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EFFECTS OF STARVATION AND ALCOHOL CONSUMPTION ON SOME METABOLIC ASPECTS IN MALE ALBINO RATS .

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Abstract

This study was conducted to examine the effects of starvation in presence or absence of alcohol consumption on some metabolic pathways in rats. The results indicated that starvation decreased blood glucose, total proteins and albumin concentrations, whereas insulin and glucagon increased. On the other hand, serum enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities were increased in response to complete starvation, but alkaline phosphatase (ALP) and gamma-glultamyltransferase (GGT) activities decreased, however, acid phosphatase was approximately unaltered. Ethanol was found to decrease serum (ALT) and (LDH) activities, while serum (GGT) was increased.

Hepatic glycogen was found to decrease in starved and alcoholic fasted rats.Aminotransferases of the liver were decreased in response to starvation. Liver succinic dehydrogenase activity increased at 1 and 3 days of fasting, but decreased insignificantly at the 5 th day of starvation.

Ethanol induced an increase in hepatic ALT activity at the 5 th day of starvation, but it decreased glycogen content, AST and succinic dehydrogenase activities in fasted rat liver. The present findings support the suggestion that starvation disturbs most of the mtabolic pathways while alcohol intake of starved animals exacerbates such a disturbance.

INTRODUCTION

It was reported that starvation causes many alterations in animal physiology and biochemistry, including the body weight (Chandrasekharan, 1969 and Inoue *et al.*, 1993), blood glucose level (Brooke, 1981), hepatic and muscle glycogen (Cleader and Robert, 1992), plasma glucagon like immunoreactivity (Takahashi *et al.*, 1992), serum and liver lipids and proteins (Mendez and Menchu, 1966 and Sassoon *et al.*, 1966), hepatic glucose- 6- phosphate dehydrogenase (McDonald and Johnson, 1965), hepatic ALT, liver AST (Stielau *et al.*, 1965) and arterial alkaline phosphatase activities (Zemplenyi, 1962).

These changes may also be affected in case of ethanol intake after starvation (Forsander *et al.*, 1965). This paper investigates the influence of starvation on different metabolic parameters in rat liver and serum with special reference to ethanol interaction.

MATERIAL AND METHODS

Animals:

Male albino rats (*Rattus norvegicus*), purchased from the Egyptian organization for Biological and vaccine production, A.R.E., weighing about 100 ± 20g, were used as experimental animals throughout the present work. Animals were housed in groups in plastic cages.

Experimental Design:

Animals were segregated into 3 groups as follows:

1- The first group received balanced diet and tap water *ad-libitum* and served as control.

2- The animals of the second group were starved and received only tap water *ad-libitum.*

3- Animals of the third group were starved and received 20% of ethanol in tap water *ad-libitum.*

Animals of different groups were sacrified after 1 , 3 and 5 days of starvation.

Collection of Serum Samples:

Blood samples were received in conical glass centrifuge tubes and were put in refrigerator till clotting. Sera were then separated using cooling centrifuge at 1000 *g* for 10 min. and kept at- 20°C till analyses.

Preparation of Tissue Homogenates:

After decapitation, animals were dissected and livers were immediately excised, blotted with filter paper and frozen in a deep freezer at-20°C till further experimental procedures. Accurately weighted hepatic portions were homogenized in an ice bath using glass homogenizer to give a final dilution of 10% tissue homogenate which is further diluted in order to be convenient for determinations of different parameters.

Biochemical Analysis:

Serum total protein and albumin contents were determined using an automatic multiparameter apparatus, ASTRA[8] synchron AS clinical system.

Blood glucose concentration was determined colourimetrically using dimension ® ES machine, The Liver Institute, Menoufia University, Egypt. Serum glucagon concentration was carried out by the method of Harris *et al.* (1979) using radioimmunoassay kits of Biomedicals Inc. Company, USA. Insulin was determined in serum according to the radioimmunoassay method of Wilson and Miles, (1977), using kits of SORIN Biomedica Company, Italy.

Serum aspartate aminotransferase (AST) [Ec. 2.6.1.1], alanine aminotransferase (ALT) [Ec. 2.6.1.2], alkaline phosphatase (ALP) [EC. 3.1.3.1], acid phosphatase [Ec. 3.1.3.2] and gamma-glutamyltransferase (GGT) [Ec. 2.3.2.2] activities were determined using an automatic multiparameter apparatus, ASTRA[8] synchron AS clinical system. Lactate dehydrogenase (LDH) [Ec. 1.1.1.2] activity in serum was determined using kits of human gesllschaft fllr biochemica lnd diagnostica mbh. Hepatic glycogen contents were quantifid colourimetrically by the method of Seifter *et al.*, (1950). Hepatic AST and ALT activities were measured by the method of Reitman and Frankel (1957), using commercial kits of Al-Gomhoria Co., Egypt Hepatic succinic dehydrogenase activity was measured colourimetrically using the method of Kun and Abood, (1949).

Statistical Analysis:

Data are presented as means ± standared deviation (S.D.) in Tables. Student's t-test was used to evaluate statistical significances of results according to Hine and Wetherill, (1975).

RESULTS

Blood parameters

Total Proteins:

As represented in (Table. 1), it can be noticed that serum total protein content decreased after 1 , 3 and 5 days of starvation by 7.31, 8.68 and 10.97%, respectivily, while starvation with ethanol intake resulted in increased these depression magnitudes (Table. 2)

Albumin:

Albumin decreased significantly in starved rats (Table. 1). Similar results were observed with ethanol intoxication (Table. 2).

Glucose:

It was found that serum glucose level decreased highly significantly by the ratios: 52.46, 52.67 and 55.14 at 1,3 and 5 days of starvation (Table. 1), similar results were observed after alcohol abuse of starved rats (Table.2).

Glucagon:0

This parameter showed highly significant increases in serum after starvation, which were amounted to 35.55, 22.42 and 96.43% of their respective control values at 1,3 and 5 days, respectively (Table. 1)

On the other hand, ethanol intake causes blood glucagon level to increase highly significantly by 27.92, 77.83 and 197.79% after 1,3 and 5 days of treatment, respectively (Table. 2).

Insulin:

Radioimmunoassay analysis of insulin has resulted in a significant increase in serum insulin content of starved rats at 3 rd day of experiment, and a highly significant ($P < 0.01$) elevation of 101.11% at 5 th day of starvation (Table. 1).

Values of serum insulin in starved rats after ethanol intoxication (Table. 2) exhibited highly significant increases amounted to 56.66 and 53.30% at 1 and 5 days of treatment, respectively.

Aminotransferases:

Serum ALT and AST activities were noticed to increase at different periods of starvation, but insignificant depression was observed in serum AST activity at 1 st day of treatment (Table 3).

On the other hand, these enzyme activities were noticed to increase at different periods of starvation of ethanolic rats however, a highly significant decrease in hepatic ALT activity was observed at 5 days of treatment (Table. 4).

Alkaline phosphatase:

This enzyme activity was decreased highly significantly with magnitudes of 31.30 and 41.29% at 3 and 5 days of starvation (Table. 3), while it diminshed at 1,3 and 5 day of ethanol intake in completely starved rats by the ratios: 44.33, 46.52 and 48.94%, respectively (Table. 4).

Acid phosphatase:

As shown in table (3), the activity of acid phosphatase was decreased at 1 and 3 days of starvation while it increased at the 5 th day of fasting (Table. 3). On the other hand this parameter decreased at the 1 st day of starvation of ethanolic rats, but increased at 3 and 5 days of treatment (Table. 4).

Gamma glutamyl transferase:

The changes in serum GGT activities following starvation were presented in table (3). It was found that GGT activities were decreased highly significantly at different periods of starvation.

Starvation of ethanol-treated rats showed highly significant decreases in the activity of this enzyme at 1 and 3 days of treatment by the values 43.55 and 38.99%, respectively (Table. 4).

Lactate dehydrogenase:

This enzyme showed highly significant ($P < 0.01$) elevations amounted to 70.92 and 43.59% at 1 and 3 days of starvation (Table. 3), while it increased only at 1 st day of ethanol intake of starved rats, then it decreased insignificantly at 3 and 5 days of treatment.

Liver parameters:

Glycogen:

Hepatic glycogen content were found to be greatly decreased under the influence of starvation or fasting with alcohol abuse (Tables 5 and 6).

Transaminases:

Aminotransferase activities in liver of starved rats were decreased highly significantly after all periods of the experiment (Table. 5).

Similarly, hepatic aminotransferases decreased in response to starvation and alcohol drinking at different periods of treatment but with an exception at day 5 of treatment where liver ALT activity was found to increase insignificantly (Table. 6).

Succinic dehydrogenase:

Starvation induced to increases in the activity of this enzyme at 1 and 3 days of fasting while it caused hepatic succinic dehydrogenase activity to decrease insignificantly at the 5 th day of fasting (Table 5).

Hepatic succinic dehydrogenase activity in starved rats was found to decrease highly significantly by the magnitudes: 42.72, 47.39 and 44.06% at 1,3 and 5 days of ethanol treatment, respectively (Teble. 6).

DISCUSSION

The results showed that blood glucose and hepatic glycogen contents were decreased after starvation with or without alcohol abuse. These results are in agreement with those reported by Morata *et al.* (1982), who referred to the decrease in gluconeogenesis process and to the enhancement of glycogenolysis in the liver because of the utiliztion of liver glycogen as a source of blood glucose during the initial period.

The depression in blood glucose during starvation is associated with an increase in serum insulin and glucagon content that was in accordance with the opinion that fasting facilitates glucose utilization (Unger *et al.*, 1963 and Tsutomu *et al.*, 1992). Pico *et al.* (1991) reported that starvation increases red cells amino acids uptake; this may partially interpreter the decrease in serum total prateins. Our results for ethanol intoxicated starved rats are in agreement with those reported by Baraona and Lieber, (1982) and Brassinne, (1979).

Ethanol was reported to increase serum ALT activity (Piedras *et al.*, 1987 and Shalan(M), 1996); where alcohol may exert its effect through alterations it induces on synthesis in the endoplasmic reticulum, intracellular translocation or possibility of solubilization at the site of plasma membrane, hence increasing the level of serum enzymes especially membrane-bound enzymes such as GGT, ALP and cytosolic enzymes such as ADH, LDH and aminotransferase enzymes (ALT and AST).

Forsander *et al.* (1965) reported that ethanol treatment of starved rats decreased the pyruvate concentration which can be referenced for the decrease in ALT and LDH activities at 5 days of ethanolic starved rats. Moderate acohol consumption resulted in small but significant elevation in GGT and urate while AST, folate and fibrinogen showed no change (Lieber, 1984 and Pikaar *et al.*, 1987).

Shalan(A), (1995) showed that following long-term alcohol intake, striking increase of serum ALP activity was observed in male albino rats fed laboratory balanced diet. However Goz *et al.* (1983) and Nishimura and Teschke (1982) demonestrated that ethanol didn't affect ALP activity. The increased serum activity of GGT at day 5 of ethanol intoxication in starved rats may result from the induction of this enzyme in the liver and/ or due to damage of hepatocytes and other organ tissues known to be rich in this enzyme such as renal tissues (Jacquemin *et al.*, 1990).

On the other hand, it was recorded that hepatic alkaline phosphatase and aminotransferase (ALT and AST) activities decreased insignificantly in response to chronic alcohol intake (Teschke *et al.*, 1983; Yamada *et al.*, 1985 and Adel-Raheem *et al.*, 1990).

The fact that present findings showed that starvation causes an increase in hepatic succinic dehydrogenase activity at 1 and 3 days, may contribute to increased rate of citric acid cycle and glycogenolytic process (Bayomy and Taie 1992). However, hepatic succinic dehydrogenase was reported to decrease in response to chronic alcohol intoxication (Shalan(M), 1996).

In conclusion the present data indicates that alcohol eggravates metabalic disturbance induced in starved rats as indicated by the chosen parameters measured in both serum and liver homogenate.

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Table (1): Effect of starvation on some blood metabolites of rats.

	Control	Duration of Starvation					
		1 day		3 days		5 days	
	Mean ± S.D.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.
Glucose a	97.2 ± 6.05 1	46.2 ± 7. 69	** -52.46	46 ± 1.58	** -52.67	43.6 ± 2.70	** -55.14
Glucagon a	188.62 ± 13.4 2	255.68 ± 20.28	** 35.55	230.92 ± 16.25	** 22.42	370.52 ± 16.48	** 96.43
Insulin a	10.73 ± 0.55 3	13.25 ± 2.48	23.48	13.27 ± 1.90	* 23.6	21.58 ± 2.67	** 101.11
Total Protein a	6. 56 ± 0.24 4	6.08 ± 0.015	* -7.31	5.99 ± 0.03	** -8.68	5.84 ± 0.21	** -10.97
Albumin a	3.46 ± 0.23 5	1.87 ± 0.02	** -45.95	1.17 ± 0.015	** -66.18	1.13 ± 0.011	** -67.34

a : n = 5. 3 : µu/ml. ** = Highly significant (P < 0.01).
1 : mg/dl. 4: gm/dl. * = Significant (P <0.05).
2 : Pg/ml. 5 : gm/dl.

Table (2): Effect of ethanol intake on some blood metabolites in starved rats.

	Control	Duration of Starvation					
		1 day		3 days		5 days	
	Mean ± S.D.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.
Glucose a	97. 2 ± 6.05 1	54.4 ± 3.36	** -44.03	52.2 ± 2.86	** -46.29	42 ± 4.63	** -56.79
Glucagon a	188.62 ±13.4 2	241.30 ±29.23	** 27.92	335.43 ± 29.24	** 77.83	561.70 ± 30.06	** 197.79
Insulin a	10.73 ± 0.55 3	16.81 ± 2.42	** 56.66	13.05 ± 2.17	* 21.62	16.45 ± 3.07	** 53.30
Total Protein a	6.56 ± 0.24 4	5.42 ± 0.31	** -17.37	4.90 ± 0.16	** -25.3	4.70 ± 0.16	** -28.35
Albumin a	3.46 ± 0.23 5	1.16 ± 0.03	** -66.47	1.04 ± 0.02	** -69.94	0.974 ± 0.011	** -71.96

a : n = 5. 3 : µu/ml. ** = Highly significant (P < 0.01).
1 : mg/dl. 4 : gm/dl. * = Significant (P < 0.05).
2 : Pg/ml. 5:gm/dl.

Table (3): Effect of starvation on some blood enzymes of rats

	Control	Duration of Starvation					
		1 day		3 days		5 days	
	Mean ± S.D.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.
AST [a]	183.6 ± 15.94 [1]	171.6 ± 2.41	- 6.99	186 ± 3.67	1.307	194.4 ± 2.70	5.88
ALT [a]	39.8 ± 1.92 [2]	58.80 ± 1.92	* 47.73	47.4 ± 3.64	* 19.09	43 ± 2.73	8.04
ALP [a]	164.2 [3] ± 12.67	146.4 ± 12.62	-10.84	112.8 ± 3.11	* -31.30	96.4 ± 2.4	* -41.29
Acid phosphatase [a]	1.17 [4] ± 0.13	0.734 ± 0.09	* -37.31	1.14 ± 0.08	-2.81	1.27 ± 0.11	8.45
GGT [a]	27.21 [5] ± 2.00	11.53 ± 0.04	* -57.66	13.72 ± 0.23	* -49.65	20.48 ± 0.33	* -24.73
Lactate dehydrogenase [a]	775.8 [6] ± 31.32	1326 ± 91.14	* 70.92	1114 ± 85.32	* 43.59	879 ± 158.11	13.03

[a] : n = 5. [3] : Iu/L. [6] : U/L.
[1] : Iu/L. [4] : U/L. * = Highly significant (P < 0.01).
[2] : Iu/L . [5]: U/L.

Table (4): Effect of ethanol intake on some blood enzymes of starved rats.

	Control	Duration of Starvation					
		1 day		3 days		5 days	
	Mean ± S.D.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.
AST [a]	183.6 [1] ± 15.94	185.6 ± 2.19	1.08	197.2 ± 2.16	7.40	207.2 ± 5.93	* 12.85
ALT [a]	39.8 [2] ± 1.92	51.6 ± 1.81	** 29.64	40.4 ± 1.14	1.50	33.4 ± 1.14	** -16.08
ALP [a]	164.2 [3] ± 12.67	91.40 ± 3.50	** -44.33	87.8 ± 1.30	** -46.52	83.83 ± 2.22	** -48.94
Acid phosphatase [a]	1.17 [4] ± 0.13	0.35 ± 0.079	** -70.11	1.24 ± 0.07	5.89	1.73 ± 0.08	** 48.42
GGT [a]	27.21 [5] ± 2.00	15.36 ± 0.24	** -43.55	16.60 ± 0.20	** -38.99	28.91 ± 0.31	6.24
Lactate dehydrogenase [a]	775.8 [6] ± 31.32	1242.2 ± 84.65	** 60.11	687 ± 114.01	-11.44	706 ± 119.07	-8.99

[a] : n = 5. [3] : Iu/L. [6] : U/L.
[1] : Iu/L. [4] : U/L. ** = Highly significant (P < 0.01).
[2] : Iu/L. [5]: U/L. * = Significant (P < 0.05).

Table (5): Effect of starvation on hepatic glycogen content, ALT, AST and succinic dehydrogenase activities of rats.

	Control	Duration of Starvation					
		1 day		3 days		5 days	
	Mean ± S.D.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.
Glycogen [a]	35.31 [1] ± 5.54	22.92 ± 1.89	** -35.08	12.00 ± 1.54	** -63.18	21.12 ± 1.19	** -40.18
ALT [a]	11.47 [2] ± 0.49	10.16 ± 0.28	** -11.42	9.09 ± 0.33	** -20.74	9.81 ± 0.104	** -14.47
AST [a]	21.42 [3] ± 2.66	6.86 ± 0.25	** -68.16	5.81 ± 0.17	** -72.87	2.97 ± 0.125	** -76.79
Succinic dehydrogenase [a]	1.65 [4] ± 0.035	1.829 ± 0.065	** 10.85	1.736 ± 0.065	* 5.21	1.619 ± 0.036	-1.87

[a] : n = 5.
[1] : mg/gm wt. tissue.
[2] : U/gm wt. tissue.
[3] : U/gm wt. tissue .
[4] : ug of dye reduced/gm of tissue/ 10 min.
** = Highly significant (P < 0.01).
* = Significant (P < 0.05).

Table (6): Effect of ethanol intake on hepatic glycogen content, ALT, AST and succinic dehydrogenase activities of starved rats.

	Control	Duration of Starvation					
		1 day		3 days		5 days	
	Mean ± S.D.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.	Mean ± S.D.	% diff.
Glycogen [a]	35.31 [1] ± 5.54	20.78 ± 0.69	** -41.14	22.25 ± 0.60	** - 36.98	27.14 ± 0.46	* - 23.25
ALT [a]	11.47 [2] ± 0.49	10.42 ± 0.21	** -9.15	11.2 ± 0.29	-2.35	11.50 ± 0.16	0.261
AST [a]	21.42 [3] ± 2.66	6.38 ± 0.29	** -70.21	5.24 ± 0.17	** -75.53	4.58 ± 0.16	** -78.86
Succinic dehydrogenase [a]	1.65 [4] ± 0.035	0.945 ± 0.061	** -42.72	0.868 ± 0.054	** -47.39	0.923 ± 0.066	** -44.06

[a] : n = 5.
[1] : mg/gm wt. tissue.
[2] : U/gm wt. tissue.
[3] : U/gm wt. tissue .
[4] : ug of dye reduced/gm of tissue/ 10 min.
** = Highly significant (P < 0.01).
* = Significant (P < 0.05).

تأثير التجويع وإستهلاك الكحول على بعض المؤشرات الأيضيه فى ذكور الجرذان البيضاء.

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الملخص العربى

تم فى هذا البحث دراسة تأثير التجويع مع تناول الكحول أو بدونه، على بعض المسارات الأيضيه فى الجرذان. تشير النتائج إلى أن التجويع يسبب إنخفاضاً فى مستوى جلوكوز الدم وكذا تركيزات البروتينات الكلية والألبيومين، بينما تزداد نسب هرمونى الإنسولين والجلوكاجون بالسيرم مقارنة بالمجموعات الضابطة.

ومن ناحية أخرى، فإن أنشطة إنزيمات السيرم وتشمل : الإنزيمات الناقلة لمجموعات الأمين واللاكتات ديهيدروجينيز تزداد إستجابة للتجويع الكامل، وعلى النقيض من ذلك تنخفض أنشطة إنزيمى الفوسفاتيز القاعدى والجاماجلوتاميل ترانسفيريز، بينما لم يتأثر نشاط إنزيم الفوسفاتيز الحامضى تقريباً.

أدى استهلاك الإيثانول إلى إنخفاض أنشطة إنزيمى الألانين أمينوترانسفيريز واللاكتات ديهيدروجينيز، بينما تسبب فى إرتفاع نشاط إنزيم الجاماجلوتاميل ترانسفيريز فى السيرم فى الحيوانات المجوعة.

لقد سجل إنخفاض فى محتوى جليكوجين الكبد فى كل من الجرذان المجوعة وكذا التى تتناول الكحول مع التجويع، كما لوحظ أن أنشطة الإنزيمات الناقلة لمجموعات الأمين تنخفض فى الكبد إستجابة لعملية التجويع، ويزداد نشاط إنزيم السكسينك ديهيدروجينيز فى الكبد فى اليوم الأول والثالث من التجويع، بينما ينخفض فى اليوم الخامس.

لوحظ أن إستهلاك الإيثانول يتسبب فى إرتفاع نشاط إنزيم الألانين أمينوترانسفيريز فى الكبد بينما يؤدى إلى إنخفاض فى محتوى الجليكوجين، وفى أنشطة إنزيمات الاسبارتات أمينوترانسفيريز والسكسينك ديهيدروجينيز فى أكباد الجرذان المجوعة.

هذا، وتدعم هذه الدراسة الإفتراضات التى تشير إلى أن عملية التجويع تحدث إضطراباً فى مختلف العمليات الأيضية وأن الكحول يؤدى إلى تفاقم هذا الإضطراب.