International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

The Protective Role of Green Tea and Ginkgo biloba Extract Against Aging Dysfunction Induced by D-Galactose in Rats

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Abstract

The current study was aimed to investigate the potential anti-aging role of Green Tea and Ginkgo biloba extract in hepatic, cardiac and renal aging dysfunctions induced by D-galactose in rats. Aging was induced by daily intraperitoneal injection of D-galactose (300 mg/kg dissolved in 1 ml DW) for 9 weeks to accelerate senescence and aging induction. The rats were randomly divided into four groups (7 rats per group). The rats of the first group (G I), were kept on normal feeding without any treatment and regarded as non aged control group, the rats of the second group(G II),were injected by D-galactose daily for 9 weeks, and regarded as induced aged group, the rats of the third group (GIII) , were injected daily with D-galactose(300 mg/kg) and, orally treated with green tea extract (200 mg/kg) daily for 9 weeks , the rats of the fourth group (G IV) were daily injected by D-galactose (300 mg/kg) and orally treated with ginkgo biloba extract (200 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 the D-galactose inducing aged rats with green tea extract(200 mg/kg) and ginkgo biloba extract (200 mg/kg), appeared a protective role against aging dysfunctions induced by D-galactose , and caused significant ($p<0.05$) decrease in the levels of hepatic and cardiac biochemical markers, in the serum levels of AST, ALT, ALP, GGT, LDH and CPK levels as compared with D-galactose induced aged rats. With respect to the renal function test parameters, 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 non treated control group rats. Green tea extract (200 mg/kg) and Ginkgo biloba extract (200 mg/kg) treatments of D-galactose injected rats, 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 protective role of Green tea extract and Ginkgo biloba extracts, in suppression of senescence markers and aging dysfunction in hepatic, cardiac and renal functions, contributes to oxidative stress and apoptosis induced by D-galactose in rats.

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Keywords: Aging, Dysfunction, D-Galactose, Induced aging, Ginkgo biloba extract, Green tea.

Introduction

D-Galactose (D-gal) known to cause the accumulation of Reactive Oxygen Species (ROS) or/and stimulates free radical production indirectly by the formation of advanced glycation end-products (AGEs) in vivo, resulting in oxidative stress (Zhang *et al*., 2005).

Repeated injection of D-galactose could induce aging-like symptoms in animals, such as alterations in biochemical markers, loss in propagating ability, retrograde changes in neural cells and memory impairments (Shen *et al*., 2002; Lu *et al*., 2006). D-

Galactose has been used to induce oxidative stress in vivo to mimic natural aging in rats, mice and Drosophila (Song *et al*., 1999; Wang *et al.*, 1999; Ho *et al*., 2003).

Several studies showed increased free radicals production in D-galactose-injection animals and suggested the account for the underlining mechanism responsible for the acceleration of aging (Zhang *et al*.,2010; Hsieh, *et al*.,2011; Banji *et al*., 2013). Also seen that overdose of D-gal can be catalyzed by aldose reductase into galactitol, which cannot be metabolized but will accumulate in the cell, leading to osmotic stress and generation of ROS which is caused dysfunction and apoptosis of cell (Wang ,1999). In addition, accumulated D-gal in animal tissues can react with amino groups of proteins and peptides to form advanced glycation end products (AGE) which has been suggested to accelerate the aging process and linked to the pathogenesis of many age-associated diseases such as diabetes, arteriosclerosis, nephropathy, infection, cardiovascular and Alzheimer's disease(Song *et al*.,1999; Hori *et al*., 2012). Therefore, D-gal. injected animals have been used for physiological and pharmacological studies in vivo on brain, cardiac, liver and renal aging dysfunctions.

Green tea is rich in polyphenols compounds, mainly epigallocatechingallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechingallate (ECG). EGCG, the major and most active component of green tea catechins, acts as an antioxidant in the biological system and is rapidly absorbed and distributed mainly into the mucous membranes of the small intestine and reach to the liver; more interestingly, it can cross the blood brain barrier (Nakagawa and Miyazawa, 1997;Weinreb *et al*., 2004). Green tea polyphenols antioxidant potential is directly related to the combination of aromatic rings and hydroxyl groups that make up their structure, and is a result of binding and neutralization of free radicals by the hydroxyl groups. In addition, green tea polyphenols stimulate the activity of hepatic detoxification enzymes, thereby promoting detoxification of xenobiotic compounds, and are also capable of chelating metal ions, such as iron, that can generate radical oxygen species (Serafini *et al*.,1996; Erba *et al*.,1999)

Previously reported that, green tae has been shown to afford significant protection against age-associated pathologies and diseases as, neurodegenerative diseases, Parkinson's disease, Alzheimer's disease, and ischemic damage (Mandel and Youdim, 2004). Several studies showed suppression action of green tae polyphenolic compounds against the formation of AGEs and decreasing the risk of age-related disease (Salah *et al*., 1999; Valcic *et al*., 1999;Yang, *et al*., 2002; Lee *et al*., 2004). Most recently GTE treatment was seen to able to reverse the impairment in hepatic, cardiac and renal functions and reducing the non desirable aging associated biochemical markers impacts in rats (Shereen *et al*., 2013).

The photochemical analysis of Ginkgo biloba extract appeared to containing 24% ginkgo-flavone glycosides (kaempferol, quercetin, and isorhamnetin derivatives) and 6%terpenoid (ginkgolides A, B, C, J and bilobalide). The flavonoid components showed to scavenge superoxide, hydroxyl radicals, and nitric oxide (NO) and protect myocardia from ischemiareperfusion injury, and the terpenoid constituents also showed their cardioprotective effects independent from the free radical-scavenging properties(Schindowski *et al*., 2001). Ginkgo biloba is with antioxidant capacity and free radical scavenger properties which could contribute to its hepatic and neuroprotective anti-apoptotic activity, it showed to reverse age related decline of neurotransmitter systems(Williams *et al*.,2004). Ginkgo biloba extract(GBE) has been shown to have a SOD and hydroxyl radical scavenging activity (Schindowski *et al*., 2001). Also GBE seen to has a protective action against the free radicals and peroxidations in liver fibrosis in aged rats (Shang-Zhen *et al*., 2005). Several earlier reports showed that EGE has particular antiaging effects on cardiomyocytes by inhibiting nonenzymatic glycation and reducing the deposition of AGEs in myocardium tissues (Jezova *et al*.,2002; Mozet *et al*.,2009 ; Schiborr *et al*., 2010) .

The current study aimed to investigate the antiaging and protective potential of green tea and Grape seed and Ginkgo biloba extracts in hepatic, cardiac and renal aging dysfunction induced by D-galactose in male rats.

Materials and Methods

Experimental Animals

Young male albino Wistar rats of 4 months age and 280 –290 g body weight, were used throughout the experiments of this study, 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 h 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 first group (**G I**) served as negative control group and kept on normal feeding without any treatment. The rats of the second group (**G II**), were daily injected (i.p.) with D-galactose (300 mg/kg b·w) for 9 weeks and served as induced aged rats control group. The rats of the third group (**GIII**), were daily injected with D-galactose (300 mg/kg b·w.) and administrated orally by green tea extract (200 mg/kg b·w/day) for 9weeks. The rats of the fourth group (**G IV**), were daily injected (i.p.) with (300 mg/kg b·w/day) of D-galactose and treated daily with single dose of Ginkgo biloba extract (200 mg/kg b·w/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*., 2006).

Green tea extract Administration

Green tea leaves were bought at private markets. Ethanol extract was prepared by maceration of grinded tea leaves (100 g) for 72 hours with 250 ml of 99.9% ethanol at ambient conditions. Collected extracts were filtered, ethanol was evaporated on rotary evaporator (RVO 200A, INGOS). The powdered ethanol extract was kept frozen (–18°C) until further use(Gramza *et al* ., 2005). Green tea Extract dissolved in double distal water and was daily administrated orally by gavage 200 mg/kg at the same time of the day for 9 weeks.

Ginkgo biloba extract Administration

Ginkgo biloba extract was purchased from Mason Vitamins Company,USA (No. 1140.90). GBE dissolved in double distal water and was daily administrated orally by gavage 200 mg/kg at the same time of the day for 9 weeks (Huang *et al*., 2005).

Blood collection

After being anaesthetized by intramuscular injection of 0.2 ml/100g 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

In response to D-galactose injection, the inducer of the aging symptoms, the body weight loses was at ratio of 24.6% in D-galactose induced aged rats. whereas in the normal non aging control rats showed a increase in body weight by ratio of 13.6% . In D-galactose induced aging rats treated with GTE and Ginkgo biloba extract alone showed a an increase in body weight by ratio of 15.1% and 21.3% respectively (Table 1). Body weight loss in D.galactose induced aging rats was significantly ($p<0.05$) minimized in animals treated with Green tea extract and Ginkgo biloba extract.

Protective Effects of the Green tea extract and Ginkgo biloba extract

Hepatic and Cardiac Functions

The effects of the D-galactose inducing aging and protective antiaging action of green and ginkgo biloba extracts 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 serum levels in rats treated with D-galactose, compared non induced aging control group. In the D-galactose induced aging group rats, showed elevated levels of serum AST, ALT, and ALP, GGT, LDH and CPK compared, to non induced aging control group. The treatment of D-galactose injected rats with green tea and ginkgo bioloba extracts, showed a protective role in decreasing in the serum levels of AST, ALT, and ALP, GGT, LDH and CPK significantly($P < 0.05$) as compared with the D-galactose induced aging control rat's values.

The renal functions test parameters changes are shown in table 3. There were significant differences ($P < 0.05$) in serum urea, ceratine, uric acid, albumin and total bilirubine of rats treated with D-galactose (300 mg/kg i.p.) when compared with non induced aging control 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 group. Treatment of the D-galactose injected rats with Green tea and Ginkgo biloba extract showed, increase significantly($P < 0.05$) 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.

Table1. Body weight Changes in normal and D-galactose induced aging albino rats treated with Green tea extract and Ginkgo biloba extract

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}	296.6 ± 8.75^{b}	286.04 ± 12.03^{bc}
B.W(g) after treatment	333.5 ± 15.77^{a}	223.38 ± 4.32^{b}	341.2 ± 26.14^{a}	347.54 ± 13.56^{bcd}
% of B.W. change	13.6	-24.6	15.1	21.3

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 GTE (200 mg/kg b·w/day), (G IV) aged group treated with GBE (200 mg/kg b·w/day).

Table-2: Protective role of the Green tea and Ginkgo biloba extracts in 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}	108.86 ± 9.33^{b}	109.84 ± 21.51^{b}
ALT(IU/L)	30.18 ± 3.82^{c}	71.64 ± 11.85^{a}	42.8 ± 14.61^{c}	61.38 ± 21.69^{ab}
ALP(IU/L)	43.66 ± 6.21^{d}	124.44 ± 11.54^{a}	54.50 ± 17.48^{bcd}	67.60 ± 10.62^{bcd}
GGT(IU/L)	9.98 ± 2.40^{d}	31.24 ± 3.898^{a}	17.84 ± 3.34^{c}	15.98 ± 4.93^{c}
LDH(IU/L)	55.34 ± 14.37^{d}	126.26 ± 15.15^{a}	46.14 ± 11.16^{de}	53.06 ± 7.80^{d}
CPK(IU/L)	112.16 ± 17.68^{a}	213.56 ± 26.37^{b}	62.10 ± 13.95^{d}	52.30 ± 14.31^{d}

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

Table-3: Protective role of the Green tea and Ginkgo biloba extracts in 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}	26.2± 6.40^{ab}	16.64± 5.18^{c}
Creat.(mg/dl)	0.488±0.087^{b}	0.701±0.129^{a}	0.544±0.14^{b}	0.46± 0.039^{b}
U.Acid (mg/dl)	1.62±0.32^{ab}	0.821±0.356^{c}	1.742± 0.26^{ab}	1.98± 0.48^{ab}
Alb(g/dl)	3.15±0.84^{c}	0.298±0.152^{d}	3.228± 0.37b^{c}	3.8± 0.23^{ab}
T.bili(mg/dl)	0.334±0.319^{a}	0.086±0.048^{b}	0.212± 0.095^{ab}	0.18± 0.083^{ab}

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

Discussion

The D-galactose-induced aging increasing the oxidative stress and inflammation, causing senescence injury. The reducing of body weight of D-galactose induced aging rats, could be consequence of many factors or physiological effects including glycation oxidative injury from this d- galactose induction model, this may explain the body weight decrease. This senescence-induced model could result in a decline in cognitive function in the liver, brain and cardiovascular damage (Lu *et al*., 2007; Buemi *et al*., 2005; Kumar *et al*., 2011). An imbalance between the formation and removal of ROS and the development of oxidative stress plays an important role in aging and age-associated suggest such as hepatic necrosis, fibrosis, renal failure and other diseases of aging (Rikans *et al*.,1997; Johnson *et al*., 1999).

The D- galactose injection induced biochemical signs of cellular hepatic, cardiac and renal injury, evidenced by increased AST, ALT, ALP, GGT, LDH, CPK and urea, ceratine, uric acid and decreased albumin and total bilirubine levels. The protective roles of green tea and Ginkgo biloba extracts administration to the D-galactose injected rats with may be promising as a therapeutic option in D- galactose induced oxidative stress in the rat cellular hepatic, cardiac and renal protective anti-aging. Treatments with green tea seed and Ginkgo biloba extracts resulted in a significant prevention of rises in the serum levels of AST, ALP, γ-GT, LDH, and CPK . This reduction suggested that green tea extract and Ginkgo biloba extract 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. The increase levels of γ-GT, AST, ALT, ALP, LDH and CPK activity in the serum are often associated with hepatocellular damage. Levels of these enzymes may increase due to cellular damage in the liver and heart. The increase of the activities of cardiac and hepatic enzymes in serum is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream.

The anti-aging and beneficial effects of green tea extract previously evident by improvement of hepaticand renal function (Shereen *et al*.,2013). The significant decrease in total biliriubin and albumin in D- galactose induced aging indicates acompromized liver secretary functions and impairment of the liver synthetic function (Nematalla *et al.*, 2011).

Urea, uric acid and serum creatinine levels rise is an indicator of renal dysfunctions, where they are not adequately excreted by the kidney. In present investigation the impairment in hepatic and renal function parameters in induced aged rats may be contributed to the results of oxidative stress associated with aging, that led to reduced hepatic and renal functions.

Green tea polyphenols significantly inhibits apoptosis of the tubular and interstitial cells in rats with cyclosporine-induced chronic nephrotoxicity, and that tea polyphenols may be useful to prevent CsA-associated kidney toxicity (Shaohua *et al.*,2003). Ginkgo biloba extracts also exhibited protective effects on vancomycin-induced nephrotoxicity, presumable through inhibition of free

oxygen radical production (Celik *et al.*,2005). Ginkgo biloba extracts also protects endotoxin-induced oxidative renal tissue damage of rats (Coskun *et al.*,2003).

Under the light of the results, concluded that green tea and Ginkgo biloba extracts 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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