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RESEARCH ARTICLE

Anti-aging Role of Grape Seed Extract and α-Lipoic Acid in D-Galactose-Induced aging Rats

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ABSTRACT

This study investigate the effects of Grape seed extract and -α-lipoic acid supplementation on hepatic, cardiac and renal biochemical aging markers of D-galactose induced aging rats. Aging was induced by intraperitoneal injection of D-galactose (300 mg/kg dissolved in 1 ml DW) every day for 9 weeks to accelerate senescence and aging induction.

The rats were randomly divided into four groups (7 male rats per each group). 1st group (G I) negative control group without any treatment, the 2nd group (G II), was D-galactose injected daily for 9 weeks and regarded as induced age control group, the 3rd group (GIII) was injected daily with D-galactose (300 mg/kg) and orally treated with Grape seed extract (200 mg/kg) daily for 9 weeks, the 4th group(G IV), was daily injected by D-galactose (300 mg/kg) and orally treated with α-lipoic acid (100 mg/kg) for 9 weeks.

The results showed significant increase ($P<0.05$) in cardiac and hepatic enzymes levels for serum aspartate amino-transferase (AST), alanine aminotransferase (ALT) , Alanine phosphatase (ALP),α - Glutamyle transferase (GGT), Lactate dehyderogenase (LDH) and Cretinine phosphokinase (CPK) in D-galactose induced aged group. The treatment of D-galactose inducing aging rats with Grape seed extract and DL-α-lipoic acid alone reversed the aging effects of D-galactose in hepatic and cardiac biochemical markers, that showed significant ($P<0.05$) decrease in the levels of AST, ALT, ALP, GGT, LDH and CPK levels as compared with D-galactose induced aged rats.

With respect to the renal biochemical markers, D-galactose (300 mg/kg body weight) injection for 9 weeks caused elevation in the levels of urea and creatinine but a decrease in uric acid, albumin and total bilirubin as compared to the no injected rats. Grape seed extract (200 mg/kg) and α-Lipoic acid (100 mg/kg) showed increase in levels of uric acid, albumin and total bilirubin with non significant decrease in the urea and creatinine levels as compared with the D-galactose induced aging control rats. In conclusion the results of the current investigation revealed the improving effects of Grape seed extract and α-lipoic acid suppresses senescence markers in D-galactose inducing aging effects in hepatic, cardiac and renal functions, contributes to oxidative stress and apoptosis in d-galactose-induced aging rats.

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INTRODUCTION

Aging is characterized by a progressive decline in function and a decrease in the body's ability to maintain homeostasis (Terry and Buccafusco, 2003). The free radical theory of aging proposed that aging is due to the accumulation of free radicals damage of unrepaired cellular components as a result of shift in the balance between the prooxidative and anti-oxidative processes in the direction of the pro-oxidative state (Cadenas and Davies, 2000).

Chronic exposure to D-galactose (D-Gal) causes an acceleration of senescence in different animal species by producing an unprecedented rise in oxidative stress and has been used as a reliable animal model for gerontological research (Ho *et al.,* 2003;Cui *et al.,* 2004, 2006; Chen *et al.*. 2006; Banji *et al*., 2013). D-Gal-treated animals have a shortened life span and exhibit symptoms

similar to those of natural aging, especially the decline in cognitive functions (Xu and Zhao 2002; Holden *et al*. 2003;Wei *et al*. 2005; Cui *et al.,* 2006; Lu *et al.,* 2010; Hua *et al.,* 2007; Sun *et al.,* 2007; Zhang *et al.,* 2007). D-Galactose (D-gal) causes the accumulation of Reactive Oxygen Species (ROS) or/and stimulates free radical production indirectly by the formation of advanced glycation end-products in vivo, thus finally resulting in oxidative stress (Zhang *et al.*, 2005). In addition, repeated injection of D-galactose could induce aging-like symptoms in animals, such as abnormal alterations in biochemical markers, loss in propagating ability, retrograde changes in neural cells and memory impairments (Shen *et al.*, 2002; Lu *et al*., 2006). Therefore, rats injected with D-gal have been used for physiology and pharmacological studies in vivo on brain, liver and renal aging. Some studies have further showed that D-gal induced aging-related changes, including increased production of ROS (Zhang *et al.*, 2007) and decreased antioxidant enzyme activities. D-gala is a reducing sugar that reacts readily with the free amines of amino acids in proteins and peptides both in vitro and in vivo to form advanced glycation endproducts (AGEs). AGE is increased during aging and has been linked to the pathogenesis of many age associated pathologies such as diabetes, arteriosclerosis, nephropathy, infection, drug toxicity, ischemic damage, neoplastic transformation and metastasis, and cardiovascular, cancer, and Alzheimer's disease (Safciuc *et al*., 2007).

Aging has been shown to result in increased superoxide anion, hydrogen peroxide, and hydroxyl radical resulting in oxidative protein damage in the liver. Liver aging is associated with morphological changes attributable to decreased hepatic blood flow, and altered enzyme function, including disturbance of physiology of liver enzyme functions (Jung *et al.*, 1996). Renal functions changes that occur with aging involving, decreased renal weight, thickening of the intrarenal vascular intima, sclerogenous changes of the glomeruli, and infiltration of chronic inflammatory cells and fibrosis in the stroma. Altered renal tubular function, including impaired handling of water, sodium, acid, and glucose, is also frequently present in old age (Muhlberg, 1999).

Grape seed extract (GSE) is a natural extract from the seeds of *Vitis vinifera* contain several active components including flavonoids, polyphenols, anthocyanins, proanthicyanidins and procyanidines, grape seeds extract (GSE) contain 70-95% standardized proanthocyanidins (Ferreira and Li, 2000). These flavonoids have demonstrated a marked spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress. Grape seed proanthocyanidin extract (GSPE) has more powerful antioxidative activity than other well-known antioxidants, including vitamin C, vitamin E, and gallic acid (Ariga, 2004). GSPE has various biological functions including antiaging, potent phytochemical antioxidants, antibacterial, antiviral, anti-inflammatory, anti-allergic, and vasodilatory actions. Various reports have shown that long term dietary supplementation of polyphenols improved the codnitive performance in aged rats (Alia *et al*., 2003; Balu *et al*., 2006; Abdelgawad *et al*., 2012).

α-lipoic acid has been described as a potent biological antioxidant, a detoxification agent, and a diabetes medicine; it has been used to improve age-associated cardiovascular, cognitive, and neuromuscular deficit. α-lipoic acid is a short chain of fatty acid containing two sulfur atoms. Lipoic acids directly eliminate ROS and regenerate oxidized intrinsic antioxidant enzymes in the process of redox coupling with dihydrolipoic acid showing potent anti-oxidants in inhibiting apoptosis of hippocampus (Packer *et al*., 2001). Lipoic acid (LA) is a thiol compound found naturally in plants and animals. Lipoamide dehydrogenases, found only in mitochondria, reduce free LA to dihydrolipoic acid, which is a potent antioxidant. Thus, LA supplementation may increase cellular and mitochondrial antioxidant status, thereby effectively attenuating any putative increase in oxidative stress with age (Arivazhagan *et al*., 2001, 2002; Suh *et al.*, 2001).

The current study aimed to investigate the antiaging and protective potential of Grape seed extract and lipoic acid in hepatic, cardiac and renal function biomarkers of induced aging in male rats.

MATERIALS AND METHODS

Experimental Animals

Young male albino Wistar rats of 4 months age and 280-290 gm body weight were used throughout these experiments and they were procured from experimental animal laboratory of College of Veterinary of Baghdad University, Iraq. Animals were housed for 10 weeks at the experimental animal housing in polypropylene cages of College of science, Salahdeen University. The rats were housed at a constant temperature of 25±1°C, humidity of 55%, and 12 hr light /dark cycle. The animals were fed standard chow and given tap water *ad libitum* throughout the experimental periods. After an acclimation period of one week, 28 rats were divided randomly into four groups (7 rats in each group). The rats of the 1st group (G I) served as negative control group without any treatment. The rats of the 2nd group (G II) were intraperitonealy injected daily with D-galactose (300 mg/kg BW) for 9 weeks and served as induced aging control group. The rats of the 3rd group (GIII) were injected with D-galactose (300 mg/kg BW/day) and administrated orally by GSE (200 mg/kg BW/day) for 9 weeks. The rats of the 4th, group (G IV), were injected with (300 mg/kg BW/day) of D-galactose and treated daily with single dose of α-lipoic acid (100 mg/kg BW/day) for 9 weeks.

Aging induction

Aging was induced by daily intraperitoneal injection of 300 mg per body weight of D-galactose (Sigma Chemical Company (St. Louis, Missouri, USA) after diluting with distilled water for 9 weeks (Lu *et al.*, 2010).

Grape seed extract administration

Grapes as large clusters with red berries were bought from a local super- market in Iraq (Erbil) and identified as *Vitis- vinifera*. Grape seeds were removed from the grapes, air dried for 1 week. The ethanolic extract was prepared by soaking 100 gm of grape seeds powdered in 300 ml ethanol (99%) shaking (24 h) then covered by a

piece of aluminum foil and kept in refrigerator. The infusion was filtered by a piece of double gauze and the filtrate was centrifuged at 3000 rpm for 10 minutes, then the supernatant (ethanol) was evaporated using a rotatory evaporator apparatus attached with vacuum pump. The 100 gm of dried grape seeds powder yield 26.7 gm ethanol. Grape seed extract dissolved in double distal water and was daily supplemented orally by gavage 200 mg/kg at the same time of the day for 8 weeks (Balu *et al*., 2006).

Alfa-lipoic administration

DL-α-lipoic acid was purchased from Sigma Chemical Company (St. Louis,Missouri, USA) and was daily supplemented orally by 100 mg/kg daily for 8 weeks (Arivazhagan *et al*., 2001).

Blood collection

After being anaesthetized by intramuscular injection of 0.2 ml/100gm of a 1 ml ketamine (50 mg) and 1 ml of xylazine (20 mg) solution, the animals were weighed. Animals were sacrificed 48 h after the last dose of the treatment, blood samples were taken of each animal under anesthesia by cardiac puncture. Serum samples were obtained by centrifuging the whole blood at 3000 rpm at 4°C for 10 minutes and the supernatants were transferred into tubes for separate biochemical assay and maintained at -80°C for biochemical analysis.

Biochemical analysis

The level of hepatic, cardiac and renal biochemical markers in serum, aspartate amino-transferase (AST), alanine aminotransferase (ALT), Alanine phosphatase (ALP), α-Glutamyle transferase(GGT), Lactate dehyderogenase (LDH) and Cretinine phosoinase (CPK), Blood Urea, Uric acid, Albumin (ALB) and Total bilirubine were estimated by the use of end point colorimetric diagnostic kit (Pars azmun Co., Tehran, Iran) through biochemical auto-analyzer: Cobas analyzer Roche, Diagnostics, Gmbh.

Statistical analysis

All the results were expressed as mean ± standard deviation (SD). Data was analyzed using one-way ANOVA followed by using Duncan's multiple range tests using SAS "Statistical Analysis System" Institute, (1988). Differences with a P-value <0.05 were considered as statistically significant.

RESULTS

Body weight indicator of aging

Table 1 show changes in body weight in D-galactose induced aging rats, non-aged and antiaging treated rats. As revealed from the table there was a body weight loses by 24.6% of D-galactose induced aged rats whereas in the normal non aging control rats group showed an increase in body weight by ratio of 13.6%. In D-galactose induced aging rats treated with GSE and α-Lipoic alone, both groups, showed an increase in body weight by ratio of 12.8%, by 12.2% respectively. Body weight loss in D-galactose induced aging rats was significantly (P<0.05) minimized in animals treated with Grape seed extract and α-Lipoic acid.

Antiaging effects of grape seed extract and α-lipoic acid

The effects of the D.galac induced aging and Grasp seed extract and α-Lipoic acid as antiaging agents in cardiac and hepatic functions parameters involved in the current study are shown in the table 2. There were significant differences (P<0.05) in AST, ALT, ALP, GGT, LDH and CPK of rats treated with D-galactose, compared to non induced aging control rats group. In the D-galactose induced aging group rats, showed elevated levels of AST, ALT, and ALP, GGT, LDH and CPK compared, to non induced aging control group. Rats treated with Grape seed extract and α-Lipoic acid showed decrease in levels of AST, ALT, ALP, GG, LDH and in CPK as compared with the D-galactose induced aging group rats.

The renal functions test parameters changes in D.galac induced aging and Grasp seed extract and α-Lipoic acid is shown in table 3. There were significant differences (P<0.05) in serum levels of urea, ceratine, uric acid, albumin and total bilirubine in rats treated with D-galactose (300 mg/kg i.p.) when compared with non induced aging control rats group. In the D-galactose induced aging group rats, there were elevated levels of urea and creatinine and a decrease in uric acid, albumin and total bilirubine compared to the non induced aging control rats group. Rats treated with Grape seed extract and α-Lipoic acid showed increase in levels of uric acid, albumin and total bilirubine with slight decrease in urea and creatinine as compared with the D-galactose induced aging control rats.

DISCUSSION

The D-galactose-induced aging increasing the oxidative stress and inflammation, causing senescence injury, this senescence-induced model could result in a decline in cognitive function in the liver, brain and cardiovascular damage (Buemi *et al*., 2005; Lu *et al*., 2010; Kumar *et al*., 2011).

Decrease in body weight occurs in induced aged rats, could be consequence of many factors or physiological effects including glycation oxidative injury from this d-galactose induction model, this may explain why the body weight decrease, which is different from induced aging.

Previously reported that an imbalance between the formation and removal of ROS and the development of oxidative stress plays an important role in aging and age-associated diseases such as hepatic necrosis, fibrosis, renal failure and other diseases of aging, (Rikans *et al*., 1997; Johnson *et al*., 1999).

The liver plays a key role in the metabolic process of itself as well as other tissues in maintaining the internal body homeostasis. Hepatic injury due to elevated level of oxidation and some toxic phytochemicals found in medicinal plants and failure to eliminate these metabolic products by the liver often results in marked distortion of the normal function, hepatic necrosis and fibrosis of the liver (Geidam *et al*, 2004).

Table1: Body weight changes in normal and D-galactose induced aging albino rats treated with Grape seed extract and α-Lipoic acid

Body weight Index	G I	G II	GIII	G IV
B.W (g) before treatment	293.74 ± 10.46^{b}	296.46±6.30^{a}	294.92±18.68^{a}	293.6±11.55^{b}
B.W (g) after treatment	333.5±15.77^{a}	223.38±4.32^{b}	332.2±22.86^{b}	329.6±25.54^{a}
% of B.W. change	13.6	-24.6	12.8	12.2

Values are expressed as mean ± SD. different letters are statistically significant (*P<0.05); (G I) non aged control, (G II) aged control group, (GIII) aged treated with GSE (200 mg/kg Bw/day), (G IV) aged group treated with α-lipoic acid (100 mg/kg BW/day).

Table 2: Antiaging affects of Grape seed extract and α-Lipoic acid on the hepatic and cardiac serum biochemical markers in male albino rats

Biochemical markers	G I	G II	GIII	G IV
AST (IU/L)	46.44±4.74^{d}	138.7±20.96^{a}	69.49±10.88^{c}	79.40±17.46d^{c}
ALT (IU/L)	30.18±3.82^{c}	71.64±11.85^{a}	31.84±3.35^{c}	55.38±9.28^{ab}
ALP (IU/L)	43.66±6.21^{d}	124.44±11.54^{a}	70.28±23.30^{bc}	52.82±13.30^{bcd}
GGT (IU/L)	9.98±2.40^{d}	31.24±3.898^{a}	10.84±2.47^{d}	7.67±1.77^{d}
LDH (IU/L)	55.34±14.37^{d}	126.26±15.15^{a}	79.36±16.32^{c}	37.46±6.46^{de}
CPK (IU/L)	112.16±17.68^{a}	213.56±26.37^{b}	105.54±6.73^{a}	101.08±20.70^{a}

Values are expressed as mean ± SD, different letters are statistically significant (*P<0.05).

Table 3: Anti-aging effects of Grape seed extract and α-Lipoic acid on the renal serum biochemical markers in male albino rats

Biochemical markers	G I	G II	GIII	G IV
Urea (mg/dl)	16.40±4.2^{c}	27.26±9.67^{ab}	27.12±3.17^{ab}	26.36±8.83^{ab}
Creat. (mg/dl)	0.488±0.087^{b}	0.701±0.129^{a}	0.543±0.060^{b}	0.554±0.14^{b}
U.Acid (mg/dl)	1.62±0.32^{ab}	0.821±0.356^{c}	1.264±0.28^{bc}	1.93±0.89^{ab}
Alb (g/dl)	3.15±0.84^{c}	0.298±0.152^{d}	3.318±0.202^{bc}	4.21±0.40^{c}
T.bili (mg/dl)	0.334±0.319^{a}	0.086±0.048^{b}	0.188±0.087^{ab}	0.184±0.077^{ab}

Values are expressed as mean ± SD, different letters are statistically significant (*P<0.05).

The γ-GT, Aspartate aminotransferase (AST) and alanine aminotransferase, alkaline phospatase (ALP), LDH and CPK activity in this senescence induction model was significantly increased after 9 weeks of D-galactose induction, and the increase in these biomarkers activity is a risk factor for chronic liver disease formation and often associated with hepatocellular damage, it's a sensitive detectors in biliary cirrhosis, hepatitis, in diseases characterized by inflammation, regeneration, intrahepatic and extrahepatic bile obstruction and in cardiovascular damage (Mayne, 1994; Gagliano *et al*.,2007).

Grape seed extract and α-lipoic acid may be promising as a therapeutic option in D-galactose induced oxidative stress in the rat cellular hepatic and cardiac protective antiaging. After 9 weeks of treatments with grape seed extract and α-lipoic acid, a significant reduction effects in the serum levels of AST, ALP, γ-GT, LDH and CPK levels were seen. This reduction suggested that grape seed extract and α-lipoic acid may likely hepatic and cardio-protective compounds hence enzymes low levels are a strong indicator of cardiac, hepatic and renal protection, prevention damage to cardiac muscle and hepatocytes and it is therefore indicative in determination of hepatocytes and myocardial injury. Several studies of evidence demonstrated that, grape seed proanthocyanidins exhibited in vivo hepato-protective and anti-fibrogenic effects against liver injury and act as free radicals scavengers and protective liver damage (Cetin *et al.*, 2008).

The significant decrease in total biliriubin and albumin with aging indicates acompromized liver excretory function and impairment of the liver synthetic function (Ho *et al*., 2003). Moreover enzymes may be released into blood plasma and serum, levels of these enzymes may increase due to cellular damage in the liver. The increase of the activities of liver enzymes in serum is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream.

Under the light of the results, concluded that grape seed extract and α-lipoic acid are a useful anti-aging therapy, especially for controlling oxidative damages, they are considered as a potent protective agent against hepatic and cardiac oxidative stress damage and act as free radicals scavengers and protective liver and heart damage .

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