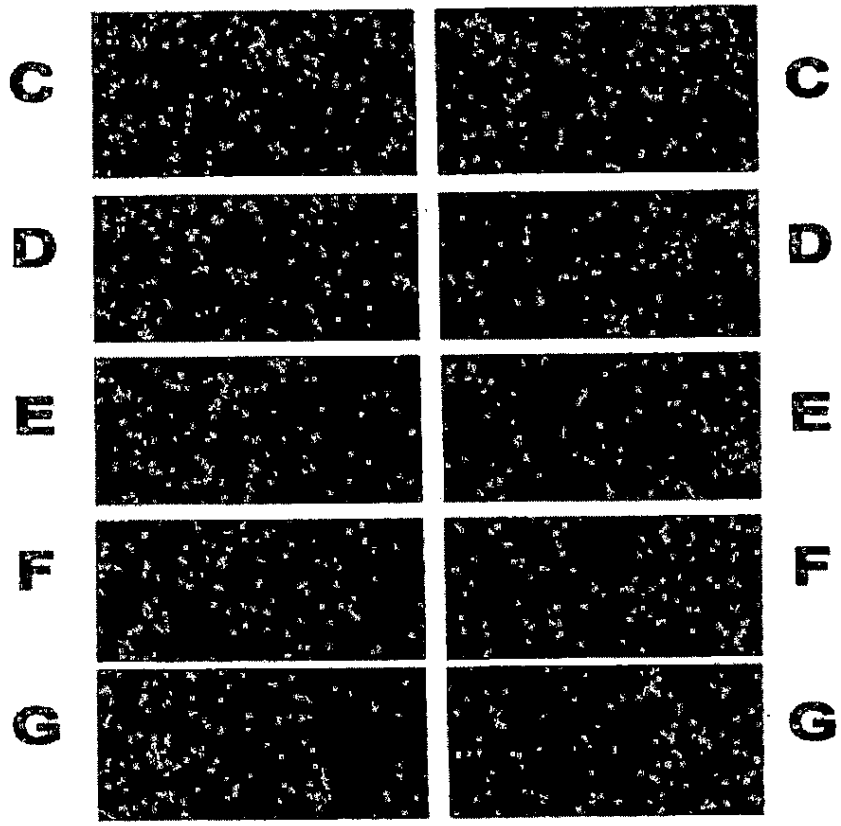

Fig. (2): Representative comet images showing different levels of damage in rat liver DNA In visual scoring: C:score 0 (unchangedDNA) and various degree of damage from minor (grade1) to severe (grade 4); D: ES15; E: ES30; F: ES45; G ESW.

DISCUSSION

The amphetamine derivative MDMA is not a designer drug or novel psychoactive substance as it has been used for decades. However, MDMA is still one of the most widely used recreational drugs and many novel psychoactive substances were designed to mimic its effects or as substitutes for MDMA in ecstasy pills. Ecstasy can be incriminated in causing the liver damage. It has been reported that liver toxicity is one of the consequences of ecstasy abuse. Many

studies showed that the hepatotoxicity induced by MDMA is a consequence of the metabolism of MDMA[30]. Various factors may play role in ecstasy-induced hepatotoxicity, namely its metabolism, the increased efflux of neurotransmitters, the oxidation of biogenic amines, and hyperthermia. MDMA undergoes extensive hepatic metabolism that produce reactive metabolites which form adducts with intracellular nucleophilic sites[31]. Many studies have indicated that each of the amphetamine derivatives (MDMA and MDA) have direct effects on liver tissue including the induction of apoptosis[32].

In the present study, parameters of liver toxicity induced by ecstasy administration were shown as a significant increases in liver tTG activity and collagen content associated with significant decreases in GSH content, SOD and Cat activities and increases in LP, LHP and CD content. Hepatic damage was indicated by significant increases of serum AST, ALT, ALP activities and bilirubin content. The results exhibited major changes in the protein pattern which included changes in the molecular weight of the control bands and the relative percentage of protein fraction as well as the total number of bands, as a result of disappearance of some original bands and appearance of other new ones. The obtained results revealed that ecstasy10 mg has no significant effect on DNA damage, while ecstasy (30mg) exerts DNA damage in different groups (fig 2) and table (12) which represented DNA damage pattern obtained by DNA comet assay of rat hepatocytes.

tTG is a unique member of the transglutaminase family as its transamidating activity is regulated by both ATP and GTP. Impairment of mitochondrial function results in a significant increase in tTG activity. This increase in tTG activity is likely due in part to decreased levels of ATP and GTP, which inhibit tTG activity and an increase in oxidative conditions, which likely increase the tTG modification of certain intracellular substrates. GTP has been shown to regulate TG activity both directly and indirectly, as the binding of GTP to TG inhibits its transamidating activity as well as causes a conformational change of the enzyme that reduces its affinity for calcium. Therefore, it is likely that a decrease in intracellular GTP

levels increases the activity of tTG[33]. The mitochondrion is one of the major sources of free radicals in cells, and there is also compelling evidence demonstrating that ecstacy treatment results in an increase in the production of free radicals. Oxidative stress can result in the modification of specific proteins, making them better substrates for TG, a mechanism that contributes to the enhanced TG modifications in response to the increase in tTG activity which may be attributed to the increased binding of the nuclear factor-kappaB (NF-κB) to the NF-κB motif of the tTG promoter, where tTG gene expression increases during hepatic injury and fibrosis[34]. The concomitant increase of both hepatic collagen and tTG activity may be explained by the dual effect exerted by the NF-κB, which is induced by oxidative stress[35]. The increase of tTG activity may be a consequence of GSH depletion and mitochondrial dysfunction[36]. Tissue fibrosis is associated with the increase of the tTG activity and accumulation of ECM (extracellular matrix) (ECM)[37]. In liver fibrosis induced in rats by carbon tetrachloride (CCl_4) and in human patients with an acute liver disease, a dramatic rise in tissue transglutaminases (tTG) activity was observed. The enzyme catalyzes the specific cross-linking of ε-amines and α-glutamyl residues among amino acids This activity leads to the cross-linking of extracellular matrix (ECM) proteins thereby increasing the deposition of such proteins and their resistance to proteolytic enzymes, which leads to tissue fibrosis[38,39,40].

Tables (3, 4) recorded a significant reduction in the levels of reduced glutathione (GSH) compared to control group. The results are in line with that of Liu and Gaston, 2010[41], who stated that, cellular GSH concentration and the GSH/GSSG ratio are reduced markedly in response to oxidative stress and much pathological condition. At the same time the decrease in NADPH, necessary for drug metabolism leads to a decrease in GSH level, as NADPH utilized to maintain GSH in the reduced form and increased the level of oxidized glutathione (GSSG)[42]. GSH can react with the end product of drug metabolism, therefore, another potential mechanism to explain the apparent loss of glutathione (GSH) in serum[43].

In humans, as well as in rats, MDMA hepatic metabolism involves N-demethylation to 3,4-methylenedioxyamphetamine (MDA), which is also a well-known drug of abuse. MDMA and MDA are O-demethylenated to N-methyl-methyldopamine (N-Me-MeDA) and methyldopamine (MeDA), respectively, both of which are catechols and can be O-methylated by Catechol-O-methyl transferase (COMT). Alternatively, N-Me-MeDA and MeDA can undergo oxidation to the corresponding O-quinones, which can form adducts with glutathione (GSH) and other thiol-containing compounds[44].

The daily administration of ecstasy at 30 mg/kg dose for 45 days was significantly decreased the activity of the antioxidant defense enzymes (CAT and SOD) in a dose dependant as compared with that of the normal rats. This depression of antioxidant enzyme activities reflects failure of the antioxidant defense mechanisms to overcome the influx of ROS induced by ecstasy administration that leads to the accumulation of free radicals and facilitate the enhancement of LPO, which in turn increases the oxidative damage of the cell membrane and alteration in dynamic permeability of membranes due to peroxidation which is followed by the release of intracellular enzymes to the blood stream, so decreased the activity of liver antioxidant enzymes[45]. The current data are in accordance with our results which reported a decreased activity of antioxidant enzymes in liver of treated rats.

In the present study, chronic administration of ecstasy10 and 30 mg/100 g body weight of for 45 days resulted in a significant increase of lipid peroxidation (MDA),LHP and CD in liver tissue as indicating in Tables (5and6) which probably due to the interaction of the potent hydroxyl radicals (OH) with the polyunsaturated fatty acids in the phospholipids portion of cell membrane initiating the lipid peroxidation chain reactions, produced greater tissue injury, therefore, liver MDA level revealed direct relationship between the severity of oxidation stress and drug toxicity[46]. The marked increase in LP,LHP ,andCD levels are likely to be a result of the inactivation of scavenger enzymes induced by reactive oxygen species (ROS)[47].

Serum AST, ALT, and ALP, γGT and bilirubin are the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction[48]. In liver injury the transport function of hepatocytes is disturbed, resulting in the leakage of plasma membrane[49]. The increase activities of AST, ALT, ALP, γGT, Bilirubin level in serum of treated rats, indicates ecstasy-induced liver impairment[50]. Bhadauria and Nirala (2009)[51] and Yuan *et al.* (2010)[52] in which hepatic markers were reportedly elevated. The elevated activities of serum AST, ALT and ALP in APAP induced liver injury indicative of cellular leakage and loss of functional integrity of cell membrane in liver[53]. The effect of ecstasy administration on serum protein pattern was found to induce severe changes in the protein regions when compared with the control pattern. The decrease of protein bands was reported by many different types of protein oxidative modification which can be induced directly by Reactive Oxygen Species (ROS) or indirectly by reactions of secondary byproducts of oxidative stress[54].

The present study investigated the effect of chronic administration of ecstasy on protein in male rats as manifested by changes in serum protein patterns and gene expression manifested by DNA damage measured by Comet assay. The present study has revealed major changes in the electrophoretic mobility of serum protein among the treated groups especially in case of ecstasy ES45group. These changes might reflect a considerable degree of oxidation to protein molecules ,which might in turn results in tertiary structural alteration that promote protein aggregation and amyloidal formation as supported by the study of Shinall *et al.*, (2005)[55].

ROS has also been described by the work of Dunlop *et al.*, (2009)[56] in which amino acids, simple peptides and proteins were exposed to oxidation by the action of hydroxyl radicals or a mixture of hydroxyl/superoxide radicals. The authors' concluded that the side chain of all amino acid residues of proteins, in particular cysteine and methionine residues of protein are susceptible to oxidation by the action of ROS. The present data has also reflected a wide range variation in serum protein patterns due to chronic daily treatment of ecstacy (30mg), which direct reflects a parallel variation in the process

of gene expression. Oxygen free radicals produced from ecstacy have shown to induce DNA breaks and DNA damage. The appearance or disappearance of protein fraction in the present study may be attributed to this damage or may be explained by the report of Bedwell *et al.*, (1989)[57], who claimed that the free radicals promote sulfhydral mediated cross linking of the labile amino acids such as methionine, histidine, cysteine and lysine causing a fragmentation of polypeptide chains in the protein molecule. Ecstacy increased lipid per oxidation, due to long term use[58].

In living cells, when the formation of intracellular reactive oxygen species exceeds the cells antioxidant capacity, oxidative stress can arise ,resulting in damage to cellular macromolecules such as proteins, lipids and DNA[59]. DNA is a particularly sensitive cellular target because of the potential to create cumulative mutations that can disrupt cellular homeostasis. In this case ,the reactive oxygen species can lead to the formation of single and double – strand breaks as well as induce chemical and structural modifications to purine and pyrimidine bases and also to 2-deoxyribose[60].

Yuan and Kaplowitz, (2009)[61] stated that drug overdose is currently the most frequent cause of acute liver failure, evaluation of the mechanisms of drug-induced liver injury indicates that mitochondria are critical targets for drug toxicity, either directly or indirectly through the formation of reactive metabolites. The consequence of these modifications is generally a mitochondrial oxidant stress and peroxynitrite formation, which leads to structural alterations of proteins and mitochondrial DNA and, eventually, to the opening of mitochondrial membrane permeability transition (MPT) pores. MPT pore formation results in a collapse of mitochondrial membrane potential and cessation of adenosine triphosphate synthesis. In addition, the release of intermembrane proteins, such as apoptosis-inducing factor and endonuclease G, and their translocation to the nucleus, leads to nuclear DNA fragmentation.

Conclusion: 10 mg/kg MDMA had minor effect on liver tissue, while 30 mg/kg MDMA significantly affected the liver tissue of male rats by induction of oxidative stress.

The pathogenesis of liver damage is not clear, however, it was suggested that an increase of ROS coupled with a decrease in body antioxidant system activity play an important role in the pathological changes, particularly in the cases of liver toxicity. Our findings pointed out the risk of increased lipid peroxidation, hepatic DNA damage and abnormality in serum protein pattern due to long term use of ecstasy. Rat Liver toxicity may cause disruption of normal cell membranes as a result of direct interaction of free radicals with cellular membranes or several endogenous protective mechanisms may limit ROS and the damage caused by them. However, this protection may be insufficient when the formation of ROS is excessive, additional protective mechanisms of dietary, antioxidants may help to maintain liver functions.

REFERENCES

1-Teixeira-Gomes, A., Costa, V. M., Feio-Azevedo, R., Duarte, J. A., Duarte-Araújo, M., Fernandes, E., ... & Capela, J. P. (2016): "Ecstasy" toxicity to adolescent rats following an acute low binge dose. BMC pharmacology and toxicology, 17 (1): 1.

2-Silva, D. D., Silva, E., Carvalho, F., & Carmo, H. (2014): Mixtures of 3, 4-methylenedioxymethamphetamine (ecstasy) and its major human metabolites act additively to induce significant toxicity to liver cells when combined at low, non-cytotoxic concentrations. Journal of Applied Toxicology, 34 (6): 618-627.

3-Downey, C., Daly, F., & O'Boyle, K. M. (2014): An in vitro approach to assessing a potential drug interaction between MDMA (ecstasy) and caffeine. Toxicology in Vitro, 28(2): 231-239.

4-Karami, M., Saeidnia, S., & Nosrati, A. (2013): Study of the hepatoprotective activity of methanolic extract of Feijoa sellowiana fruits against MDMA using the isolated rat liver perfusion system. Iranian journal of pharmaceutical research: IJPR, 12(1): 85.

5-Kelly, B. C., Wells, B. E., Pawson, M., LeClair, A., & Parsons, J. T. (2014):Combinations of prescription drug misuse and illicit drugs among young adults. Addictive behaviors, 39(5): 941-944.

6- Cerretani, D., Bello, S., Cantatore, S., Fiaschi, A. I., Montefrancesco, G., Neri, M., & Turillazzi, E. (2011): Acute administration of 3, 4-methylenedioxymethamphetamine (MDMA) induces oxidative stress, lipoperoxidation and TNFα-mediated apoptosis in rat liver. Pharmacological research, 64(5): 517-527.

7- Da Silva, D. D., Carmo, H., Lynch, A., & Silva, E. (2013): An insight into the hepatocellular death induced by amphetamines, individually and in combination: the involvement of necrosis and apoptosis. Archives of toxicology, 87(12): 2165-2185.

8- Hu, L., Li, L., Xu, D., Xia, X., Pi, R., Xu, D., ... & Song, Y. (2014):Protective effects of neohesperidin dihydrochalcone against carbon tetrachloride-induced oxidative damage in vivo and in vitro. Chemico-biological interactions, 213: 51-59.

9-Custódio, J. B., Santos, M. S., Gonçalves, D. I., Moreno, A. J., Fernandes, E., Bastos, M. L., ... & Fernandes, M. A. (2010): Comparative effects of 3, 4-methylenedioxymethamphetamine and 4-methylthioamphetamine on rat liver mitochondrial function. Toxicology, 270(2): 99-105.

10-Lewi Paul J; Marsboom and Robert P., (1981): Toxicology, Experimental, Rats as Laboratory Animals, Data Collection, Toxicology, Tables, Elsevier/North-Holland Biomedical Press, Amsterdam (ed.), 3.

11-Huff, C., Bhide, N., Schroering, A., Yamamoto, B. K., & Gudelsky, G. A., (2013): Effect of repeated exposure to MDMA on the function of the 5-HT

transporter as assessed by synaptosomal 5-HT uptake. Brain research bulletin, 91: 52-57.
12-Paget GE and and Barnes JM., (1964): Evaluation of Drug Activities and Pharmacometries. Editors: RR Laurence and Bacharach AL, Academic Press, London, (1): 135-166.
13-de Macédo, P., Marrano, C., & Keillor, J. W. (2000): A direct continuous spectrophotometric assay for transglutaminase activity. Analytical biochemistry, 285 (1): 16-20.
14-Henry RJ., (1974): Clinical Chemistry, In Principle and Techniques, Second Edition, New York, Harper & Row, (2): 422-431.
15-Woessner, J. F. (1961): The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Archives of biochemistry and biophysics, 93(2):440-447.
16-Beutler, E., Duron, O., & Kelly, B. M. (1963): Improved method for the determination of blood glutathione. The Journal of laboratory and clinical medicine, 61 (5): 882-890.
17- Masayasu, M., & Hiroshi, Y. (1979): A simplified assay method of superoxide dismutase activity for clinical use. Clinica Chimica Acta, 92 (3): 337-342.
18-Sinha AK., (1972):Colorimetric Assay of Catalase, Anal Biochem, 47 :389-394.
19- Yoshioka, T., Kawada, K., Shimada, T., & Mori, M. (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. American journal of obstetrics and gynecology, 135(3): 372-376.
20-Jiang ZK; Hunt JV and Wolf SP., (1992): Detection of Lipid Hydroperoides Using Fox Method, Anal Biochem., 202:384-389.
21-Rechnagel RO and Gglende EA.,(1984): Spectrophotometric Detection of Lipid Conjugated Dienes, Methods Enzymol., 105 :.331-337.
22-Nowak D; Pierscinski G and Drzewoski J., (1995): Ambroxol Inhibits Doxorubicin-Induced Lipidperoxidation in Heart of Mice, Free Radic Biol Med., 19 (5): 659-63.
23- Teitz NW., (1976): Fundamental of Clinical Chemistry,Chem Acta., 70 :602-609.
24-Reitman SandFrankel S., (1957): A colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases, Amer J Clin Path., 28: 56-63.
25-Szasz G., (1976): Reaction-Rate Method for Gamma Glutamyltransferase Activity in Serum, J Clin Chem., 22: 2051-2055.
26-Perry, B. W., Doumas, B. T., Bayse, D. D., Butler, T., Cohen, A., Fellows, W.,... & Louderback, A. (1983): A candidate reference method for determination of bilirubin in serum. Test for transferability. Clinical chemistry, 29(2):297-301.
27-Laemmli, UK., (1970): Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4, Nature, 227: 680-685.

28-Speit, G. and Hartmann, A., (2005): The Comet Assay: a Sensitive Genotoxicity Test for the Detection of DNA Damage, Methods Mol. Biol., 291: 85-95.
29- Collins AR., (2004):The Comet Assay for DNA Damage and Repair: Principles, Applications, and Limitations, Mol Biotechnol., 26 (3): 249-261.
30-Liechti M1., (2015): Novel Psychoactive Substances (Designer Dugs): Overview and Pharmacology of Modulators of Monoamine Signaling, Swiss Med Wkly., 14,145: w14043.
31-Cerretani D; Bello S; Cantatore S; Fiaschi AI; Montefrancesco G;Neri M, Pomara C; Riezzo I; Fiore C; Bonsignore A; Turillazzi E and Fineschi V.,(2011): Acute Administration of 3,4-Methylenedioxymethamphetamine (MDMA) Induces Oxidative Stress, Lipoperoxidation and TNFα-Mediated Apoptosis in Rat Liver, Pharmacol Res., 64 (5): 517-27.
32- Silva, D. D., Silva, E., & Carmo, H. (2014): Combination effects of amphetamines under hyperthermia-the role played by oxidative stress. Journal of Applied Toxicology, 34 (6): 637-650.
33-Zhang, J., Antonyak, M. A., Singh, G., & Cerione, R. A. (2013): A mechanism for the upregulation of EGF receptor levels in glioblastomas. Cell reports, 3 (6): 2008-2020.
34-D'Argenio, G., Amoruso, D. C., Mazzone, G., Vitaglione, P., Romano, A., Ribecco, M. T., ... & Caporaso, N. (2010): Garlic extract prevents CCl 4-induced liver fibrosis in rats: the role of tissue transglutaminase. Digestive and Liver Disease, 42 (8): 571-577.
35-Tatsukawa, H., Fukaya, Y., Frampton, G., Martinez-Fuentes, A., Suzuki, K., Kuo, T. F., ... & Iismaa, S. (2009): Role of transglutaminase 2 in liver injury via cross-linking and silencing of transcription factor Sp1. Gastroenterology, 136 (5): 1783-1795.
36- Kojima, S., Kuo, T. F., Tatsukawa, H., & Hirose, S. (2011): Induction of cross-linking and silencing of Sp1 by transglutaminase during liver injury in ASH and NASH via different ER stress pathways. Digestive Diseases, 28 (6): 715-721.
37-Huang, C. S., Lee, Y. R., Chen, C. S., Tu, S. H., Wang, Y. J., Lee, C. H.,... & Wu, C. H. (2010): Long-term ethanol exposure causes human liver cancer cells to become resistant to mitomycin C treatment through the inactivation of bad-mediated apoptosis. Molecular carcinogenesis, 49 (8): 728-738.
38-Singla, A., Griggs, N. W., Kwan, R., Snider, N. T., Maitra, D., Ernst, S. A.,... & Omary, M. B. (2013): Lamin aggregation is an early sensor of porphyria-induced liver injury. J Cell Sci, 126 (14): 3105-3112.
39- Elli, L., Bergamini, C. M., Bardella, M. T., & Schuppan, D. (2009): Transglutaminases in inflammation and fibrosis of the gastrointestinal tract and the liver. Digestive and Liver Disease, 41(8):541-550.
40-Sekhar, R. V., Patel, S. G., Guthikonda, A. P., Reid, M., Balasubramanyam, A., Taffet, G. E., & Jahoor, F. (2011): Deficient synthesis of glutathione underlies

oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation. The American journal of clinical nutrition, 94 (3): 847-853.
41-Liu, R. M., & Pravia, K. G. (2010): Oxidative stress and glutathione in TGF-β-mediated fibrogenesis. Free Radical Biology and Medicine, 48 (1): 1-15.
42-Auchère, F., Santos, R., Planamente, S., Lesuisse, E., & Camadro, J. M. (2008): Glutathione-dependent redox status of frataxin-deficient cells in a yeast model of Friedreich's ataxia. Human molecular genetics, 17 (18): 2790-280 2.
43-Kiang, T. K., Teng, X. W., Surendradoss, J., Karagiozov, S., Abbott, F. S., & Chang, T. K. (2011): Glutathione depletion by valproic acid in sandwich-cultured rat hepatocytes: role of biotransformation and temporal relationship with onset of toxicity. Toxicology and applied pharmacology, 252 (3): 318-324.
44-Goni-Allo, B., Mathúna, B. Ó., Segura, M., Puerta, E., Lasheras, B., de la Torre, R., & Aguirre, N. (2008). The relationship between core body temperature and 3, 4-methylenedioxymethamphetamine metabolism in rats: implications for neurotoxicity. Psychopharmacology, 197 (2): 263-278.
45-Hfaiedh, N., Murat, J. C., & Elfeki, A. (2012): A combination of ascorbic acid and α-tocopherol or a combination of Mg and Zn are both able to reduce the adverse effects of lindane-poisoning on rat brain and liver. Journal of Trace Elements in Medicine and Biology, 26 (4): 273-278.
46-Padurariu, M., Ciobica, A., Dobrin, I., & Stefanescu, C. (2010): Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. Neuroscience letters, 479 (3): 317-320.
46-Nazia Uzma1; B. Santhosh Kumarand and Syeda Anees., Red Wine Ameliorates CCl4 – Induced Acute Liver Injury in Rats, AJBS .,1 (1), 2011, pp. 1-7.
47-Kerman, M., & Senol, N. (2012): Oxidative stress in hippocampus induced by 900 MHz electromagnetic field emitting mobile phone: Protection by melatonin. Biomedical Research, 23 (1): 147-151.
48-Johnkennedy, N., Adamma, E., Austin, A., & Chukwunyere, N. E. (2010): Alterations In Biochemical Parameters Of Wister Rats Administered With Sulfadoxine And Pyrimethamine (Fansidar R). Al Ameen J Med Sci, 3 (4): 317-321.
49-Rajesh, M. G., & Latha, M. S. (2004): Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. Journal of Ethnopharmacology, 91 (1): 99-104.
50-reejith, G., Jayasree, M., Latha, P. G., Suja, S. R., Shyamal, S., Shine, V. J., ... & Vilash, V. (2014): Hepatoprotective activity of Oxalis corniculata L. ethanolic extract against paracetamol induced hepatotoxicity in Wistar rats and its in vitro antioxidant effects. Indian J Exp Biol., 52 (2): 147-52.
51-Bhadauria, M., & Nirala, S. K. (2009): Reversal of acetaminophen induced subchronic hepatorenal injury by propolis extract in rats. Environmental toxicology and pharmacology, 27 (1): 17-25.

52-Yuan, H. D., Jin, G. Z., & Piao, G. C. (2010): Hepatoprotective effects of an active part from Artemisia sacrorum Ledeb. against acetaminophen-induced toxicity in mice. Journal of ethnopharmacology, 127 (2): 528-533.
53-Abraham, P. (2005):Oxidative stress in paracetamol induced pathogenesis:(I) renal damage. Indian J Biochem Biophys, 42 (1): 59-62.
54-Berlett, B. S., & Stadtman, E. R. (1997): Protein oxidation in aging, disease, and oxidative stress. Journal of Biological Chemistry, 272 (33): 20313-20316.
55-Shinall, H., Song, E. S., & Hersh, L. B. (2005): Susceptibility of amyloid β peptide degrading enzymes to oxidative damage: a potential Alzheimer's disease spiral. Biochemistry, 44 (46): 15345-15350.
56-Dunlop, R. A., Brunk, U. T., & Rodgers, K. J. (2009): Oxidized proteins: mechanisms of removal and consequences of accumulation. IUBMB life, 61 (5): 522-527.
57-Bedwell, S., Dean, R. T., & Jessup, W. (1989): The action of defined oxygen-centred free radicals on human low-density lipoprotein. Biochemical Journal, 262 (3): 707-712.
58-Silva, D. D., Silva, E., & Carmo, H. (2014): Combination effects of amphetamines under hyperthermia-the role played by oxidative stress. Journal of Applied Toxicology, 34 (6): 637-650.
59-Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007):Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology, 39 (1): 44-84.
60-Powell, C. L., Swenberg, J. A., & Rusyn, I. (2005): Expression of base excision DNA repair genes as a biomarker of oxidative DNA damage. Cancer letters, 229 (1): 1-11.
61-Yuan, L. & Kaplowitz, N. (2009): Glutathione in liver diseases and hepatotoxicity. Molecular aspects of medicine, 30 (1): 29-41.

ملخص

تسمم الكبد الناتج عن تعاطى الاكستاسى

نادية جمال زكى
ليلى عبد القوى

الاكستاسى هو أحد أنواع المنشطات المشتقة من الأمفيتامين، وهو من المواد المؤثرة على الحالة النفسية التى تستخدم بكثرة بين أوساط الشباب. ويعتبر الكبد هدفا سهلًا للتسمم بالمنشطات، ولكن تبقى الآليات التى تشارك فى تسمم الكبد غير مفهومة. ولهذا السبب، كان من الأهمية أن ننبه إلى خطورة إدمان وتعاطى هذه المواد على أنسجه الكبد، ونتعرف على الآليات التى تشارك بها فى تسمم الكبد. لذلك فقد تناولت هذه الدراسة تأثير تعاطى كلا من الجرعات المنخفضة والجرعات العالية (١٠ و ٣٠ مج/ كج) من الاكستاسى عن طريق الفم يوميًا لمده ٤٥ يومًا على وظائف وأنسجة الكبد فى ذكور الجرذان.

ثم حقن ذكور الجرذان يوميًا عن طريق الفم (بصفر مج/ كج) (المجموعة الضابطة) و ١٠ و ٣٠ مج/ كج (المجموعة المعالجة) لمده ١٥ و٣٠ و٤٥ يومًا متتالية، تلاه فترة انسحاب ١٥ يومًا. وتم بعد ذلك تقييم الاختبارات الكيموحيوية فى مصل الدم والكبد.

أجريت التجارب على عدد ١٠٠ من ذكور الجرذان البيضاء تزن حوالى (١٠٠-١٥٠ مج)، تم تقسيمها إلى ثلاث مجموعات: كل مجموعة ٢٠ جرذًا، مجموعة ضابطة، ومجموعتين للمعالجة تم حقنها بـ ١٠، ٣٠ مج/ كجم من وزن الجسم من الاكستاسى (الجرعة المزمنة) من خلال أنبوب تغذية عن طريق الفم لمدة ١٥ و ٣٠ و ٤٥ يومًا؛ وأعقب فترة ٤٥ يومًا من إعطاء العقار ١٥ يومًا من الانسحاب.

ذبحت جميع الحيوانات بعد ٣٠ دقيقة من العلاج وتم عمل بعض القياسات الكيموحيوية والنسيجية فى مصل الدم والكبد.