Research Article

Effect of Dehydroepiandrosterone (DHEA) on Adenylate Energy Pool in Partially Hepatectomized and/or Propagated-Cirrhotic Liver of Rats.

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ABSTRACT

The present study aims to evaluate the effect of dehydroepiandrosterone (DHEA) on adenylate nucleaotide pool including ATP, ADP, AMP, total adenylate (TA), adenylate energy charge (**AEC**) phosphate potential and inorganic phosphate (Pi) in partially hepatectomized, propagated- cirrhotic and cirrhotic -partially hepatectomized rats. Male Wistar albino rats (100: 120g) rats were used in this study and DHEA hormone was administered orally by gastric intubation at a dose 300 mg/kg body weight daily for two weeks. The present biochemical results reveal disturbances in adenylate nucleaotide pool in hepatectomized, cirrhotic and cirrhotic-hepatectomized rats and attenuation of these findings upon treatment with DHEA. Thus, it could be concluded that, DHEA treatment markedly ameliorate the adenylate pool in different therapeutic groups.

Keywords: Dehydroepiandrosterone (DHEA), hepatectomized rats, cirrhotic, adenylate energy pool.

INTRODUCTION

Dehydroepiandrosterone (DHEA) is naturally occurring adrenal steroid in mammals which can be synthesized *via* cholesterol and is metabolized to androsten-edione and estrogens. The decline in their production is the most characteristic age-related change in the adrenal cortex.[1]

It was reported that, DHEA has various biological effects, such as antiobesity, antidiabetic and anticarcinogenic when administered to mice and rats. In addition, DHEA *in vivo* plays a role in peroxisome proliferators activated receptor alpha induced DNA synthesis in rats after partial hepatectomy.[2] Moreover, DHEA facilitates liver regeneration after partial hepatectomy.[3]

Actually, liver regeneration is a fundamental mechanism by which the liver can withstand injury. Changes in the morphology and physiology of organs and tissues such as the liver might be due to the accumulation of oxidative oxygen species (ROS) and reactive nitrogen species (RNS).

Hepatocytes are very rich in mitochondria and have a high respiratory rate, so they are exposed to large amount of ROS and permanent oxidative stress which may be involved in the hepatocyte dysfunction observed in the setting of fulminate hepatic failure.[4]

In addition, mitochondria isolated from rat liver during the early phase of liver regeneration, after partial hepatectomy showed marked decrease in the rate of ATP synthesis, increase of malondialdehyde and of oxidized protein production, decrease of the content of intra–mitochondrial glutathione and of protein thiols on mitochondrial proteins as well as increase of glutathione bound to mitochondria proteins by disulfide bonds. These observations suggested an increase production of ROS in liver mitochondria following partial hepatectomy.[5]

Dimethyl nitrosamine (DMN) through metabolic activation by cytochrome P450 2E1 exerts hepatotoxicity and tissue injury. Liver injuries induced by multiple DMN treatments lead to hepatic necrosis, fibrosis, and eventually cirrhosis.[6]

Thus, the present results aim to evaluate the level of ATP, ADP, AMP, TA, phosphate potential and inorganic phosphate in partially hepatectomized, cirrhotic and cirrhotic–hepatectomized rats and the effect of DHEA hormone in amelioration the level of adenylate nucleotide pool in different therapeutic groups.

MATERIALS AND METHODS

Chemicals

All chemicals used in the present study were of high analytical grade, products of Sigma (USA), DHEA manufactured by Natrol, Inc. Chatsworth (USA).

Animals

Male Wistar albino rats (100: 120g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature (26±3°C) with access of water, diet and *ad libitum*.

Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt and performed for being sure that the animals not suffer at any stage of the experiment.

Partial Hepatectomy

Rats were partially hepatectomized at 9-10 am according to the method of Higgins and Anderson.[7]

Induction of Cirrhosis

Liver cirrhosis was initiated by intraperetonial injection of dimethyl nitrosamine at a dose of 1 ml (diluted 1:100 with 0.15 M sterile NaCl) per 100 gm body weight. The injections were given on three consecutive days of each week for a period of 3 weeks.[8]

Route of Administration

DHEA suspended in H_2O and given orally by gastric intubation in a dose of 300 mg/kg body weight daily for two weeks.[3]

Experimental Design

A total of 190 adult Wistar albino rats, weighing 100-120 g. were randomly divided into 7main groups, fed on standard diet and tap water as follows:

Group (1): Normal healthy untreated control group (10 rats).

Group (2): Untreated 33% partially hepatectomized rats (40 rats), sacrificed, after one, three days, one and two weeks (10 rats each), respectively.

Group (3): Untreated propagated-cirrhotic rats (10 rats), liver cirrhosis was initiated for one month, and then rats were sacrificed immediately.

Group (4): untreated cirrhotic- hepatectomized rats (40 rats), cirrhotic rats post one month of imitation were subjected to one third partial hepatectomy and then were sacrificed, after one, three days, one and two weeks respectively (10 rats each).

Group (5): Treated hepatectomized rats with DHEA (40 rats) and sacrificed also, after the same intervals respectively (10 rats each).

Group (6): Treated cirrhotic rats (10 rats) treated with DHEA for two weeks and then sacrificed.

Group (7): Treated cirrhotic-hepatectomized rats (40 rats), treated with DHEA and scarified after one, three days, one and two weeks of treatment (10 rats at each interval).

Enzymatic determination of adenosine nucleotides (ATP, ADP and AMP) and inorganic phosphate (Pi) in tissue extracts of Albino rat liver

Preparation of tissue extract

Adenosine nucleotides were extracted from liver of albino rats using trichloroacetic acid (7% TCA) according to the method of **Wijsman.**[9] 0.25 g from rats liver tissue (V_1) were homogenized in 5 ml ice-cold 7% TCA (V_2). The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant (V_3) was neutralized with 0.25 M sodium hydroxide to pH 6.[5-7]. The volume of the neutralized

extract was measured (V4). The neutralized solution was allowed to stand for 10 minutes in ice-cold water, the precipitate was discarded and the supernatant was used for measuring ATP, ADP, AMP and Pi. From V_1, V_2, V_3 and V_4 the dilution factor (F) can be calculated according to Lamprecht and Trautschold[10] from the following equation:

$$F = \frac{V_2 \times V_4}{V_1 \times V_3}$$

Determination of ATP with hexokinase and glucose-6-phosphate dehydrogenase (G-6-P-dehydrogenase)

The enzymatic determination of adenosine triphosphate (ATP) by spectrophotometric method involving pyridine nucleotides has come into general use because of its simplicity according to Lamprecht and Trautschold.[10]

Determination of ADP and AMP

ADP and AMP in tissue extract of albino mice were determined according to the method of Jaworek et al.[11]

Calculation of Adenylate Energy Charge (AEC)

Adenylate energy charge has been proposed as a measure of the energy potentially available from the adenylate system for cellular metabolism and can be calculated from the following equation according to **Atkinson and Walton.**[12]

$$AEC = \frac{\frac{1}{2}[ADP]+[ATP\}}{[ATP]+[ADP]+[AMP]}$$

and is expressed without dimension.

Determination of inorganic phosphates (Pi)

Inorganic phosphates (Pi) was determined in the same extract in which ATP, ADP and AMP were determined. The method used was that of Fiske and Subbarow.[13]

Calculation of phosphate potential

Phosphate potential is an alternative index used to indicate the free energy status of the tissues and can be calculated from the concentrations ratio of [ATP], [ADP] and [Pi] according to Van Waarde et al.[14]

$$Phosphate\,potential = \frac{[ATP]}{[ADP][Pi]}$$

is expressed without dimensions.

Statistical Analysis

Statistical analysis is performed using two ways analysis of variance (ANOVA) combined with Co-state computer program and Post Hoc (LSD). Unshared letters between groups are significant at p value ≤ 0.05.

RESULTS

Effect of DHEA treatment on ATP, ADP and AMP, levels, adenylate energy charge (AEC), total adenylate (TA) level, inorganic phosphate level and phosphate potential in different therapeutic groups

As compared to normal healthy rats, partially hepatectomized rats showed insignificant decrease in AMP level after one, three days and two weeks, while significant decrease ($p \leq 0.05$), post one week of operation was demonstrated with percentage decrease reached to 48.45% (Fig.1). ADP level showed significant increase with time intersection reached to its highest percentage increase two weeks post-surgery (1480.00%) (Fig.2). However, ATP level showed significant ($p \leq 0.05$),decrease with time intersection recorded more or less similar lower levels one and two weeks post operation(72.56% and 71.39%, respectively) (Tables 1,2 and Fig 3). Whereas, AEC, recorded significant decrease with time intersection and showed its lowest value two weeks post operation with percentage decrease amounting to 26.23% (Fig. 4). Also, TA showed significant decrease at 1st, 3rd day and 1st week with percentages 60.39, 48.69 and 51.52%, respectively, although, two weeks post operation exhibited insignificant change as compared to normal control group (Fig 5). With regard to, inorganic phosphate and phosphate potential, significant decrease ($p \leq 0.05$) was noticed in the concentration of inorganic phosphate with different intervals recorded the lowest concentration level after one day (80.20%). While, phosphate potential demonstrated insignificant change after one day, one week and two weeks post operation, while significant decrease was observed after three days with percentages decrease 86.66 and 60.00%, respectively as compared to normal healthy rats (Table 1, 2 and Figs.6,7).

In addition, as compared to normal control rats, propagated -cirrhotic rats showed insignificant decrease in AMP level, while ADP level recorded significant ($p \leq 0.05$) increase amounting to 486.39%. In contrast, ATP level demonstrated significant decrease reached to 27.85 % (Table 1).Moreover, AEC and TA showed insignificant change, while significant ($p \leq 0.05$), decrease was detected in inorganic phosphate level and phosphate potential with percentages decrease amounting to 42.86% and 50.00 % as compared to normal healthy rats (Table 2 and Figs 1-7).

Concerning, cirrhotic–partially hepatectomized rats, insignificant change was recorded in AMP level one, two days and one week post operation, while two weeks showed significant increase with percentage increase reached to 118.87 % . While, ADP level exhibited significant increase with the time intersection recorded the highest percentage increase after two weeks (619.00%). In contradictory, ATP showed significant depletion with effect of time, recorded the lowest percentage decrease after two weeks (45.30%) (Table1 and Figs 1-3). Regarding to AEC, it showed significant

decrease after one, three days and two weeks with percentages 35.80, 11.40 and 18.40%, respectively, while insignificant change in AEC was detected after one week (Fig. 4). TA and inorganic phosphate showed significant increase ($p \leq 0.05$), after one day with percentages increase reached to 79.50 and 38.78%, respectively, while, significant decline was demonstrated in TA after three days (41.80%) and one week (42.80%), followed by insignificant change after two weeks(Fig. 5). However, inorganic phosphate level recorded insignificant change after three days and two weeks, while one week showed significant decrease with percentage of 39.52 %, as compared to normal control rats (Fig. 6). Furthermore, phosphate potential declared significant decrease after one, three days and two weeks with percentages decrease 83.6, 83.33 and 73.33%, respectively, while, insignificant change was detected after one week (Table2 and Fig. 7).

Furthermore, treatment of partially hepatectomized rats with DHEA showed significant ($p \leq 0.05$), decrease in AMP level after one day of DHEA treatment with percentage decrease amounting to 78.30%, while significant increase was detected after three days, one and two weeks of treatment with percentages increase reached to 58.49, 31.32 and 59.43 %, respectively (Fig.1) . Also, ADP exhibited significant increase after one, three days and one week post treatment with percentages increase 186.05, 418.32 and 395.64%, respectively, while insignificant change was observed in ADP level two weeks post DHEA treatment (Fig.2) . Remarkable significant decrease in ATP level was detected in partially hepatectomized rats treated with DHEA after one and three days (37.99 and 31.51 %, respectively), while insignificant change was observed after one week of treatment. Whereas, significant increase (10.09%), was noticed post two weeks as compared to normal healthy rats (Table 1 and Fig.3). Considering AEC, insignificant change was noticed with time intersection post DHEA treatment (Fig. 4). However, significant decrease was observed in TA level at 1st day post treatment with percentage decrease reached to 33.77 %, then, insignificant change was detected (Fig. 5). On the contrary, significant increase in inorganic phosphate was recorded one day post treatment with percentage increase 27.83%, and insignificant change was noticed after other durations (Fig.6). Treatment of partially hepatectomized rats with DHEA showed also, insignificant change in phosphate potential after one, three days and one week post treatment, while, two weeks after treatment showed significant increase ($p \leq 0.05$), reached to 106.63%, as compared to normal healthy rats (Table 2 and Fig. 7).

It was found that, cirrhotic rats after two weeks of DHEA treatment, exhibited significant increase($p \leq 0.05$) in AMP, ADP, ATP and TA with percentages increase amounting to 115.967, 967.44, 12.333 and 55.53%, respectively, while AEC and phosphate potential recorded significant decrease of 6.55 and 73.06%,respectively as compared to

normal control group. Although, insignificant change was noticed in inorganic phosphate level as compared to normal healthy rats (Table 1 and Figs 1-7).

Moreover, treatment of cirrhotic–partially hepatectomized rats with DHEA showed significant(p≤0.05), increase in AMP level after three days and one week of treatment with percentages increase reached to 104.30 and 30.30%, respectively while, insignificant change post one day and two weeks of DHEA treatment (8.74 and 9.37%,respectively), was detected . Also, ADP level showed significant (p≤0.05), increase with time intersection recorded the minimum percentage of increase after three days (116.40%), while the highest percentage was recorded post two weeks of treatment (512.63%). However, ATP showed significant decrease (p≤0.05) after one and three days of DHEA treatment with percentages decrease reached to 36.81 and 25.67%, respectively. Insignificant change was observed in ATP level after one week of treatment followed by significant increase after two weeks of treatment with percentage increase 9.56%, as compared to normal control group

(Table 1 and Figs 1-3). With regard to AEC, it showed insignificant change with the time intersection, as compared to normal control group (Fig. 4). TA level showed significant decrease after one day (27.63%), followed by insignificant change after three days and one week as compared to normal control group. While, significant increase (26.68%), was noticed in TA level after two weeks of DHEA treatment as compared to normal control group (Fig.5). Inorganic phosphate showed significant decrease in cirrhotic –partially hepatectomized rats treated with DHEA with the time intersection recorded percentages decrease amounting to 46.10, 77.40, 76.80 and 57.90%, after one, three days, one and two weeks respectively (Fig. 6). While, phosphate potential demonstrated insignificant change one day post treatment as compared to normal control ,then significant increase was detected after three days, one and two weeks with percentages increase reached to 243.33, 136.67 and 156.67%, respectively as compared to normal healthy rats (Table 2 and Fig. 7).

**Table 1:** Effect of DHEA treatment on AMP, ADP and ATP levels in normal and different therapeutic groups

Time	Groups Parameters	AMP				ADP				ATP			
1 Day	1)Normal	1.06	±	0.157	def	0.57	±	0.013	ij	12.03	±	1.068	f
	2)PH	0.81	±	0.02	efg	1.35	±	0.014	gh	3.42	±	0.001	l
	3) Cirr.-PH	1.29	±	0.04	cde	2.15	±	0.002	a	2.43	±	0.011	a
	4)Cirr.-PH with tt.	1.16	±	0.03	def	1.44	±	0.08	gh	7.60	±	0.09	j
	5)PH with tt	0.23	±	0.06	hi	1.64	±	0.10	fgh	7.46	±	0.01	b
3 Days	1)Normal	1.06	±	0.157	def	0.57	±	0.013	ij	12.03	±	1.068	e
	2)PH	0.93	±	0.495	defg	3.64	±	0.482	de	2.64	±	0.091	d
	3) Cirr.-PH	0.97	±	0.012	defg	2.72	±	0.031	def	4.51	±	0.232	k
	4)Cirr.-PH with tt.	2.18	±	0.083	ab	1.24	±	0.064	gh	8.94	±	0.312	i
	5)PH with tt	1.68	±	0.271	bc	2.97	±	0.023	def	8.24	±	0.561	b
1 week	1)Normal	1.06	±	0.157	def	0.57	±	0.013	ij	12.03	±	1.068	b
	2)PH	0.55	±	0.015	gh	2.98	±	0.17	def	3.30	±	0.242	bc
	3) Cirr.-PH	1.39	±	0.113	cd	1.31	±	0.03	gh	5.35	±	0.102	j
	4)Cirr.-PH with tt.	1.39	±	0.06	bc	2.26	±	0.072	ef	11.58	±	1.111	b
	5)PH with tt	1.39	±	0.041	bc	2.84	±	0.021	def	11.20	±	1.041	b
2 weeks	1)Normal	1.06	±	0.157	def	0.57	±	0.013	ij	12.03	±	1.068	a
	2)PH	0.80	±	0.567	efg	9.06	±	0.903	b	3.44	±	0.201	f
	3)Cirr.-PH.	2.32	±	0.891	a	4.12	±	0.383	d	6.58	±	0.219	c
	4)Cirr.	0.73	±	0.06	fgh	3.36	±	0.982	def	8.68	±	0.801	j
	5)Cirr.-PH with tt.	1.17	±	0.084	def	3.51	±	0.061	de	13.18	±	0.29	de
	6)Cirr.withtt	2.30	±	0.045	a	6.12	±	0.204	c	13.51	±	0.93	g
	7)PH with tt	1.69	±	0.029	bc	0.94	±	0.008	hij	13.24	±	1.13	b

Data are means ± SD of 10 rats in each group; Unshared letters between groups are significant at P ≤ 0.05; Data are expressed in µmol /g wet weight; PH with tt: Partially hepatectomized rats with treatment; Cirr. with tt: Propagated cirrhotic rats with treatment.; Cirr.- PH. with tt: Propagated cirrhotic-partially hepatectomized rats with treatment.; PH: Partially hepatectomized rats.; Cirr.: Propagated cirrhotic rats.; Cirr.-PH: Propagated cirrhotic - partially hepatectomized rats.

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
288

Table 2: Effect of DHEA treatment on adenylate energy charge, totaladenylate, inorganic phosphate levels and phosphate potential in normal and different therapeutic groups

| Time	Groups Parameters	AEC				TA				Inorganic phosphate				Phosphate potential			
1 Day	1)Normal	0.81	±	0.02	bcd	14.09	±	0.11	de	31.05	±	0.002	cde	0.30	±	0.0072	de
	2)PH	0.73	±	0.02	f	5.58	±	0.01	i	6.12	±	0.03	jk	0.41	±	0.003	cd
	3) Cirr.-PH	0.52	±	0.05	i	25.30	±	0.01	a	43.09	±	2.76	a	0.54	±	0.001	f
	4)Cirr.-PH with tt.	0.82	±	0.05	bcd	10.20	±	0.03	f	16.73	±	0.04	hi	0.32	±	0.67	de
	5)PH with tt	0.89	±	0.05	bcd	9.33	±	0.05	fg	39.69	±	13.71	ab	0.29	±	0.065	ef
3 Days	1)Normal	0.81	±	0.02	bcd	14.09	±	0.11	de	31.05	±	0.002	cde	0.30	±	0.0072	de
	2)PH	0.64	±	0.10	gh	7.23	±	2.49	hi	10.63	±	0.96	jk	40.00	±	0.001	cd
	3) Cirr.-PH	0.72	±	0.00	f	8.20	±	0.04	gh	31.68	±	0.087	cd	0.05	±	0.678	f
	4)Cirr.-PH with tt.	0.77	±	0.001	def	12.36	±	0.07	e	7.00	±	0.87	jk	1.03	±	0.003	a
	5)PH with tt	0.76	±	0.03	c	12.88	±	1.88	e	24.82	±	3.054	defg	0.27	±	0.073	ef
1 week	1)Normal	0.81	±	0.02	bcd	14.09	±	0.11	de	31.05	±	0.002	cde	0.30	±	0.0072	de
	2)PH	0.72	±	0.08	f	6.83	±	2.75	hi	15.57	±	6.930	hi	0.23	±	0.0018	def
	3) Cirr.-PH	0.75	±	0.01	f	8.05	±	0.05	gh	18.78	±	0.87	ghi	0.22	±	0.003	def
	4)Cirr.-PH with tt.	0.83	±	0.06	bc	15.24	±	0.00	d	7.18	±	0.98	jk	0.71	±	0.07	b
	5)PH with tt	0.82	±	0.08	bc	15.43	±	1.29	d	22.73	±	1.338	fghd	0.24	±	0.086	def
2 weeks	1)Normal	0.81	±	0.02	bcd	14.09	±	0.11	de	31.05	±	0.002	cde	0.30	±	0.0072	de
	2)PH	0.60	±	0.08	h	13.30	±	0.07	e	19.77	±	0.78	ghi	0.27	±	0.008	d
	3)Cirr.-PH.	0.66	±	0.01	g	13.02	±	1.28	e	24.26	±	4.581	efg	0.08	±	0.004	f
	4)Cirr.	0.81	±	0.09	cde	12.76	±	0.01	e	17.74	±	0.67	ghi	0.15	±	0.007	f
	5)Cirr.-PH with tt.	0.85	±	0.08	abc	17.85	±	1.52	c	13.06	±	1.67	ij	0.77	±	0.06	b
	6)Cirr.withtt	0.76	±	0.05	ef	21.92	±	0.02	b	28.61	±	0.912	cdef	0.08	±	0.01	f
	7)PH with tt	0.86	±	0.02	ab	15.87	±	1.06	d	35.21	±	1.54	bc	0.62	±	0.512	bc

Data are means ± SD of 10 rats in each group.; Unshared letters between groups are significant at P ≤ 0.05; Inorganic phosphate is expressed as µmol /g wet weight. Adenylate energy charge (AEC) and phosphate potential are without dimension.; PH with tt: Partially hepatectomized rats with treatment.; Cirr. with tt: Propagated cirrhotic rats with treatment.; Cirr.-PH with tt: Propagated cirrhotic- partially hepatectomized with treatment.; PH: Partially hepatectomized rats.; Cirr.: Propagated cirrhotic rats.; Cirr.-PH: Propagated cirrhotic -partially hepatectomized rats.
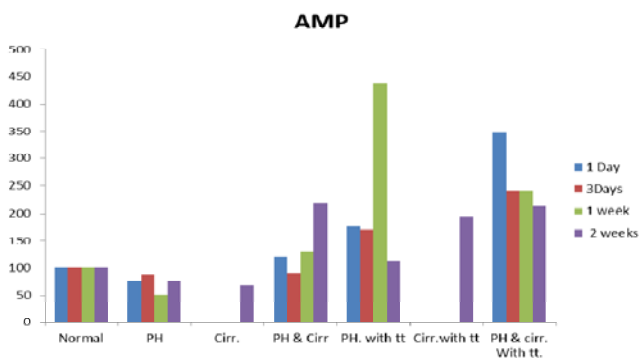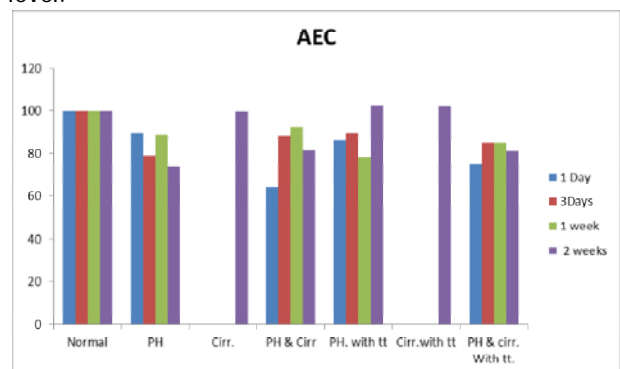

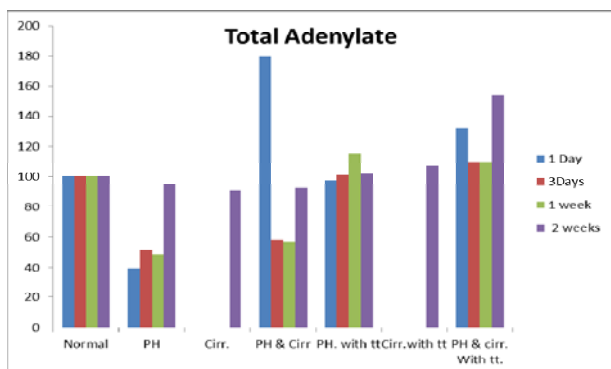**Figure 1:** Percentage change of DHEA treatment on AMP level
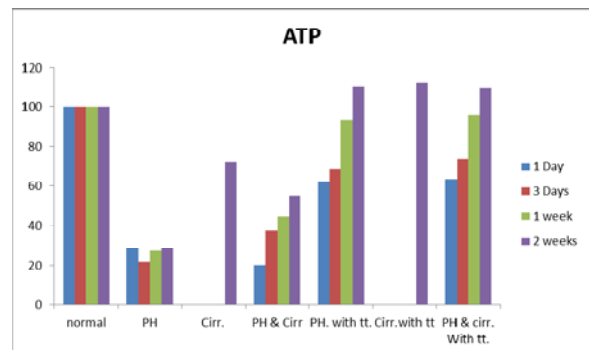
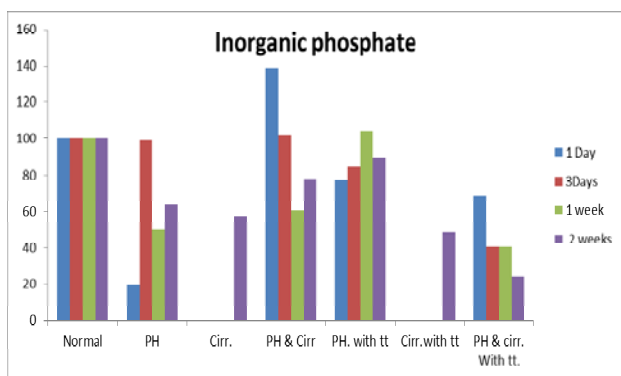**Figure 3:** Percentage change of DHEA treatment on ATP level.
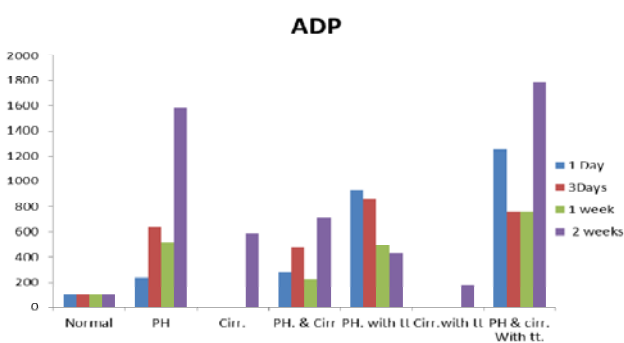
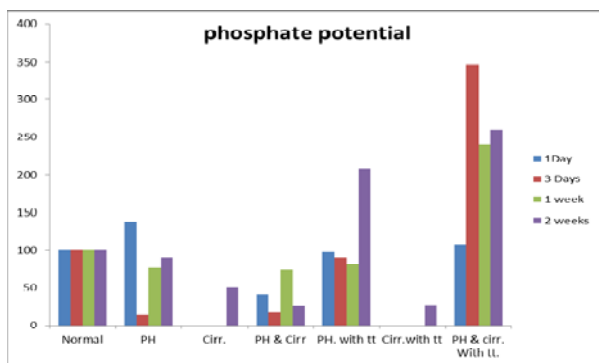**Figure 2:** Percentage change of DHEA treatment on ADP level

Figure 4: Percentage change of DHEA treatment on AEC level

Figure 5: Percentage change of DHEA treatment on total adenylate level.

Figure 6: Percentage change of DHEA treatment on inorganic phosphate

Figure 7: Percentage change of DHEA treatment on phosphate potential

DISCUSSION

Liver regeneration after two-thirds partial hepatectomy (PH) in rodents has become a useful paradigm of studying regenerative organ growth. The popularity of the model is based on two important aspects. First, the removal of the resected tissue is not associated with massive necrosis. The resected hepatic tissues are amenable to "clean" removal due to the multilobular structure of rat and mouse liver. Thus, regeneration of the residual lobes from its very beginning is mediated by processes relevant only to liver tissue and not to necrosis or acute inflammation. In contrast, in models involving necrosis of lobular zones induced by toxins (such as CCl_4, Di-methyl nitrosamine), the events of first day after toxic injury are dominated by acute inflammation of the necrotic zones. Polymorphonuclear leukocytes and macrophages

infiltrate the necrotic area to remove dead hepatocytes. *Second,* because PH stimulates immediate initiation of regeneration without complications from inflammatory situations, and because PH can be performed in a few minutes, the regenerative phenomena can be precisely timed, with a reference (time 0) point from the time of the performance of PH. These two attributes of the model are the major reason for its usefulness and enhanced popularity.[15] Hepatic fibrosis, which arises from overproduction of extracellular matrix, including collagens, is a prepathologic state of cirrhosis that occurs as a consequence of severe liver damage in diverse chronic liver diseases. Fibrogenesis with loss of liver function leads to development of cirrhosis. Dimethylnitrosamine (DMN) through metabolic activation by cytochrome P450 2E1 exerts hepatotoxicity and tissue injury. Liver injuries induced by multiple DMN treatments lead to hepatic necrosis, fibrosis, and eventually cirrhosis.[6]

Because of liver function is usually impaired in patients with cirrhosis, and because cirrhotic livers are less able to regenerate, it is important to stimulate both the regeneration and function of the remnant cirrhotic liver after hepatectomy.

Liver injury is characterized by oxidative stress, inflammation and fibrosis. The use of drugs for cirrhosis treatment might be focused to attack the causes of oxidative stress and inflammation preventing cirrhosis complications and apoptosis.[16]

The ameliorations of DHEA are attributed to a multifunctional steroid produced in the adrenal cortex. It can also be synthesized in the central and peripheral nervous system[17], and is involved in a wide range of biological effects in humans and rodents. Together with its sulphate ester (DHEA-S),they are the most abundant steroids in humans that can act both directly or through its metabolites (including androstenediol and androstenedione), which can undergo further conversion to produce primarily testosterone and estradiol.[18] Several beneficial effects have been observed in relation to the administration of DHEA (mainly in animals), including improvement of vascular function[19], cardioprotective, enhancement of glucose uptake in adipocytes and hepatocytes[20], prevention of oxidative tissue damage, and action as an antioxidant agent, all of which provide a promising strategy for the treatment of hepatic disorders and nephropathy.[21] DHEA supplementation in rodents has produced antioxidant effects in a wide variety of protocols for oxidative stress.[22] Thus, the ameliorative effects of DHEA may be related to its antioxidant effect that preserved liver functions and architectures.[23]

Regarding to adenylate nucleotide pool, the present results reveal that partially hepatectomized rats exhibited insignificant decrease in AMP level after one, three days and two weeks, while significant decrease post one week of operation was demonstrated. ADP level showed significant increase with time intersection reached to its

International Journal of Pharmaceutical Sciences Review and Research
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290

highest percentage two weeks post-surgery. However, ATP level showed significant decrease with time, recorded the lowest level two weeks post operation. Whereas, AEC recorded significant decrease with time intersection and showed its lowest value two weeks post operation. Also, total adenylate, showed significant decrease with the time intersection. With regard to, inorganic phosphate and phosphate potential, significant decrease was noticed in inorganic phosphate with the effect of time, showed the lowest percentage decrease, at two weeks post operation, while phosphate potential demonstrated insignificant change at different durations post operation as compared to the normal healthy rats .In addition, cirrhotic rats showed an insignificant decrease in AMP level, while ADP level recorded significant increase. In contrast, ATP level demonstrated significant decrease. Moreover, AEC , total adenylate and phosphate potential showed insignificant decrease, while significant decrease was detected in inorganic phosphate concentration as compared to the normal healthy rats. Cirrhotic - partially hepatectomized rats, showed an insignificant change in AMP level one, two days and one week post operation, while two weeks showed significant increase in AMP level. While, ADP level exhibited significant increase with the time intersection. In contradictory, ATP showed significant depletion with the effect of time .Whereas, AEC showed significant decrease at different durations recorded the lowest percentage decrease after one week. Total adenylate and inorganic phosphate showed significant increase after one day. While, significant decline was demonstrated in total adenylate after three days and one week, followed by insignificant change after two weeks. However, inorganic phosphate recorded insignificant change after three days and significant decline at one week and insignificant change post two weeks as compared to normal control rats. Furthermore, phosphate potential declared significant decrease at one, three days and two weeks, while one week recorded insignificant change.

It's of a great importance to mention that, mitochondria, the main supplier of cellular energy, are functionally impaired during the early phase of liver regeneration after partial hepatectomy. Mitochondrial dysfunction is accompanied by a higher release of ROS, oxidative damage for membrane proteins, lipids and a decreased ATP production.[24] Previous studies have demonstrated that cellular apoptosis consumes large amounts of nicotinamide adenine dinucleotide (NAD), and the process to resynthesize NAD, results in a decrease of cellular ATP level.[25] Our data showed that ATP content was dramatically decreased in the livers of different experimental models. These situations were correlated with elevated levels of oxidative stress and increased expression of pro-apoptotic genes.[25]

The energy charge level, which indicates a metabolically available energy pool, is normally maintained at a constant level. In this dynamic steady state, a rise in energy expenditure in the cells would result in a decrease of the energy charge. Also, it is considered as an index of such cellular energy status for understanding the range of equilibrium between energy generating and energy utilizing reactions within cells.[26]

It was found that, AMP nucleosides acts as a regulator of intracellular levels of AMP. Regulation of the activity is a consequence of alterations in the ratio of ATP, AMP and Pi. Regulation of cellular energy metabolism may be brought about by changes in the ATP/ADP ratio, which is considered to reflect the metabolic status of the cell, a low ratio indicates reduced metabolism. ATP/AMP ratio reflects the energy requirement of a cell and rate of ATP utilization. Cells which require a large change in the rate of glycolysis to satisfy energy demands theoretically possess a high ATP/AMP ratio.[27]

In Okatani et al.[28] study, marked reduction in respiratory control index was detected in hepatic mitochondria from ischemia / reperfusion and explained this observation to a reduced State 3 respiration and an increased State 4 respiration. The decrease in ADP/O in mitochondria from ischemia/reperfusion was likely due to uncoupling as a result of membrane damage. Ischemia / reperfusion and almost in general liver injury, a marked drop in the pH indicating a decrease in ATP synthesis and an uncoupling of oxidative phosphorylation. The increase in State 4 respiration in mitochondria during liver injury may also be explained by their uncoupling. The stimulation of uncoupling induced by ischemia/reperfusion or cytotoxicity was supported by increase in ATPase activity. It was suggested that, the coupling mechanism for energy transfer reactions of the electron transport system may be altered during ischemia/reperfusion and liver injuries. Higher levels of oxygen free radicals are also found when the respiratory chain is inhibited.[29] A large body of evidence suggested that a channel formed in mitochondrial membranes, identified as the permeability transition pore, is involved in cell damage associated with ischemia/reperfusion and several liver injuries.[30] This channel increases the permeability of the inner mitochondrial membrane to solutes.[31] Permeability transition pore opening is triggered by the association of calcium overload with an inducer such as oxidative stress or high phosphate concentrations; both these conditions are encountered during ischemia/reperfusion and liver injuries.[28]Treatment of partially hepatectomized rats with DHEA, significant (p≤0.05), decrease in AMP level was detected 1st day of DHEA treatment, while significant increase was recorded at 3rd day, one and two weeks of treatment with percentages of improvement reached to 68.70,79.00,83.40%, respectively. Also, ADP exhibited significant increase at 1st, 3rd days and one week post treatment with percentages of improvement 34.80%, 118.00%, 25.50% respectively, while insignificant change was observed in ADP level two weeks post DHEA treatment . Remarkable significant increase in ATP level was noticed post two weeks with percentage of improvement 81.40% as compared to normal healthy rats.AEC showed insignificant change with time

intersection post DHEA treatment, while, significant decrease was observed in TA level at 1st day with percentage of improvement reached to 19.60%, then, insignificant change was detected. On the contrary, significant increase in inorganic phosphate was recorded one day and two weeks post treatment with percentage of amelioration 108.10%, an insignificant change was noticed in inorganic phosphate level after three days, while significant reduction was recorded post one week. Treatment of partially hepatectomized rats with DHEA showed also, insignificant change in phosphate potential at 1st, 3rd days and one week post treatment, while, two weeks after treatment showed significant increase (p≤ 0.05), reached to 116.8%, as compared to normal healthy rats.

It was found that, cirrhotic rats after two weeks of DHEA treatment, exhibited significant increase(p≤0.05) in AMP,ADP, ATP and TA with percentages of improvement amounting to 147.10, 481.30,40.10 and 64.90%, respectively, while AEC and phosphate potential recorded significant decrease as compared to normal control group. Although, insignificant change was noticed in inorganic phosphate level as compared to the normal healthy rats.

Moreover, treatment of cirrhotic –partially hepatectomized rats with DHEA showed insignificant change of AMP level post two weeks of DHEA treatment with a percentage of improvement 115.00%.Also, ADP showed significant (p≤0.05), increase with time intersection recorded its lowest level after three days with percentage of improvement 258.10 %,while the percentage of improvement reached to 106.30% post two weeks of treatment. However, ATP showed significant increase at 2nd week post treatment with percentage of improvement 54.80% as compared to normal control group. With regard to AEC, demonstrated insignificant change with the time intersection as compared to normal control group. TA level showed significant increase after two weeks of DHEA treatment with percentage of improvement 34.30% as compared to normal control group. Inorganic phosphate exhibited significant decrease in cirrhotic –partially hepatectomized rats treated with DHEA with the time recorded percentages of enhancement reached to 84.80, 597.90, 37.30 and 36.00% after one, three days, one and two weeks respectively. While, phosphate potential demonstrated insignificant change one day post treatment as compared to normal control, then significant increase was detected after three days, one and two weeks with percentages of improvement reached to 329.90, 164.90 and 234.30%, respectively as compared to normal healthy rats.

The enhanced levels of adenylate nucleotide pool in different experimental groups treated with DHEA may be explained on the basis of, increased ADP-phosphorylation rates, resulted in stimulation of respiratory activity and increase in the energy potential of the liver.[32] It is possible that DHEA treatment-induced changes in the

respiratory activities in mitochondria that could influence the cellular reactive oxygen species (ROS). Thus, treatment with DHEA leads to stimulation of respiratory activity and increase in the energy potential of the liver mitochondria. However, the effects were dose dependent and tissue specific. Also, higher dose (2.0 mg) of DHEA had adverse effects.[32] In addition, treatment with DHEA resulted in an increase in the contents of cytochrome aa_3b and ATPase activity. It is well established that crucial polypeptide of cytochrome oxidase, cytochrome b and FoF1 ATPase are coded by mitochondrial DNA.[33] It may be suggested that, DHEA action may be mediated by activating specific mitochondrial genes coding for polypeptide subunits of cytochrome aa1, b and FoF1 ATPase. In this connection, the presence of dexamethasone binding site in COX II region of mitochondrial genome has been demonstrated.[32] It would be interesting to know if similar DHEA binding site(s) exists on mitochondrial genome. Likewise, increase in the glutamate dehydrogenase and succinate reductase activities suggested that DHEA action may also be specific for activating these nuclear genes.[23] One mechanism indicated that, DHEA decreases the methylation of hepatic DNA by NDMA (Dimethyl nitrosamine), without inhibiting the metabolic activation of the carcinogen. This may be attributed to DHEA increased cellular protein content, which in turn serves as a trap for the electrophilic form of NDMA, thus reducing damage to cellular DNA. The significance of the results of this study is the decreased methylation of DNA in rats treated with DHEA. The present results supported the hypothesis that DHEA modulates NDMA–induced hepato-carcinogenesis.[23]

It has been reported that treatment with DHEA elevated the concentration of NAD and NADP in liver[34], and enhanced the rate of malic enzyme gene transcription.[35] Hypertrophy of hepatocytes following treatment with DHEA has been attributed to proliferation of peroxisomes and mitochondria.[36] Antioxidant effects of DHEA have also been demonstrated.[37]

Hormone replacement therapy with DHEA is a frequently discussed topic, especially in Alzehimar's disease(AD) and general liver disease.[38] DHEA is a well-known inhibitor of glucose 6-phosphate dehydrogenase (G6PD), and it is hypothesized that reduced G6PDH activity has a beneficial effect on age related disease development and longevity.[39] Because NADPH is a key co-factor in the activity of many antioxidative and reductive enzymes[40], its depletion may result in an impairment of fuel utilization and consequently the onset of the diseases. In addition to its other roles, NADPH is a co-factor for the enzyme CYP7B1, which is abundant in brain and liver tissues and responsible for 7a-hydroxylation of DHEA.[41] In a good agreement with the present findings Liu and Dillon[19], found that, mitochondria, isolated from rat livers during the early phase of liver regeneration (7–24)hours after partial hepatectomy), showed: (i) decrease in the rate of ATP synthesis; (ii) increase of malondialdehyde

and of oxidized protein production; (iii) decrease of the content of intra-mitochondrial glutathione and protein thiols of mitochondrial proteins; (iv) increase of the glutathione bound to mitochondrial proteins by disulfide bonds. These observations suggested an increase production of oxygen radicals in liver mitochondria, following partial hepatectomy, which can alter the function of the enzymes involved in the oxidative phosphorylation.

CONCLUSION

It could be concluded that, fluctuation in adenylatenucleotide pool level was achieved in hepatectomized, propagated-cirrhotic and cirrhotic - partially hepatectomized rats and attenuation of these findings upon treatment with DHEA. Thus, DHEA treatment markedly ameliorate the adenylate pool including the level of ATP, ADP, AMP, phosphate potential, inorganic phosphate and adenylate energy charge in different therapeutic groups.

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