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The potential effect of octreotide on liver fibrosis induced by alcohol in albino rats

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Abstract: **Fibrosis is an excessive wound healing response to chronic liver injury characterized by the accumulation of an extracellular matrix (ECM) rich in fibrillar collagens, mainly type I and III collagen. Activated hepatic stellate cell (HSC) is the main fibrogenic cell. HSC activation is crucial in liver fibrogenesis. Although liver fibrosis is reversible, cirrhosis and HCC are generally irreversible Stress response is known as behavioral and metabolic changes to maintain body homeostasis that is caused by internal or external sources like physical or psychological stimuli known as stressors. Animal immobilization or restraint stress is known to be an applicable, easy, and convenient model to induce both psychological and physical stress.**

Aim of the Work: **To assess the potential effect of octreotide on liver fibrosis induced by alcohol in albino rats and observe the effects of octreotide on progression of liver fibrosis induced by alcohol in comparison with silymarin as a standard hepato-protective effect.**

Patients and Methods: **This was an experimental study was conducted in physiology department of faculty of medicine. Rats were classified into 4 groups: Group (1) (control): rats were received saline. Group (2) (ethanol): rats were received ethanol 1 ml/100 g orally twice a week for 8 weeks. Group (3) (octreotide): rats were hypodermically injected with octreotide diluted with saline (10 µg/kg) twice a day plus ethanol for 8 weeks. Group (4) (silymarin): rats were received silymarin (100 mg/kg/day) orally plus ethanol for 8 weeks. The duration of the study ranged from 6- 12 months.**

Results: **There was a statistically significant difference between groups at baseline and at the end as regard ALT. There was a statistically significant difference between groups at baseline and at the end as regard AST. There was a statistically significant difference between groups at baseline and at the end as regard alkaline phosphatase. There were statistically significant differences between groups at baseline and at the end as regard Gamma-glutamyl transferase. There were statistically significant differences between groups at the end as regard bilirubin.**

Conclusion: **Suggest that Octreotide could efficiently protect rats against stress-induced hepatic changes which mayaccompany liver fibrosis induced by alcohol.**

Keywords: **Hepatic stellate cell; Extracellular matrix; SSTRs; (TGF)-β.**

1. INTRODUCTION

Alcohol is a dietary component which is usually consumed for its psychophysical and mood-altering effects. Long-term alcohol consumption may cause damage to vital organs including cardio-vascular, endocrine, gastrointestinal, and central nervous systems (**Rachdaoui, 2017**).

According to the World Health Organization report of 2005, approximately 2 billion people world-wide consume alcohol, and about 76 million of them have been estimated to be suffering from alcohol consumption disorders. The most extensively investigated aspect of ethanol on health is alcoholic liver disease (ALD), which is one of the major causes of

illness and death worldwide **(Liu et al, 2010)**. ALD morphological features include fatty liver (steatosis), hepatitis, and alcoholic fibrosis and cirrhosis **(Smathers et al, 2011)**.

The mechanisms contributing to the pathogenesis of ALD include direct hepatotoxicity, production of reactive oxygen species (ROS) induced by ethanol and its metabolites, activation of innate immunity and complement system with subsequent production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) **(Breitkopf et al, 2009)**.

Fibrosis is an excessive wound healing response to chronic liver injury characterized by the accumulation of an extracellular matrix (ECM) rich in fibrillar collagens, mainly type I and III collagen. Activated hepatic stellate cell (HSC) is the main fibrogenic cell. HSC activation is crucial in liver fibrogenesis **(Hernandez-Gea and Friedman, 2011)**. Although liver fibrosis is reversible, cirrhosis and HCC are generally irreversible **(Hernandez-Gea and Friedman, 2011)**.

Therefore, one of the main therapeutic strategies may be preventing the progression of steatosis to fibrosis and other advanced hepatic lesions. Alcoholic liver fibrosis results from oxidation of ethanol to the highly reactive compound acetaldehyde, which is the main metabolite of alcohol as it stimulates HSC activation to produce several ECM components, including type I collagen **(Altamirano, 2011; Ceni et al, 2014)**.

As response to acetaldehyde-induced stimulation, HSCs change from quiescent vitamin A-storing cells to activated myofibroblast- like cells, which proliferate and become fibrogenic **(Luo et al, 2013)**. Somatostatin is secreted in two biologically active forms: a 14 amino acid peptide (somatostatin14) and a 28 amino acid peptide (somatostatin-28). Both forms bind with high affinity to all five receptor subtypes **(Hankus, 2016)**. The widely used synthetic analog, octreotide, binds with high affinity only to somatostatin receptors (SSTRs) subtypes 2 and 5 and with lower affinity to sst3. In earlier studies we have shown that octreotide profoundly affects the production of pro-inflammatory cytokines, pro and anti-fibrotic agents by rat Kupffer cells ***(Tsagarakis, 2011).*** Previous studies have demonstrated that somatostatin exerted its inhibitive action on activated HSCs by means of somatostatin receptors subtypes, especially SSTR2 ***(Aziz et al, 2018).*** Meanwhile, a plethora of studies demonstrated that octreotide could alleviate liver fibrosis through the inhibitory effect on the activation of HSCs by down-regulating the expression of transforming growth factor (TGF)-β **(Wang et al, 2013; Zhang et al, 2018).**

However, further mechanisms underlying the protective effect of octreotide on alcoholic liver fibrosis remain to be explored. Therefore, the present study aims to investigate the effects of octreotide on the progression of alcoholic liver fibrosis and to evaluate inflammation, HSC activation and in the mechanism of anti-fibrotic effect of octreotide.

AIM OF THE WORK

To assess the potential effects of octreotide on liver fibrosis induced by alcohol in albino rats and observe the effects of octreotide on progression of liver fibrosis induced by alcohol in comparison with silymarin as a standard hepat oprotective effect.

2. PATIENTS AND METHODS

Study setting: Study was conducted in physiology department of faculty of medicine AL. Azhar university.

Sample size: The sample size was calculated according to these values produced a minimal sample size of 28 rats were enough to find such a difference. Assuming a drop-out ratio, the sample size will be 32 rats, subdivided into four groups, 8 rats in each group. Based on MedCalc® version 12.3.0.0 program & quot; Ostend, Belgium & amp; quot; was used for calculations of sample size, statistical calculator based on 95% confidence interval and power of the study 80% with α error 5%, According to a previous study ***(Sayed et al, 2017)*** showed that the results indicated that DEC, when combined with HDN, blunted EtOH-induced necroinflammation and elevation of liver injury parameters in serum. Besides, attenuated EtOH- induced liver fibrosis, demonstrated by hepatic histopathology scoring and 4- hydroxyproline content. The mechanisms behind these beneficial effects of both DEC and HDN were also elucidated. So it can be relied upon in this study, based on this assumption,

Study design: Prospective study.

Experimental animals: Experiment was done on 32 healthy adult male Sprague rats weighting 100-150 grams. Rats had been housed in fully ventilated cages (8 per cge) with sawdust bedding material, the size of the cage was (60 cm length * 60 cm width *2.5 cm height) maintained under standard condition a 12:12 h light and dark cycle with room temperature around 22–24°C, humidity from 60-65% with free access to diet and purified drinking water. Acclimiatization was done for 10-15 days to accommodate for the new surrounding media. All of the procedures regarding the care and use of animals experimentation in this study were complied with the guide for the care and use of laboratory animals published by the US National Institutes of Health.

Drugs and chemicals:

- Ethanol (from AL-Gomhoreya for Chemicals Co. (Cairo, Egypt))
- Octreotide (from Shanghai TASH Biotechnology Co., Ltd. Shanghai, china)
- Silymarin (Purchased from Bulk Sigma (St. Louis, MO).

Experimental Design:

1. **Group 1 (control):** rats had been receiving saline and don't receive any medications.

Induction of liver fibrosis by Ethanol: To induce liver fibrosis, rats will receive 1 ml/ 100 g ethanol (25%, vol. /vol.) orally twice a week for 8 weeks according to the method of Safer et al, 2015.

2. **Group 2 (ethanol):** rats had been receiving ethanol 1 ml/100 g orally twice a week for 8 weeks.

3. **Group 3 (octreotide):** rats had been receiving hypodermically injected with octreotide diluted with saline (10 µg/kg) twice a day plus ethanol for 8 weeks ***(Zhang et al, 2018).***

4. **Group 4 (silymarin):** rats had been receiving silymarin (100 mg/kg/day) orally plus ethanol for 8 weeks ***(Soto et al., 1998).***

Parameters: At the end of experimental period (8 weeks), the following measurements had been done:

1. **Body weight.**

2. **Liver enzymes such as alanine transaminase (ALT):** Serum alanine aminotransferase was determined according to the method of ***Bergmeyer et al. (1986).***

3. **Aspartate transaminase (AST):** Serum aspartate aminotransferase was determined according to the method described by ***Bergmeyer et al. (1986)***.

4. **Alkaline phosphate (ALP):** Serum akaline phosphatase was determined according to the method of ***Tietz et al. (1983)***.

5. **Nitrophenylphosphate 4-Nitrophenoxide+Phosphate+H2O**

6. **Gamma Glutamyl transferase (GGT):** Determination of serum γ-glutamyl transpeptidase activity γ-glutamyl transpeptidase was determined according to the method of ***Shaw et al, (1983).***

7. **Serum total bilirubin (TBIL):** Determination of serum total bilirubin concentration: Total bilirubin was determined according to the method of***Pearlman and Lee, (1974).***

8. **Determination of hepatic lipid peroxides contents (measured as malondialdehyde):** Malondialdehyde was determined according to the method of***Yoshioka et al, (1979).***

9. **Total cholesterol:** Total cholesterol was determined according to the method of ***Trinder et al, (1984).***

10. **Serum Triglycerides:** triglycerideses were determined according to the method of***McGowan et al. (1983).***

11. **Estimation of biomarkers of oxidative stress:** a) Catalase: Catalase was determined according to the method of ***Aebi (1984).*** b) Superoxide Dismutase (SOD): SOD was determined according to the method ***Nishikimi et al., (1972).***

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12. Rats had been sacrificed.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

The used tests were

1. **Chi-square test:** For categorical variables, to compare between different groups
2. **Fisher's Exact or Monte Carlo correction:** Correction for chi-square when more than 20% of the cells have expected count less than 5
3. **F-test (ANOVA):** For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons
4. **Paired t-test:** For normally distributed quantitative variables, to compare between two periods.
5. **Kruskal Wallis test:** For abnormally distributed quantitative variables, to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons.
6. **Wilcoxon signed ranks test:** For abnormally distributed quantitative variables, to compare between two periods.

3. RESULTS

Table (1): Comparison between four groups as regard to rat'sALT

ALT (IU/l)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p Value
Before					
Min.–Max.	45.0 – 52.0	45.0 – 51.0	43.0 – 55.0	43.0 – 55.0	**0.351**
Mean ± SD.	48.88 ± 2.70	48.25 ± 2.25	50.25 ± 4.77	51.25 ± 4.03	
Median	49.50	49.0	51.50	52.0	
p1		**0.985**	**0.868**	**0.555**	
p2			**0.683**	**0.355**	
p3				**0.944**	
After					
Min.–Max.	45.0 – 52.0	278.0 – 412.0	119.0 – 175.0	189.0 – 243.0	**<0.001***
Mean ± SD.	48.88 ± 2.70	355.63±53.04	147.0 ± 19.06	209.75±16.13	
Median	49.50	364.0	144.50	210.0	
p1		**<0.001***	**<0.001***	**<0.001***	
p2			**<0.001***	**<0.001***	
p3				**0.001***	
Increase					
Mean ± SD.	0.0 ± 0.0	307.38±52.76	96.75 ± 19.05	158.50±16.66	
p0	–	**<0.001***	**<0.001***	**<0.001***	

p0: p value for **Paired t-test** for comparing between **before** and **after** in each group p: p value for **ANOVA test** for comparing between **the four studied groups.** p1: p value for **ANOVA test** for comparing between **group 1** and **other groups** p2: p value for **ANOVA test** for comparing between **group 2** and **other groups** p3: p value for **ANOVA test** for comparing between **group 3** and **group 4.** *: Statistically significant at $p \le 0.05$ Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

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Table (2): Comparison between four groups as regard to rat's AST

AST (IU/l)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p Value
Before					
Min.–Max.	86.0 – 133.0	70.0 – 114.0	77.0 – 102.0	79.0 – 98.0	**0.002***
Mean ± SD.	106.38±15.22	85.88 ± 14.78	84.50 ± 8.37	85.50 ± 6.35	
Median	**101.0**	**80.50**	**82.0**	**84.50**	
p1		**0.009***	**0.005***	**0.008***	
p2			**0.995**	**1.000**	
p3				**0.998**	
After					
Min.–Max.	86.0 – 133.0	300.0 – 420.0	110.0 – 170.0	180.0 – 240.0	**<0.001***
Mean ± SD.	106.38±15.22	383.38±42.73	142.88±20.24	208.0 ± 17.82	
Median	**101.0**	**402.50**	**142.50**	**210.0**	
p1		**<0.001***	**0.046***	**<0.001***	
p2			**<0.001***	**<0.001***	
p3				**<0.001***	
Increase					
Mean ± SD.	0.0 ± 0.0	297.50±42.66	58.38 ± 17.10	122.50±17.28	
p0	–	**<0.001***	**<0.001***	**<0.001***	

p0: p value for **Paired t-test** for comparing between **before** and **after** in each group p: p value for **ANOVA test** for comparing between **the four studied groups.** p1: p value for **ANOVA test** for comparing between **group 1** and **other groups** p2: p value for **ANOVA test** for comparing between **group 2** and **other groups** p3: p value for **ANOVA test** for comparing between **group 3** and **group 4.** *: Statistically significant at $p \le 0.05$ Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

Table (3): Comparison between four groups as regard torat's TGs

TGs (mg /dl)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p Value
Before					
Min.–Max.	22.0 – 65.0	32.0 – 69.0	39.0 – 71.0	32.0 – 62.0	**0.929**
Mean ± SD.	49.38 ± 15.47	48.0 ± 13.17	51.13 ± 10.53	47.13 ± 11.39	
Median	54.50	42.50	50.0	44.50	
p1		**0.996**	**0.993**	**0.985**	
p2			**0.961**	**0.999**	
p3				**0.923**	
After					
Min.–Max.	22.0 – 65.0	159.0 – 211.0	100.0 – 130.0	114.0 – 144.0	**<0.001***
Mean ± SD.	49.38 ± 15.47	188.75±16.69	110.12±10.99	124.50 ± 9.58	
Median	54.50	194.0	107.0	122.50	
p1		**<0.001***	**<0.001***	**<0.001***	
p2			**<0.001***	**<0.001***	
p3				**0.169**	
Increase					
Mean ± SD.	0.0 ± 0.0	140.75±15.42	59.0 ± 12.14	77.38 ± 13.75	
p0	–	**<0.001***	**<0.001***	**<0.001***	

p0: p value for **Paired t-test** for comparing between **before** and **after** in each group p: p value for **ANOVA test** for comparing between **the four studied groups.** p1: p value for **ANOVA test** for comparing between **group 1** and **other groups** p2: p value for **ANOVA test** for comparing between **group 2** and **other groups** p3: p value for **ANOVA test** for comparing between **group 3** and **group 4.** *: Statistically significant at $p \le 0.05$ Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

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Table (4): Comparison between four groups as regard torat's GGT

GGT (IU/L)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p Value
Before					
Min.–Max.	2.10 – 3.70	2.79 – 3.06	2.45 – 3.20	2.78 – 3.49	**0.620**
Mean ± SD.	2.99 ± 0.49	2.92 ± 0.11	2.94 ± 0.25	3.11 ± 0.25	
Median	3.02	2.94	2.96	3.04	
p1		**0.969**	**0.982**	**0.876**	
p2			**1.000**	**0.634**	
p3				**0.678**	
After					
Min.–Max.	2.10 – 3.70	107.0 – 135.0	34.0 – 69.0	70.90 – 99.0	**<0.001***
Mean ± SD.	2.99 ± 0.49	123.0 ± 9.53	52.89 ± 11.41	85.13 ± 8.66	
Median	3.02	123.0	50.50	85.25	
p1		**<0.001***	**<0.001***	**<0.001***	
p2			**<0.001***	**<0.001***	
p3				**<0.001***	
Increase					
Mean ± SD.	0.0 ± 0.0	120.08 ± 9.50	49.95 ± 11.54	82.02 ± 8.62	
p0	–	**<0.001***	**<0.001***	**<0.001***	

p0: p value for **Paired t-test** for comparing between **before** and **after** in each group p: p value for **ANOVA test** for comparing between **the four studied groups.** p1: p value for **ANOVA test** for comparing between **group 1** and **other groups** p2: p value for **ANOVA test** for comparing between **group 2** and **other groups** p3: p value for **ANOVA test** for comparing between **group 3** and **group 4.** *: Statistically significant at $p \le 0.05$ Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

Table (5): Comparison between four groups as regard to rat'sMDA

MDA (nmol/L)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p Value
Before					
Min.–Max.	8.20 – 11.0	8.70 – 10.90	8.50 – 10.20	8.60 – 10.20	**0.462**
Mean ± SD.	9.63 ± 1.01	9.94 ± 0.80	9.40 ± 0.69	9.36 ± 0.62	
Median	9.80	10.05	9.50	9.25	
p1		**0.860**	**0.941**	**0.911**	
p2			**0.540**	**0.483**	
p3				**1.000**	
After					
Min.–Max.	8.20 – 11.0	27.70 – 34.20	15.10 – 18.20	19.30 – 22.30	**<0.001***
Mean ± SD.	9.63 ± 1.01	30.89 ± 2.30	16.64 ± 0.94	20.84 ± 1.11	
Median	9.80	30.65	16.75	20.75	
p1		**<0.001***	**<0.001***	**<0.001***	
p2			**<0.001***	**<0.001***	
p3				**<0.001***	
Increase					
Mean ± SD.	0.0 ± 0.0	20.95 ± 2.97	7.24 ± 0.91	11.48 ± 1.29	
p0	–	**<0.001***	**<0.001***	**<0.001***	

p0: p value for **Paired t-test** for comparing between **before** and **after** in each group p: p value for **ANOVA test** for comparing between **the four studied groups.** p1: p value for **ANOVA test** for comparing between **group 1** and **other**

groupsp2: p value for **ANOVA test** for comparing between **group 2** and **other groups**p3: p value for **ANOVA test** for comparing between **group 3** and **group 4.** *: Statistically significant at $p \leq 0.05$ Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

Table (6): Comparison between four groups as regard torat's catalase

Catalase(nmol/L)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p Value
Before					
Min.–Max.	22.0 – 28.0	23.0 – 29.0	22.0 – 32.0	21.0 – 30.0	**0.629**
Mean ± SD.	25.0 ± 1.77	26.25 ± 2.12	26.88 ± 3.80	26.0 ± 3.34	
Median	25.0	26.50	28.0	26.0	
p1		**0.821**	**0.570**	**0.898**	
p2			**0.972**	**0.998**	
p3				**0.929**	
After					
Min.–Max.	22.0 – 28.0	8.0 – 11.0	19.0 – 22.0	14.0 – 18.0	**<0.001***
Mean ± SD.	25.0 ± 1.77	9.88 ± 1.13	20.63 ± 1.19	16.13 ± 1.25	
Median	25.0	10.0	21.0	16.0	
p1		**<0.001***	**<0.001***	**<0.001***	
p2			**<0.001***	**<0.001***	
p3				**<0.001***	
Decrease					
Mean ± SD.	0.0 ± 0.0	16.38 ± 2.26	6.25 ± 4.37	9.88 ± 4.09	
p0	–	**<0.001***	**0.005***	**<0.001***	

p0: p value for **Paired t-test** for comparing between **before** and **after** in each group p: p value for **ANOVA test** for comparing between **the four studied groups.** p1: p value for **ANOVA test** for comparing between **group 1** and **other groups**p2: p value for **ANOVA test** for comparing between **group 2** and **other groups**p3: p value for **ANOVA test** for comparing between **group 3** and **group 4.** *: Statistically significant at $p \leq 0.05$ Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

Table (7): Comparison between four groups as regard torat's bilirubin

Bilirubin(mgl/dL)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p Value
Before					
Min.–Max.	0.30 – 0.70	0.20 – 0.70	0.20 – 0.70	0.20 – 0.70	**0.620**
Mean ± SD.	0.48 ± 0.15	0.39 ± 0.17	0.39 ± 0.20	0.46 ± 0.17	
Median	0.45	0.35	0.35	0.45	
p1		**0.742**	**0.742**	**0.999**	
p2			**1.000**	**0.820**	
p3				**0.820**	
After					
Min.–Max.	0.30 – 0.70	3.10 – 4.40	1.40 – 2.40	2.40 – 3.20	**<0.001***
Mean ± SD.	0.48 ± 0.15	3.94 ± 0.40	1.99 ± 0.32	2.88 ± 0.27	
Median	0.45	4.05	1.95	2.90	
p1		**<0.001***	**<0.001***	**<0.001***	
p2			**<0.001***	**<0.001***	
p3				**<0.001***	
Increase					
Mean ± SD.	0.0 ± 0.0	3.55 ± 0.50	1.60 ± 0.44	2.41 ± 0.33	
p0	–	**<0.001***	**<0.001***	**<0.001***	

p0: p value for **Paired t-test** for comparing between **before** and **after** in each group p: p value for **ANOVA test** for comparing between **the four studied groups.** p1: p value for **ANOVA test** for comparing between **group 1** and **other groups** p2: p value for **ANOVA test** for comparing between **group 2** and **other groups** p3: p value for **ANOVA test** for comparing between **group 3** and **group 4.** *: Statistically significant at p ≤ 0.05 Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

Table (8): Comparison between four groups as regard torat's weight

Weight (gm)	Group (1)(n=8)	Group (2)(n=8)	Group (3)(n=8)	Group (4)(n=8)	p value
Baseline					
Min.–Max.	120.0 – 134.0	129.0 – 177.0	133.0 – 160.0	130.0 – 156.0	**0.002***
Mean ± SD.	125.12 ± 5.17	146.0 ± 18.31	143.63 ± 9.87	143.75 ±10.07	
Median	123.0	137.50	141.50	147.50	
p1		**0.003***	**0.001***	**0.002***	
p2			**0.852**	**0.894**	
p3				**0.957**	
31 – 10 weeks	119.88 ± 0.99	115.63 ± 6.16	118.63 ± 1.60	119.25 ± 1.28	0.095
7 – 11 weeks	137.0 ± 6.26	129.0 ± 4.0	130.0 ± 2.0	136.13 ± 3.48	0.002*
14 – 11 weeks	155.50 ± 7.07	147.25 ± 6.18	140.75 ± 1.04	150.75 ± 4.53	0.001*
21 – 11 weeks	175.13 ± 8.54	165.63 ± 5.80	152.88 ± 3.80	167.75 ± 6.45	<0.001*
28 – 11 weeks	193.0 ± 11.24	184.88 ± 6.60	169.63 ± 3.25	183.50 ± 5.95	<0.001*
5 – 12 weeks	209.13±12.83	202.88 ± 5.33	180.38 ± 8.37	197.38 ± 4.87	<0.001*
12 – 12 weeks	223.0 ± 10.74	222.13 ± 3.64	188.25 ± 7.98	213.0 ± 3.89	<0.001*
19– 12 weeks	236.38±10.35	244.88 ± 4.97	204.0 ± 10.14	227.13 ± 3.83	<0.001*
26 – 12 weeks					
Min.–Max.	230.0 – 259.0	253.0 ± 263.0	204.0 – 237.0	239.0 – 251.0	<0.001*
Mean ± SD.	249.25 ± 9.07	258.37 ± 3.50	221.88±11.69	244.25 ± 4.74	
Median	249.50	258.50	224.0	244.0	
p1		0.080	0.002*	0.330	
p2			<0.001*	0.006*	
p3				0.036*	
Increase (26 – 12 weeks, Baseline)					
Mean ± SD.	124.13±13.80	112.38±17.05	78.25 ± 6.50	100.50±11.58	
p0	**0.012***	**0.012***	**0.012***	**0.012***	

p0: p value for **Wilcoxon signed ranks test** for comparing between **Baseline** and **26 – 12 weeks** in each group. p: p value for **Kruskal Wallis test** for comparing between **the four studied groups** p1: p value for **Kruskal Wallis test** for comparing between **group 1** and **other groups** p2: p value for **Kruskal Wallis test** for comparing between **group 2** and **other groups** p3: p value for **Kruskal Wallis test** for comparing between **group 3** and **group 4.** *: Statistically significant at p ≤ 0.05 Group 1: Control Group 2: Ethanol Group 3: Octreotid Group 4:Silymarin

4. DISCUSSION

The prevalence of alcoholic liver disease (ALD) is difficult to define because it is influenced by many factors including genetic (eg, predilection to alcohol abuse, gender) and environmental (eg, availability of alcohol, social acceptability of alcohol use, concomitant hepatotoxic insults) factors. In the United States, it is estimated that 67.3% of the population consumes alcohol and that 7.4% of the population meets the criteria for alcohol abuse. The use of alcohol varies widely throughout the world with the highest use in the U.S. and Europe. Men are more likely to develop ALD than women

because men consume more alcohol. However, women are more susceptible to alcohol hepatotoxicity and have twice the relative risk of ALD and cirrhosis compared with men. Elevated body mass index is also a risk factor in ALD as well as nonalcoholic fatty liver disease (**Chacko & Reinus, 2016**).

Ethnicity and genetics are important factors related to ALD. Cirrhosis mortality is higher in men of Hispanic, Native Americans, and native Alaskans origin compared with white populations. Genetic factors such as the 1148M substitution of the patatin-like phospholipase domain containing 3 (PNPLA3) gene appear to be associated with a more severe fatty liver and a poor prognosis (**Trépo et al, 2016).**

Somatostatin is a phylogenetically ancient, multigene family of peptides with two bioactive products, somatostatin-14 and somatostatin-28, acting as both neurotransmitters and neurohormones. Octreotide, the first synthetic somatostatin analogue introduced for clinical use, has been used for the treatment of secreting pituitary adenomas, metastatic islet-cell and carcinoid tumors, somatostatin receptor-positive neuroendocrine tumors and adenocarcinomas, and refractory diarrhea syndromes. Moreover, octreotide has been regularly used in cirrhotic patients, either for the arrest of esophageal bleeding or for the treatment of hepatocellular carcinoma, and might be useful as a long-term administration for the prevention of variceal bleeding (**Samonakis et al, 2002**).

The main aim of this study was to assess the potential effect of octreotide on liver fibrosis induced by alcohol in albino rats and observe the effects of octreotide on progression of liver fibrosis induced by alcohol in comparison with silymarin as a standard hepatoprotective effect.

An experimental study was conducted in physiology department of faculty of medicine. Rats were classified into 4 groups: Group (1) (control): rats were received saline. Group (2) (ethanol): rats were received ethanol 1 ml/100 g orally twice a week for 8 weeks. Group (3) (octreotide): rats were hypodermically injected with octreotide diluted with saline (10 µg/kg) twice a day plus ethanol for 8 weeks. Group (4) (silymarin): rats were received silymarin (100 mg/kg/day) orally plus ethanol for 8 weeks. The duration of the study was 8 weeks.

The main results of this study were as following:

As regard ALT, there was very high statistically significant increasing in value in group (2) followed by group (4) then group (3) while group (1) show increasing but without statistically significant differences. There were statistically significant differences between groups at baseline and at the end. As regard AST, there was very high statistically significant increasing in value in group (2) followed by group (4) then group (3) while group (1) show increasing but without statistically significant differences. There were statistically significant differences between groups at baseline and at the end. As regard ALP, there was very high statistically significant increasing in value in group (2) followed by group (4) then group (3) while group (1) show increasing but without statistically significant differences. There were statistically significant differences between groups at baseline and at the end.

Guo et al, 2015 revealed that as compared with the cirrhotic group, the octreotide-treated group exhibited significantly reduced serum levels of ALT , AST, total bilirubin and the extent of hepatic fibrosis, whereas the cobalt protoporphyrin group exhibited significantly aggravated hepatic function and fibrosis ($P<0.05$).

Study conducted with guinea pigs (Cavia porcellus) examining hepatic fibrosis induced through the administration of ethanol (4g/kg of weight/d) for 90 d revealed a significant increase in the activities of alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transpeptidase in the serum of the ethanol group and significant reduction reduction of lesion markers (ALT, AST, and γ-glutamyl) after silymarin treatment (**Abhilash et al, 2013**).

The present study showed that as regard cholesterol, there was very high statistically significant increasing in value in group (1) followed by group (4) then group (3) while group (1) show increasing but without statistically significant differences. There were statistically significant differences between groups at baseline and at the end. As regard triglycerides, there was very high statistically significant increasing in value in group (2) followed by group (4) then group (3) and group (1). There were statistically significant differences between groups at baseline and at the end. As regard gamma glutamyl transferase, there was very high statistically significant increasing in value in group (2) followed by group (4) then group (3) while group (1) show no change in values. There were statistically significant differences between groups at baseline and at the end.

Our results were supported by study of **Li et al, 2016** as they reported that TG, TC and LDL-C, were significantly increased in the obese group compared to the control group. Octreotide treatment significantly reduced the levels of these parameters ($p<0.05$). As the good cholesterol, the HDL-C concentration was lower in the obese group compared to that in the control group ($p<0.05$); however, octreotide was not effective in increasing the HDL-C levels.

In hypercholesterolemia, silymarin inhibits 3-hydroxy-3- methylglutaryl coenzyme A (HMG-CoA) reductase, reducing cholesterol synthesis. In chronic liver diseases, silymarin acts through different mechanisms and complex biological interactions able to produce benefits in various pathologies, some of which are systemic and can involve the liver. Researchers have studied for a long time the biological effects that natural products such as silymarin have on pathologies such as viral hepatitis, alcoholic liver disease (ALD), metabolic hepatitis, as well as on the common end stages of hepatopathies, that is, cirrhosis and HCC, on which silymarin carries out an important biological action ***(Jaggi and Singh, 2016).***

The liver is the target organ for stress. Extreme stimuli induce significant changes in the tissue structure. Kupffer cells, hepatic stellate cells (HSCs), and endothelial cells are potentially more exposed to oxidative stress. With regard to HSCs, the pr0oliferation and collagen synthesis of HSCs are triggered by lipid peroxidation caused by oxidative stress. HSC activity has been evaluated by measuring hepatic expression of glial fibrillary acidic protein (GFAP) to indicate the magnitude of fibrosis and necro- inflammatory activity. GFAP is a more useful marker of early HSC activation, and it is a member of intermediate filaments maintaining cell's mechanical strength and structure which were detected between sinusoidal endothelial cells and hepatocytes (**Li et al, 2015).**

The current study showed that as regard MDA, there was very high statistically significant increasing in value in group (2) followed by group (4) then group (3) while group (1) show increasing but without statistically significant differences. There were statistically significant differences between groups at baseline and at the end. As regard catalase, there was very high statistically significant decreasing in value in group (2) followed by group (3) then group (4) while group (1) show no change so no statistically significant differences. There were statistically significant differences between groups at baseline and at the end. As regard SOD, there was very high statistically significant decreasing in value in group (2) followed by group (4) then group (1) while group (1) no change so there is no statistically significant differences. There were statistically significant differences between groups at baseline and at the end.

In the study of **Aziz et al, 2018**, Eighteen adult male albino rats were randomly divided into three equal groups: control, IS (immobilization stress), and Octreotide-treated stressed groups. Octreotide (40 µg/kg body weight, subcutaneously) was administrated twice daily for 8 days during the exposure to IS, there was significantly increased the plasma levels of ALT, AST, and hepatic MDA with significantly decreased in the hepatic TAC (Total antioxidant capacity) level when compared with the control group.

Treatment with Octreotide significantly reduced these levels in the stressed group with significantly higher level of hepatic TAC; however, these levels still significantly differ from the control group.

These results were previously confirmed by **Schaalan and Nassar, (2011)** who reported that the anti-oxidant effects of Octreotide could be attributed to its sulfhydryl group and its capacity to induce other free radical scavenging systems.

In previous studies, the inherent hepatoprotective and antioxidant activity of silymarin was shown to be caused by its control of free radicals (FR), which are produced by the hepatic metabolism of toxic substances such as Et-OH, acetaminophen (Paracetamol), or carbon tetrachloride. The FR damage cellular membranes and cause lipoperoxidation (LPO). The cytoprotective effect in liver is also caused by the inhibition of the cyclooxygenase cycle, leukotrienes, and the production of FR in Kupffer cells in mice. These affects reduce inflammation, and it has been suggested that silymarin also performs the following functions: protecting against genomic injury, increasing hepatocyte protein synthesis, decreasing the activity of tumor promoters, stabilizing mast cells (**Trouillas et al, 2008).**

Silymarin has both hepatoprotective and regenerative actions. The mechanism of action is a reduction of the FR formed by toxins that damage the cell membranes (LPO) and competitive inhibition through hepatocyte external cell membrane modification. Silymarin forms a complex that impedes the entrance of toxins into the interior of liver cells. Additionally, silymarin metabolically stimulates hepatic cells and activates the RNA iosynthesis of ribosomes to stimulate protein formation (**AbouZid, 2012)**

In a study published by **(Sandoval et al., 2008)** the authors observed a silymarin protection effect in rat hepatic cells when they used it as a comparison factor to measure liver weight/animal weight % (hepatomegaly). The hepatomegaly was reduced compared to other groups that were administered antioxidant substances. There was no significant difference observed between the silymarin group and the silymarin-alcohol group. This result suggests liver protection by silymarin. Silymarin enhances hepatic glutathione generation by elevating cysteine availability and inducing cysteine synthesis while inhibiting its catabolism to taurine. The regulation of cysteine synthesis may subsequently contribute to the antioxidant defense. Silymarin reduced collagen accumulation by 30% in biliary fibrosis induced in rats (**Jung et al, 2013).** A study in humans reported a slight increase in the survival of patients with cirrhotic alcoholism compared with untreated controls. Silymarin is perhaps the most frequently used natural compound for the treatment of hepatic diseases worldwide due to its antioxidant, anti-inflammatory, and anti-fibrotic activities (**Bergheim et al, 2005).**

In the study in our hands, as regard bilirubin, there was very high statistically significant increasing in value in group (2) followed by group (4) then group (3) while group (1) show no change in values. There were statistically significant differences between groups at the end. As regard weight in 1st week, there was very high statistically significant increasing in value in group (3) followed by group (4) then group (2) and group (1). There were statistically significant differences between groups at all differences time.

In the study of **Li et al, 2016**, body weight gain was calculated by the difference between final body weight at the end of the experiments and original body weight in all rats within the 176-day period. The final body weight and body weight gain of the octreotide-treated group were significantly decreased compared to the obese group ($p<0.05$). However, Lee's index did not differ significantly between these two groups. In addition, octreotide was effective in attenuating the increased abdominal fat and fat/body weight ratio in obese rats.

In another study conducted by **Wang et al, 2020**, following drug administration, BDL-Silymarin rats exhibited a little higher body weight than BDL rats from 1 to 4 week. Compared with both BDL and BDL-Silymarin rats, the body weight in the BDL- Bicyclol rats were always higher and from 3 to 4 week they were significantly increased. After 4 weeks, the liver index of the BDL rats significantly increased, reaching 185.7% of that in the sham control and it was antagonized by treatment with silymarin or bicyclol to 71.2 and 61.3%, respectively.

5. CONCLUSION

The findings of the study suggest that Octreotide could efficiently protect rats against stress-induced hepatic changes which may accompany liver fibrosis induced by alcohol. Further studies may be needed to identify the molecular mechanisms underlying the etiology of this Octreotide protection.

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