

Modulatory Effects of Artichoke Leaf Extract on Nandrolone Decanoate-Induced Biochemical Alterations in Rats

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Abstract: Anabolic androgenic steroids (AAS) have been associated with several side effects range from hypogonadism to cardiac and hepatic dysfunction and alteration of blood lipid levels. Artichoke leaves extract (ALE) exhibits hepatoprotective, antioxidant, hypolipidemic and hypocholesterolemic effects. The present study was conducted to evaluate for the first time whether the use of ALE in concomitant with high dose of nandrolone decanoate (ND) which is one of the AAS compounds, can improve some or most of the possible adverse effects of the drug. Fifty Wistar albino male rats were equally divided into five groups, ten each; the first group (G1) received saline by oral gavage daily. The second group (G2) was intramuscularly injected with vehicle alone (sesam oil) once weekly. The third group (G3) received an intramuscular injection of ND (20 mg/kg) once weekly, the fourth group (G4) received an oral daily dose of 1 gm/kg b.w. of ALE. The fifth group (G5) was given a combination of oral ALE in concomitant with ND the same as in G3 and G4. The duration of the experiment was 6-weeks. Blood samples were collected after two and six weeks and liver tissue samples were collected at the end of experimental period from the control and treated groups. The serum samples were assayed for glucose, lipid profile, AST, ALT, albumin, calcium and iron). Some antioxidants and oxidative stress indices (GSH, SOD, MDA and NO) were determined in the liver homogenates. Results revealed that all ND-treated animals exhibited a significant increase of body weight gain (BWG) and serum glucose, serum triacylglycerols (TAG), total cholesterol, LDL, ALT and AST and hepatic MDA content, a significant decrease in HDL and calcium, hepatic GSH content without significant change in serum albumin, iron, SOD and NO concentrations compared to control oil. On the other hand, the administration of ALE with ND could significantly modulate these abnormalities in the tested parameters. The study reflects the ameliorating effect of the ALE on the ND-induced alteration in the lipid profile, glucose level and hepatic tissues. We can conclude that ALE has antioxidative, hepatoprotective and hypolipidaemic activities that may be attributed to its constituents of polyphenolic and flavonoids.

Key words: Anabolic Androgenic Steroids • Artichoke Leaf Extract • Lipid Profile • Liver Function • Oxidative Stress

INTRODUCTION

Anabolic Androgen steroids (AAS) include both natural compounds and synthetic analogues of testosterone [1]. One of the most commonly used AAS is nandrolone decanoate (ND); a synthetic long acting 19-nortestosterone derivative which exhibits greater anabolic -to-androgenic ratio. It is the most popular AAS prescribed for treatment of weight loss.

Despite numerous adverse side effects are present, AAS are still used widely as a therapy for a variety of clinical conditions such as the treatment of anemia, growth stimulation, appetite stimulation, osteoporosis, to counteract many wasting diseases and other catabolic conditions and improvement of age related problems [2]. For several years testosterone and its derivatives have been extensively and illegally used by body builders to improve physical performance [3].

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Adverse side effects of AAS range from hypogonadism to cardiac and hepatic dysfunction and alteration of blood lipid levels [4]. In addition, the AAS abuse causes mood alterations [5], aggression [6] and may produce dependence [7]. However, severe side effects depend on a variety of factors, from which, the type, the dose and the duration of administration, as well as the gender of the person taking the drug. Younger individuals and women show greater risk of side effects [8], prolonged use of AAS at high dose also show great risk [3].

Cynara scolymus, L. (Artichoke) belonging to the Family *Asteraceae*, is used as an edible vegetable owing to its nutritive value and medicinal properties [9]. It is a cultivated plant that grows in Egypt and many other countries. The aqueous extract of *Cynara scolymus*, L. leaves contains several polyphenolic compounds, flavonoids and sesquiterpenes (cynarin, luteolin, isochlorogenic acid, chlorogenic acid, caffeic acid and quinic acid) as reported by Gebhardt [10]. Also it contains many biologically active compounds responsible for its medicinal properties. Where, a total of 15 amino acids were found in *Cynara scolymus* leaves. Nine of these were essential: valine, threonine, methionine, isoleucine, leucine, lysine, phenylalanine, histidine and arginine [11].

The results of several clinical studies showed the efficacy and safety of *Cynara scolymus*, L. extracts as a hepatoprotective, antioxidant, hypolipidemic and hypocholesterolemic [12]. These effects indicated that the liver is the main target organ [13]. However, other effects of *Cynara scolymus*, L. extracts such as spasmolytic, carminative and antiemetic were also reported [14].

Numerous studies have shown that dietary flavonoids protect against vascular diseases, hyperlipidemia and reduce the risk of myocardial infarction [15]. One of the possible mechanisms in this case could be founded on antioxidative activity that has been attributed to flavonoids, chlorogenic acid and cinnarine; all of these are antioxidants in vitro [16].

In view of the importance of oxidative stress and hyperlipidemia in atherogenesis, we have now decided to study the biochemical influence of extract from artichoke on the possible side effects that may result from the high dosage of ND treatment. Therefore, it was the first time to use ALE in concomitant with supraphysiological dose of ND as a trial in order to improve the possible side effects resulting from AAS.

MATERIALS AND METHODS

Animals: Fifty adult Male albino Wistar rats each weighs 100-120 g, were obtained from Helwan farm of laboratory animals Cairo; Egypt and acclimatized for 2 weeks before the onset of experiment. The animals were left free to access water and were fed on uniformly basal diet (20 % Protein, 4.6 % Fat, 6.4 % Fiber, 6.5 % Ash, 50.5 % Nitrogen free extract, 12 % moisture). They were housed individually in metal cages under normal laboratory conditions.

Chemicals: The concentration of serum glucose, total cholesterol, HDL-cholesterol, Triacylglycerols and albumin were measured spectrophotometrically as described earlier [17] using kits supplied by *Human Company, Germany*. ALT, AST, calcium and iron kits were purchased from *Spinereact Company, Spain* and were determined as described earlier [17]. Hepatic concentrations of reduced glutathione (GSH), Super oxide dismutase (SOD), Lipid peroxide (MDA) and nitric oxide (NO) were determined using kits supplied by *Biodiagnostic Company, Egypt* [18-21]. All other chemicals used were of analytical grade and were obtained from *Sigma* (St. Louis, MO, USA).

Tested Materials:

- Nandrolone decanoate (ND) (Deca-Durabolin[®] produced by *Nile Co., Egypt, under license from N.V. Organon, Oss, Holland*) was purchased from a local pharmacy in a form of injection. It is an AAS with a chemical formula (C₂₈ H₄₄ O₃). The drug was diluted to the selected dose in a Sesame Oil.

Plant Material: *Cynara Scolymus*(Artichoke) leaves were obtained from the farm of Sadat City, Egypt. The samples were identified at the Botany Department, Faculty of Agriculture, Cairo University; Egypt.

Artichoke Leaves Extract Preparation: *Cynara Scolymus* leaves have cleaned; shade dried, powdered using blender into fine powder. The powdered plant material (1 kg) was extracted by percolation with methanol 95 % according to the method described by Awaad and Zain [22]. Successive addition of methanol to plant material was carried out till complete exhaustion of the leaves. The methanolic extract was concentrated under reduced

pressure using (*Rota Vapour apparatus; Switzerland*) till dryness and then weighed (yield 163 gm extract). The residue was dissolved in distilled water and filtered. The filtrate was evaporated to dryness. The dried mass was diluted with distilled water just before oral administration.

Grouping: The rats were randomly assigned into five groups (10 rats each) and treated as follows:

G1: Administered saline by stomach tube, used as a negative control saline group.

G2: Given a weekly dose of 0.25 ml sesame oil (vehicle) by deep I/M injection. It was considered as negative control oil group.

G3: I/M injected with ND at a dose of 20 mg./kg. b.w. once a week according to Tylicki *et al.* [23].

G4: Given an oral daily dose of 1 gm/kg b.w. of ALE according to Sae'nz Rodriguez *et al.* [24].

G5: Was administered a combination of oral ALE at a dose of 1 gm/Kg. b.w. daily in concomitant with 20 mg./kg. b.w. ND weekly by I/M injection. The experimental period stilled for 6 successive weeks.

The rats in all groups were weighed every week and body weight gain % was calculated according to the method of Chapman *et al.* [25] using the following formula:

$$\text{BWG \%} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

Sampling and Tissue Preparation

Blood Samples: At the end of 2nd and 6th weeks of treatment, blood samples were collected from animals at fasting state (in order to minimize the feeding-induced changes in lipid pattern and to measure fasting blood glucose level). Part of the obtained sera was quickly assayed for fasting blood glucose level, the remainder was stored at -20° C till used for estimation of various biochemical parameters including TAG, total cholesterol, high and low density lipoproteins (HDL), (LDL), albumin, AST, ALT, iron, calcium.

Liver Samples: At the end of experiment, liver was quickly excised after dissection of the animals, usually 0.5 gm, were homogenized in ten volumes of (ice -cold phosphate buffer saline PH: 7) using Teflon homogenizer

(Glass-Col, Terr Haute, USA). The homogenate was kept in deep freezer at -20°C for MDA, GSH, SOD and NO assay.

Statistical Analysis: Statistical analysis was carried out using Graph Pad In stat software (version 3, ISS-Rome, Italy). One way analysis of variance (ANOVA) test followed by Tukey-Kramer (TK) multiple comparisons post test were used. The values are expressed as mean±standard error (SE). The *p* values below 0.05 were considered statistically significant.

RESULTS

There were no significant variations between G1, G2 and G4 in all tested parameters (at $p \leq 0.05$).

Our data in table (1) showed a prominent significant increase in BWG after ND and/or ALE intake compared to either G1 or G2 (at $p \leq 0.05$). Furthermore, the combination of ALE with ND resulted in a marked significant increase of BWG compared to G1 and G2 all over the experimental periods. Regarding the effect of ND on serum albumin level, G3 showed no significant variations in serum albumin concentration compared to G2 all over the experimental periods although, a slight decrease was observed. The plant induced a significant increase in serum albumin level compared to G1 and G3 at the last period ($p \leq 0.05$) (Table, 1).

Concerning the effect of ND on fasting glucose (Table 1), the G3 showed significant increase (at $p \leq 0.05$) in serum glucose level during all experimental periods compared to G2. The administration of ALE with ND could significantly decrease serum glucose level compared to G3. In all groups serum glucose level showed a significant increase at the end of experimental period.

Table (2) showed that, ND treatment induced a significant elevation in serum TAG (at the last period only), total cholesterol, LDL-cholesterol levels all over the experimental periods while the contrast occurred in special to HDL level compared to G2 (at $p \leq 0.05$). Furthermore, the plant took the values of total cholesterol, TAG, LDL and HDL back towards normal levels after its administration with ND when compared to G1, G2 and G3. As expected, a significant increase in HDL and a significant decrease in LDL levels were noticed in G4 compared to G1 group (at $p \leq 0.05$) only at the end of experiment.

Concerning the effect of treatments on liver function tests (Table 3), G3 showed a significant increase in serum ALT activity (at all periods), AST activity (at the end of

Table 1: Body weight gain (BWG) (g), serum albumin (g/dl) and fasting Serum glucose concentration (mg/dl) in control and treated rats at different periods

		Control Saline group	Control oil group	ND-treated group	ALE-treated group	ALE+ ND-treated group
After two weeks	BWG	22.60±1.56 ^{ax}	23.90±1.36 ^{ax}	36.30±1.77 ^{bx}	26.35±2.32 ^{acx}	34.80±1.94 ^{bcx}
	Albumin	4.55±0.45 ^a	4.85±0.37 ^a	4.00±0.33 ^a	4.34±0.52 ^{ax}	4.18±0.77 ^a
	Fasting glucose	63.39±6.58 ^{acx}	55.31±5.71 ^{ax}	74.45±6.44 ^{cx}	52.01±4.43 ^{ax}	54.33±5.64 ^{ax}
After six weeks	BWG	62.00±3.59 ^{ay}	64.90±5.68 ^{ay}	77.60±3.01 ^{by}	92.10±1.86 ^{cy}	84.10±2.17 ^{bey}
	Albumin	4.64±0.57 ^a	4.58±0.41 ^a	4.06±0.34 ^a	7.73±0.10 ^{by}	4.30±0.50 ^a
	Fasting glucose	76.47±3.10 ^{ax}	77.46±2.58 ^{ay}	102.30±3.89 ^{by}	73.08±4.46 ^{ay}	87.53±4.35 ^{ay}

Table 2: Serum triacylglycerol (TAG), total cholesterol, HDL and LDL concentrations (mg /dl) in control and treated rats at different periods

	Parameter	Control Saline group	Control oil group	ND-treated group	ALE-treated group	ALE+ND-treated group
After two weeks	TAG	40.23±2.56 ^a	40.14±2.02 ^a	54.20±4.21 ^a	41.33±3.75 ^a	42.80±4.01 ^a
	Cholesterol	64.74±5.27 ^a	65.54±10.04 ^a	95.23±8.52 ^b	61.52±5.58 ^a	77.02±4.80 ^{ab}
	HDL	26.76±2.76 ^{ax}	26.30±1.69 ^{ax}	9.020±0.33 ^{bx}	27.01±3.50 ^{ax}	15.07±1.36 ^{bx}
	LDL	27.43±2.34 ^a	28.89±8.08 ^a	74.29±8.23 ^c	24.04±5.19 ^a	51.32±2.92 ^b
After six weeks	TAG	44.71±1.32 ^a	42.39±2.74 ^a	66.00±5.59 ^b	45.91±3.85 ^a	45.29±3.87 ^a
	Cholesterol	80.86±4.58 ^a	82.75±9.94 ^a	115.5±7.92 ^b	71.01±7.67 ^a	88.37±4.53 ^a
	HDL	31.43±2.87 ^{ax}	33.65±3.08 ^{ax}	20.66±1.76 ^{by}	43.42±3.47 ^{cy}	28.96±2.58 ^{ay}
	LDL	39.94±2.46 ^a	39.57±7.08 ^a	80.72±5.03 ^c	18.41±3.52 ^b	47.21±2.95 ^a

In rows: Means have different superscripts (a,b,c) indicate significant variation ($P \leq 0.05$). In columns: Means have different superscripts (x,y) indicate significant variation ($P \leq 0.05$)

Table 3: Serum ALT, AST activities (U/L), Serum iron and calcium concentrations (mg/dl) in control and treated rats at different periods.

		Control Saline group	Control oil group	ND-treated group	ALE-treated group	ALE+ ND-treated group
After two weeks	ALT	56.030±3.12 ^{ab}	52.93±2.62 ^a	67.570±3.89 ^{bx}	52.57±2.81 ^a	51.67±4.47 ^a
	AST	137.1±11.27 ^a	140.4±7.60 ^a	162.2±5.21 ^a	135.0±3.44 ^a	151.6±14.16 ^a
	Calcium	13.37±0.69 ^a	12.81±1.55 ^a	11.91±0.45 ^{ax}	13.72±0.72 ^a	13.61±0.58 ^a
	Iron	158.8±15.83 ^a	150.3±1.22 ^a	167.6±16.33 ^a	186.4±15.94 ^a	180.2±16.55 ^a
After six weeks	ALT	54.730±4.94 ^a	50.700±4.63 ^a	92.5±8.54 ^{by}	52.29±3.29 ^a	50.75±3.48 ^a
	AST	130.3±9.76 ^a	127.7±6.06 ^a	177.7±15.13 ^b	124.6±10.61 ^a	141.1±7.47 ^a
	Calcium	12.07±1.26 ^a	11.94±0.78 ^a	7.157±0.55 ^{by}	15.20±0.89 ^c	10.84±1.19 ^a
	Iron	154.0±16.48 ^a	153.3±8.87 ^a	134.1±13.09 ^a	207.0±16.73 ^b	176.4±16.59 ^{ab}

In rows: Means have different superscripts (a,b,c) indicate significant variation ($P = 0.05$). In columns: Means have different superscripts (x,y) indicate significant variation ($P \leq 0.05$)

Table 4: Hepatic GSH, SOD, MDA and NO concentrations in control and treated rats after six weeks of treatment.

	Control Saline group	Control oil group	ND-treated group	ALE-treated group	ALE+ ND-treated group
GSH (mg/g t)	13.15±1.09 ^a	13.17±0.67 ^a	9.12±0.96 ^b	13.04±0.82 ^a	12.86±1.00 ^{ab}
SOD (U/mg t)	3.236±0.51 ^a	3.014±0.33 ^a	3.909±0.49 ^a	3.886±0.37 ^a	4.629±0.26 ^a
MDA (nmol/g t)	4.598±0.30 ^a	4.573±0.46 ^a	7.71±0.85 ^b	3.682±0.39 ^a	4.580±0.56 ^a
NO (u.mol/L)	32.80±2.81 ^a	33.81±2.71 ^a	39.75±3.28 ^a	32.65±1.13 ^a	35.72±1.93 ^a

Means have different superscripts (a,b) indicate significant variation ($P \leq 0.05$)

experiment only) compared to G2 (at $p \leq 0.05$). The combination group exhibited a significant decrease in both serum ALT (at all periods) and AST (at the last experimental period) activity compared to G3. These results may explain the protective effect of ALE on the liver tissue against the possible side effect of ND.

At the end of experiment, serum calcium concentration was significantly decreased by administration of ND alone but returned towards normal values after administration of plant extract with ND drug. Furthermore, the plant extract could significantly increase

both serum iron and calcium concentrations with a higher level than those of G1 and G3 (Table 3).

Concerning the effect of ND on hepatic oxidants\ antioxidants status (Table, 4), statistical analysis revealed a significant increase of hepatic MDA and a significant decrease of hepatic GSH contents ($p \leq 0.05$) compared to G2. The plant extract could return these values to the control values as noticed in hepatic tissues of rats receiving a combined ALE and ND drug. ND treatment induced no effect on both hepatic NO content and SOD activity.

DISCUSSION

Anabolic steroids seem to be effective in two ways: the first, they convert a negative nitrogen balance to a positive one by improving the use of ingested protein and increasing protein synthesis in skeletal muscle. Anabolic steroids are believed to exert their effects by binding to androgen receptors, that are present in the reproductive tract as well as in many non reproductive tissues, including bone, skeletal muscle, brain, liver, kidney, prostate and adipocytes [2, 26] which then translocate to binding sites on chromatin, promoting gene transcription, stimulating production of mRNA and ultimately increasing protein synthesis [27]. The second, Steroids compete for glucocorticosteroid receptors, leading to decreased protein catabolism. Thus, the anabolic action of androgens is mediated directly through androgen-receptor binding and also indirectly by their antiglucocorticoid action [28].

In the present study, ND treatment exerted a significant increase of BWG compared to rats in G2 (Table 1). Nandrolone decanoate treatment resulted in greater increase in fat-free mass than placebo [29]. Moreover, Schols *et al.* demonstrated a substantial beneficial effect of ND on lean body mass, superior to that achieved with nutritional intervention alone [30]. The present results support the concept that steroid and possibly all anabolic steroids have an ability to counteract the catabolic action of corticosteroids that is greater than their androgenic activity.

An interesting feature of the present study was that the BWG of rats treated with ALE alone or in concomitant with ND was higher than that of control rats (all over the experimental periods) and higher than that of ND-treated rats (G3) (at the end of experiment). In other words, the use of AAS appears to be effective in increasing BWG when taken in conjunction with Artichoke. On the other hand, the gradual increment of BWG throughout the different experimental periods may be attributed to the progress of experimental rats in age.

Our finding concerning the effect of ALE in elevation of BWG may agree with the concept that the flavonoids might be able to act as growth hormones in animals. These results have been observed by [31] who found that, artichoke increased growth rate.

Considering that albumin is the most abundant protein in plasma, we assayed the concentration of albumin in serum of ND-treated rats. In the present study, no significant differences in the mean values of serum albumin concentration were noted between rats of the G3

and those of G2 all over the experimental periods although, a slight decrease was observed. Recent studies have reported that higher doses of testosterone in healthy men (receiving 600 mg of testosterone weekly for 10 weeks) increase body weight, fat-free mass, muscle size and strength [32]. The slight decrease of albumin level detected in the present study especially after two weeks of treatment could be a consequence of the increased anabolism and utilization of nitrogen of ingested protein in tissue building.

In our work, ALE-treated group(G4) showed a significant increase in serum albumin at the end of experiment compared to all other groups and compared to the first experimental period in the same group and this might be attributed to the more anabolic effect of flavonoids after long period of intake so the BWG was increased in G4 at the end of experimental period than other groups and this agree with Baker *et al.* [33] who suggested that, Flavonoids can act as growth hormones in animals and increase growth rate [31]. Also the hepatoprotective activity of artichoke [34] may play a role by increasing biosynthesis of proteins (including albumin) by liver. But this hepatoprotective effect of the plant extract was not observed when the plant extract was given with ND and thus this result may explain slight changes occurred in hepatocytes and decreased ability of proteins synthesis by liver after prolonged administration of ND.

Concerning the effect of ND on serum fasting glucose, treatment with ND caused significant increase in serum fasting glucose level during all experimental periods compared to the control oil (G2), ALE-treated (G4) and combination (G5) groups. On contrary, the reverse occurred after administration of ALE with ND (Table 1).

Altered glucose tolerance with increased insulin resistance, as well as, decreases of thyroid hormones are the commonest non-gonadal endocrine side effects of AAS reported in male power lifters [2]. In this respect, Hagawane *et al.* [35] concluded that Ketotic buffaloes, treated with Nandrolone laurate 125 mg intramuscularly once, showed significant elevation of blood glucose level and Ambore *et al.* [36] found that, a single parenteral treatment with the anabolic steroid nandrolone phenylpropionate along with glucose is more effective than glucose alone in treating clinical and subclinical ketosis where the treatment significantly increased blood sugar and decreased ketones. Additionally, supraphysiologic doses of androgens cause insulin resistance in humans [37]. These results may indicate that anabolic steroids have diminished glucose tolerance, which is likely to be secondary to insulin resistance.

Regarding to the effect of plant extract, ALE, the present results showed that the administration of ALE in concomitant with ND counteracted the effect of ND where it significantly decreased serum fasting glucose levels all over the experimental periods. These obtained results may reflect the improving effect of the plant extract in modulating some of undesired effects of ND administration. The obtained results in the current investigation concerning serum fasting glucose levels were in accordance with data reported by Mahmoodabadi [38] that concluded that ALE or inulin feeding decrease plasma glucose due to the effect of extract in repairing damaged pancreatic tissue in diabetic rats and induce insulin secretion from the β -cells [39].

Administration of testosterone or synthetic anabolic steroids is associated with increased risk of hypertension, alterations in lipid profile, accelerated coronary artery disease, thrombosis, myocardial infarction, heart failure and sudden death [40]. Regarding to the effect of ND on serum lipid profile, our data showed that, ND- treatment induced a significant elevation in serum triacylglycerols (at the last period only), total cholesterol, LDL-cholesterol levels all over the experimental periods while the reverse occurred in HDL level compared to control oil group (G2). These results agreed with George who found an increase in serum TAG and total cholesterol level in AAS abusers [41]. Gold *et al.* also reported higher levels of total cholesterol by AAS treatment [29]. Ammar *et al.* found that nandrolone treatment produced a significant decrease in HDL levels and a significant increase in LDL levels in rabbits treated for 12 weeks (10 mg/kg/week) and 16 weeks (50 mg/kg/week) respectively [42]. In human, Achar *et al.* stated that AAS can decrease HDL and increase LDL levels [43].

In the present study, the marked rise in total cholesterol level was attributed mainly to the elevation in LDL level in rats treated with ND as shown in Table (2). AAS lower HDL levels primarily by induction of the HDL-catabolizing enzyme, hepatic triglyceride lipase (HTGL), which is 1 of 2 heparin- elutable lipolytic enzymes. HTGL is localized to the luminal surface of hepatic endothelium and presumably catabolizes HDL via its phospholipase activity. During AAS therapy, HTGL activity has been reported to show a statistically significant increase [44]. In this respect, Baldo-enzi *et al.* suggested that serum LDL levels may increase through the induction of the enzyme HTGL and catabolism of very low density lipoprotein [45]. Hepatic triglyceride lipase induction may also catabolize HDL and reduce its serum levels. On the other hand, estrogens suppress HTGL and

increase HDL levels [46]. Since nandrolone is a poor substrate for aromatase [47], thus nandrolone is less metabolized to estrogen than testosterone. Consequently, nandrolone has higher ability to induce HTGL which may explain our results in which nandrolone has an increasing effect on LDL and decreasing effect on HDL levels. It is worth mentioned that, the marked increased levels of total cholesterol and LDL, decreased levels of HDL in ND-treated rats may reflect a special attention towards an increased risk of cardiovascular diseases [48].

In the present investigation, the plant extract could return the higher values of total cholesterol, TAG and LDL as well as the lower values of HDL to the near normal levels after its administration with ND when compared to control (saline and oil) and ND-treated groups (G3). As expected, a significant increase in HDL and a significant decrease in LDL levels were noticed in ALE-treated group (G4) compared to control saline (G1) group, this was prominent after long period of administration. Our results concerning the improving effect of ALE on serum lipid profile come in agreement with Mahmoodabadi who observed the protection of artichoke extract against hyperlipidemia in diabetic rats [38]. In addition, clinical studies on ALE demonstrated that, because of its content of flavonoids such as luteolin, it retarded LDL oxidation [16, 49].

Previous studies indicated that, the ALE increased biliary secretion leading to an increased cholesterol elimination and/or inhibition of 3-hydroxy- 3-methylglutaryl coenzyme A (HMG-CoA) reductase activity which in turn resulting in a decreased cholesterol biosynthesis, so ALE has choleric, diuretic and hypocholesterolemic activities [50]. ALE has antioxidant properties [51]. These properties are thought to operate through a reduction in de novo cholesterol synthesis via the inhibition of HMG CoA reductase, an increase in cholesterol elimination in bile secretions and an inhibition of LDL oxidation [52, 53]. In fact, it has been widely accepted that increased oxidation of LDL would be a crucial event in the progression of atherosclerosis [54]. However, numerous reports supporting the beneficial effects of antioxidants preventing the LDL oxidation,

It is currently believed that oxidative stress and inflammation play a significant role in atherogenesis. Artichoke extract exhibits hypolipemic properties and contains numerous active substances with antioxidant properties [53]. These lipid abnormalities resulted from nandrolone treatment in our experiment is harmful and represent a major risk factor for the vascular system and

should be prohibited. ALE seems to reduce the risk of atherosclerosis by preventing the oxidation of LDL [16, 53] and by inhibiting hepatic cholesterol biosynthesis [55] and reducing serum levels of cholesterol. The latter property may be due to cholera-induced elimination and inhibition of hepatic biosynthesis of cholesterol [10].

In conclusion, we believe that the present results stand for a protective effect of extract from artichoke leaves against oxidative stress induced in LDL. This effect could be exploited for the treatment of atherosclerosis and its consequences.

Liver side effects are the most common and serious ones associated with AAS use, including sub cellular changes, hyperplasia and adenoma or hepatocellular carcinoma [56]. The results obtained at the last experimental period showed that chronic administration of ND to rats led to a significant increase in serum ALT and AST activities compared to control oil (G2) group and this increment showed progression with the prolongation of term therapy. Toxic effects of AAS on the liver have often been described [57] and include increased enzyme activities, a cholestasis jaundice and peliosis hepatis adenoma and even unconfirmed case reports of carcinoma [58]. Furthermore, many reports of hepatic dysfunction secondary to AAS therapy may have been based solely on elevations in AST and ALT levels and thus may have overestimated AAS-induced hepatotoxicity [59].

The increased ALT and AST values above the upper limit of reference might be interpreted as late sequelae of the high dose of AAS. So we can say that the prolonged administration and high dose of ND had a bad effect on hepatocytes. The mechanism by which these hepatic abnormalities occur might be the formation of reactive metabolites which may lead to increasing permeability, damage and injuries of the cells as the enzyme ALT is located in the cytoplasm and the enzyme AST is located mainly in organelles such as mitochondria [60]. Increased activities of ALT and AST suggested damage of both hepatic cellular and mitochondrial membranes in ND-administered rats.

On contrary, the administration of ALE in concomitant with ND led to a significant decrease in ALT and AST compared to (G3) and this means that ALE lowers the aminotransferase levels. These results came in agreement with Jimenez-Escrig *et al.* [34] who postulated that ALE showed hepato protective properties by acting as an antioxidant and showed the efficacy and safety of artichoke extracts in the treatment of hepatobiliary dysfunction. In addition, Neveen found that ALE could reduce the activities of AST and ALT enzymes in

rats with hepatotoxicity [61]. From the previously mentioned studies and from our results, we can conclude that, ALE has a hepatoprotective effect by making the liver more tolerant to the effect of reactive metabolites of AAS.

At the end of experiment, a significant decrease of serum calcium concentration and a slight decrease of serum iron concentration were observed by administration of ND alone but these values returned to normal after administration of plant extract with ND drug. This result was in accordance with the data previously reported by Chen *et al.* [62] who reported that, the 19-nortestosterone implantation caused a decrease in blood Calcium and Phosphorus concentrations but increased tibia ash and Phosphorus content. Also this result agreed with Aithal *et al.* [63] who demonstrated that, the plasma calcium level in ND-treated rabbits was significantly lower than that of control.

ND is a bone marrow stimulant that increases the bone density by stimulating bone formation so ND helps to increase the mineral density in osteopenic bone [64]. Nandrolone decanoate has been shown to promote absorption of calcium from intestines and increase bone mineral content in women [65], stimulate endosteal bone formation in elderly dogs [66]. From the previously mentioned documents, we can say that such decrease in Ca levels could be attributed to inhibition of bone resorption and increased mineral deposition in the bone [67].

Regarding to the effect of ND on serum iron, The literatures stated that androgen are able to stimulate erythropoiesis dependent and independent on the GH-IGF-I system during male puberty [68] respectively. Moreover, Navarro *et al.* [69] found that ND increase levels of insulin-like growth factor type 1 (IGF-1) which stimulates erythropoiesis. In addition, Claustres and Sultan [70] postulated that androgens stimulate erythropoiesis in bone marrow cultures. The effect of ND in increasing serum iron may be not clearly observed in serum of treated rats owing to its incorporation into haemoglobin synthesis and consequent erythropoiesis. In other words, the serum iron may be directed to the lymphoid tissues for erythropoiesis. Concerning the effect of ALE, it was evident that there was a marked significant rise of serum calcium and iron concentrations after administration of plant extract, ALE, especially at the end of experimental period of treatment, in comparison to that of control saline group (G1) ($P \leq 0.005$). In agreement with the present findings, Kaur and Gupta [71] who concluded that the intake of inulin and oligofructose enhances

gastrointestinal mineral absorption such as calcium, magnesium and iron. Maria *et al.* suggested that the artichoke increase iron absorption from gastro intestinal tract and serum iron concentration increased accordingly [31]. In addition, Yeung *et al.* [72] hypothesized that prebiotics, such as inulin, enhance iron absorption through the fermentation of prebiotics by colonic microbiota which decrease the pH of the luminal content and improve iron solubility, promote reduction of Fe (III) to Fe (II), stimulate proliferation of epithelial cells to expand the absorptive surface area by the production of short-chain fatty acids and potentially stimulate expression of mineral-transport proteins in epithelial cells. Furthermore, Alamanni *et al.* [73] reported that artichoke is a rich source of minerals including iron after evaluating its chemical composition. Also these concepts may explain why the plant extract could increase serum calcium and iron concentrations after intake either alone or in combination with high dose of ND. Otherwise such rise in the serum calcium and iron levels noticed in combination group still lower than those of ALE-treated group (G4). It may be related to the effect of ND in delivering the calcium to bones for bone formation and the soluble iron to tissues for stimulation of erythropoiesis and this explanation may be supported by the finding of enhanced BWG recorded in the present investigation.

Although oxygen is necessary for proper cell function, cellular processes involving oxygen to create energy also result in oxidation. ROS are by-products of aerobic cellular metabolism and antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, play a crucial role in order to circumvent their deleterious effects. The imbalance between ROS generation and the intracellular levels of antioxidant defenses leads to oxidative stress, a condition that has been associated with apoptosis, neurodegenerative diseases premature aging, immune dysfunction and a myriad of diseases including cancer, cardiovascular disease and ischemia-reperfusion injury [74].

Concerning the effect of treatment with ND, Our results revealed a significant elevation of hepatic MDA and a significant decrease of hepatic GSH contents. These results agreed with data reported by Tugyan *et al.* [75]. These abnormalities were reversed by administration of plant extract with ND drug, thus reflecting an improving effect of ALE on hepatic oxidative status.

One of the possible mechanisms of extract from artichoke (*Cynara scolymus*) could be founded on antioxidative activity that was attributed to flavonoids, chlorogenic acid and cinnarine (caffeoylquinic acid derivative) [16]. In addition, Gebhardt and Fausel

previously demonstrated that, ALE prevented the loss of intracellular GSH caused by butyl hydroperoxide using primary cultures of rat hepatocytes [76].

So, from the present study, we can conclude that ALE has antioxidative and hepatoprotective activities by acting as an antioxidant owing to its components of flavonoids [55, 77].

CONCLUSION AND RECOMMENDATIONS

Clinically, the present findings indicate that a high dose of ND cause hyperlipaemia, atherogenicity as well as hepatic toxicity. Therefore the abuse of AAS is harmful to the vascular system and liver and should be prohibited.

The study also reflects the improving effect of the plant extract in modulating some of undesired effects of ND administration concerning lipid profile, serum glucose and liver status. ALE has antioxidative, hepatoprotective and hypolipaeamic activities that may be attributed to its constituents of polyphenolic and flavonoids. It is worthy mentioned that, Appreciable increase in body weight observed in rats treated with ND and ALE was probably attributed to anabolic effect of Nandrolone deconoate and artichoke that increase muscle gain, bone density and haemopoiesis.

In conclusion, the present results approved the protective effect of artichoke leaves extract against hepatic toxicity, hyperlipaemia and oxidative stress induced in LDL that may occur as a consequence of higher values of LDL resulted from treatment with high dose of nandrolone. This effect could be exploited for the treatment of atherosclerosis and its consequences.

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