doi.org/10.36721/PJPS.2020.33.4.SUP.1795-1803.1

Effect of lithium chloride on d-galactose induced organs injury: Possible antioxidative role

Noreen Samad[1*], Kainat Bilal[1], Farzana Yasmin[2,3], Saima Khaliq[4], Aqal Zaman[5] and M Mazhar Ayaz[6]

[1]Department of Biochemistry, Faculty of Science, Bahauddin Zakariya University, Multan, Pakistan
[2]Department of Food Engineering, NED University of Engineering and Technology, Karachi, Pakistan
[3]Department of Biomedical Engineering, NED University of Engineering and Technology, Karachi, Pakistan
[4]Department of Biochemistry, Federal Urdu University of Science and Technology, Karachi, Pakistan
[5]Institute of Pure and Applied Sciences, Bahauddin Zakariya University, Multan, Pakistan
[6]Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

Abstract: The aging process is concerned with oxidative stress and causing malfunction of various organs such as the liver, kidney and heart. Lithium (Li) salts have shown anti-manic, anti-suicidal, and antioxidant properties. The current study is aimed to evaluate the possible inhibitory effects of various doses (10, 20 & 40mg/ml/kg) of Lithium chloride (LiCl) on D-galactose (D-gal)-produced aging model and explore the underlying mechanism. In the study 40 male rats were randomly alienated into 8 groups i.e. saline, LiCl (10, 20 & 40mg/ml/kg), D-gal and D-gal+LiCl (10, 20 & 40 mg/ml/kg). D-gal was given at a dosage of 300mg/ml/kg and animals received their respective treatment for 6 weeks [*intraperitoneally* (*I.P*), once daily]. After 2 weeks animals were decapitated and organs (liver, kidney, and heart) were removed for antioxidant assays. Blood was also collected for biochemical parameters. LiCl substantially decreased oxidative strain marker and increased enzymatic antioxidants in the liver, kidney, and heart of D-gal treated rats. LiCl also decreased serum alanine aminotransferase (ALT), aspartate transaminase (AST), creatine, urea, CK-MB, triglyceride, cholesterol, low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) in D-gal treated animals. High dose (80mg/ml/kg) of LiCl observed as the most effective dose against D-gal induced alterations. These finding LiCl inhibits D-gal induced liver, kidney and heart damages via its antioxidant potential.

Keywords: Lithium chloride, D-galactose, organs, oxidative stress, antioxidant enzymes, blood parameters.

INTRODUCTION

Aging; a natural process that is impossible to escape. This naturally occurring process of aging influences the efficiency of all organs and systems (Zhang *et al.,* 2020). The minute difference in the capability of organs associated with age is mostly seen during the third and fourth decennium of life (Boss and Seegmiller 1981). The downturn of physiological functions associated with age is pretty perceptible, however, the rate at which physiological reserves decline is different among different organs but is quite uniform within the system (Lazarus *et al.,* 2019). So, aging prevails at the same rate for different age groups, the only difference is the accumulation of more age-related changes within the older group (Knight and Nigam 2008). The difference between age-associated physiological decline and lessen threshold for the onset of ailment due to advancing age must be made clear because uncertainty can have left several disorders untreated. Persons with advancing age undergo physiological reserve decline leading towards a high mortality rate (Thoppil and Riabowol 2020) (Hasty *et al*., 2003). Physiological inability associated with aging is magnified with the pervasiveness of concurring diseases. This situation is worsened by the fact that aging people somewhat lose repairability and become more vulnerable to pathological conditions (Siervo *et al.,* 2016). Aging influences organs in different ways. There have been many theories to explain underlying mechanisms of aging, that reactive oxygen species (ROS) play a pivotal role in the advancement of aging (Ciacka *et al.,* 2020) (Baranov and Baranova 2017). This theory is strengthened by the chronic diseases associated with aging like cancer, neuropathy, and diabetic retinopathy apparently all these pathophysiological conditions involve an excessive accumulation and wear and tear caused by ROS (Zhen *et al.,* 2016).

D-galactose (D-gal) is a reducing sugar and also known as a corporal nutrient. It is naturally present in the body and other dietary stuff i.e., milk, yogurt, fruits, nuts, and vegetables (Acosta and Gross 1995). D-gal is converted into Advanced Glycation End-products through non-enzymatic addition of amines from free amino acids. The normal level of D-gal is metabolized to give energy to the cell; however, in the excess amount, it is accumulated and metabolized to pro-oxidative metabolites which lead to ROS induced oxidative strain (Minhá Doan *et al.,* 2015). D-gal has reportedly been involved in mimicking age-related effects. Besides, it causes oxidative stress resulting in organ injury due to accumulated ROS (Chen *et al.,* 2018). Aging is induced as a result of stress-induced

**Corresponding author:* e-mail: noreen.samad@bzu.edu.pk

metabolic mechanisms, however exotically given D-gal accelerates the organ aging (Sikora *et al.* 2011).

Lithium in the form of salts i-e Lithium chloride (LiCl), has extensively been used as a mood stabilizer and a medication to treat mania (Ettenberg *et al.* 2020). Despite the development of other mood stabilizers, Li has been used for more than 60 years. Initially, Li was thought to increase life-span due to lower suicide risk, but now it is known that it has some additional potential to reduce mortality rate (Barjasteh-Askari *et al.,* 2020). Although it is used to treat bipolar disorder since ages and has anti-inflammatory potential (Fernandes *et al.* 2019). It is also known as an antioxidant, due to its protective activity against oxidative stress as it lowers lipid peroxidation and enhances antioxidants by these ways it improves cell viability (Allagui *et al.* 2009). Several parameters affect the antioxidant potential of Li among those; the dose of Li, duration of exposure and target cell type play the most significant role (Dietrich-Muszalska *et al.* 2015).

In existing data, limited reports have shown the inhibitory effects of LiCl on the D-gal induced aging of organs. To underlying its mechanism in the current study we hypothecated that the antioxidant potential of LiCl could be inhibiting the physiological damage of various organs (liver, kidney, heart) caused by D-gal induced oxidative stress. LiCl with dosages 10, 20 and 40mg/ml/kg were given to rats used as an aging model, to determine the effectual dose of LiCl for inhibition of D-gal induced physiological reserve decline.

MATERIALS AND METHODS

Animals

In the present work 40 adult male Albino Wistar rats (weighing 180-220 gm, eight weeks old) were locally bred and utilized in this study. Rats were housed in an individual cage (with static racks) to keep away from social contact effect because social contact can influence behavioral analysis of animals and given standard rodent diet and drinking water under controlled temperature (20±5°C) and 12-hour light/dark cycle. Before starting the experimental work, rats were experienced to 7 days of acclimation time and different conduct measures to diminish the stress of newness and treatment. All experiments were approved by Institutional Ethical Committee (D-1981-018-Biochem; Dated April 10, 2018).

Chemicals and reagents

D-galactose, Lithium Chloride, Sodium azide, Thio-barbituric-Acid, Tri-chloro-acetic-acid, Hydroxylamine-hydrochloride, Di-thio-bis-nitrobenzoic-acid, Nitro-blue-tetrazolium, and other reagents were procured from Co. Sigma.

Experimental protocol

40 male rats were separated into 8 sets (n,5); (a) saline (0.9% NaCl; I ml/kg) (b) LiCl; (10mg/ml/kg) (c) LiCl; (20mg/ml/kg) (d) LiCl; (40mg/ml/kg) (e) D-gal (f) D-gal*LiCl (10mg/ml/kg) (g) D-gal*LiCl (20mg/ml /kg) (h) D-gal*LiCl (40mg/ml/kg). Saline injected batch was given 1 ml/kg, whereas D-gal was given 300/mg/ml/kg per dose. Saline solution was used to dissolve all drugs. Drugs were administered *intraperitoneally* for 6 weeks. After 6 weeks, animals were decapitated then organs (*Liver, kidney and heart*) and blood were preserved in cold saline solution at -4°C for further antioxidant assay and blood parameters.

Biochemical Parameters

The liver, kidney, and heart were cleaned in an isotonic solution. Homogenate of 10% (weight/Volume) was managed by 0.10 Molar PO_4 buffer [pH7.4] from each organ and centrifuged at 4°C for ten minutes. The method of Chow and Tappel (1972) was used for the analysis of MDA with some changes in the procedure. Activity of catalase was determined by the method Samad *et al.* (2018). While % inhibition of SOD and activity of GPx were estimated by the procedure of Pari and Latha (2004) and Flohe and Gunzler (1984) respectively. Other biochemical parameters (urea, creatinine, CK-MB, triglyceride, low-density lipoprotein, high-density lipoprotein, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT)), were calculated in serum by Automated Biochemical Analyzer EPM-168, while troponin-I analyzed by using ELISA kit.

STATISTICAL ANALYSIS

Post hoc analysis subsequent 2 -way ANOVA, (mean $\pm$ SD) was performed for statistical analysis using SPSS V. 23.0. $p<0.05$ taken as significant.

RESULTS

Table 1 displays the properties of LiCl on oxidative stress markers and antioxidant enzymes in liver of D-gal and saline treated rats. All the data evaluated by *2-way-anova*. Data for lipid peroxidation revealed substantial effect of D-gal {$f_{(1,32)}$=27.56, $p<0.01$}, Li {$f_{(3,32)}$=56 .29, $p<0.01$}, and interaction between D-gal x Li {$f_{(3,32)}$=12.13, $p<0.01$}. Post hoc analysis showed that D-gal increased MDA levels than control animals. Administration of Li (10 and 20mg/ml/kg) decreased lipid peroxidation than their control animals. Level of MDA also decreased following in Li (10, 20 & 40mg/ml/kg)*D-gal than D-gal treated animals.

Data of SOD showed substantial effect of D-gal {$f_{(1,32)}$=49.48, $p<0.01$} and Li {$f_{(3,32)}$=24.12, $p<0.01$}, while insignificant interface of D-gal x Li {$f_{(3,32)}$ = 1.599, $p>0.05$}. Post hoc investigation shown that D-gal substantially reduced SOD activity than control one.

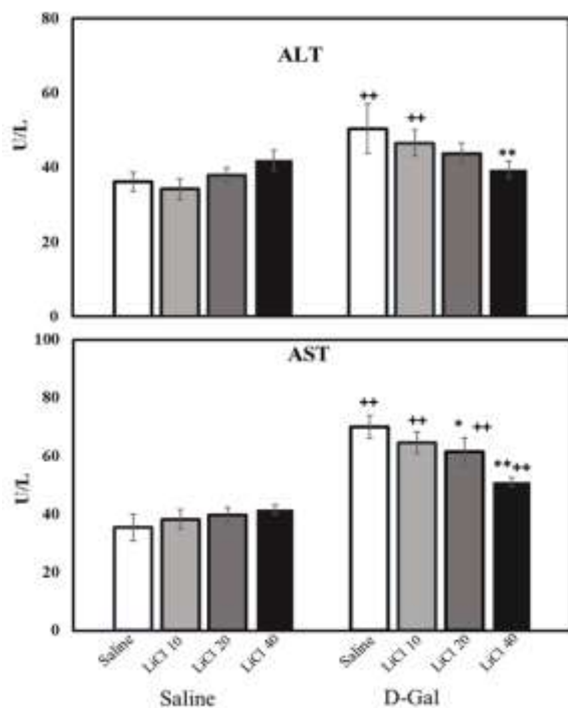

Fig. 1: Effect of LiCl on serum ALT and AST in saline and D-gal injected animals (mean± SD). 2 way-Anova followed by post hoc test expressed $*p<0.05$, $**p<0.01$, vs. respective control and $+p<0.05$, $++p<0.01$ vs. control and Li injected rats

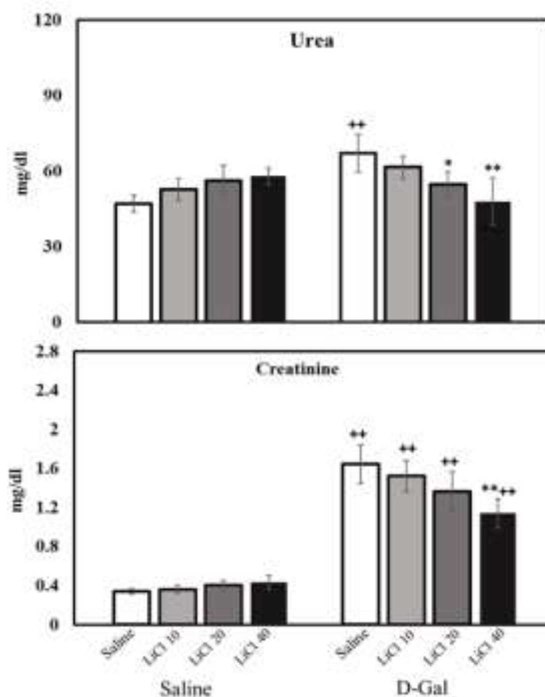

Fig. 2: Effect of LiCl on serum creatinine and urea in saline and D-gal injected rats (mean± SD). 2 way-Anova followed by post hoc test expressed $*p<0.05$, $**p<0.01$, vs. respective control and $+p<0.05$, $++p<0.01$ vs. control and Li injected rats

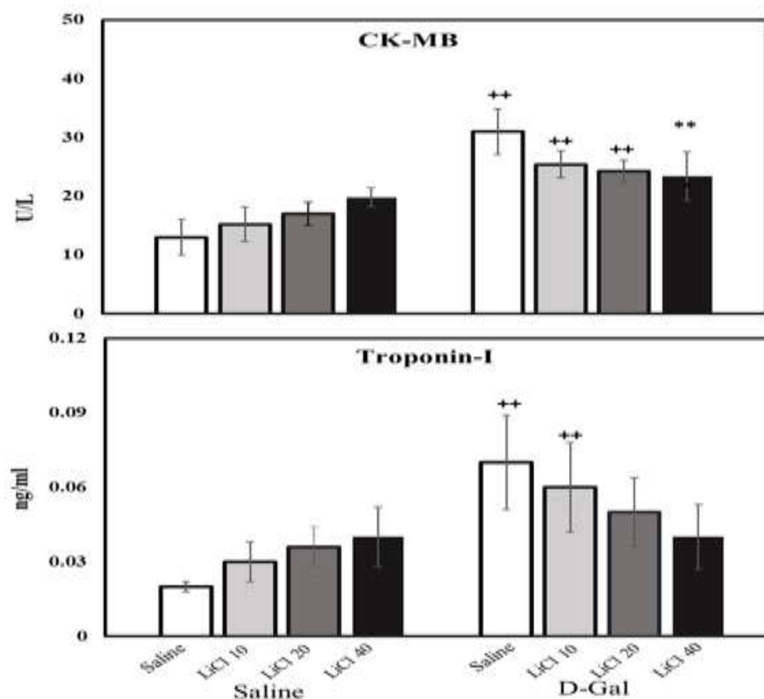

Fig. 3: Effect of LiCl on serum CK-MB and troponin-I in saline and D-gal treated rats (mean± SD). 2 way-Anova followed by post hoc test expressed $*p<0.05$, $**p<0.01$, vs. respective control and $+p<0.05$, $++p<0.01$ vs. control and Li injected rats

Table 1: Effect of LiCl on D-gal induced oxidative stress marker and antioxidant enzymes in Liver of rats (n=5, for each group).

	Saline LiCl				D-Gal LiCl			
	Saline (ml/kg)	10	20 (mg/ml/kg)	40	Saline (ml/kg)	10	20 (mg/ml/kg)	40
MDA (mM/gm)	6.59±0.97	**2.58±1.09	*3.90±1.55	5.83±1.94	$^{++}$12.71±1.39	**3.16±0.96	**4.54±0.69	**6.67±0.96
SOD (%)	84.70±7.76	89.13±5.08	93.94±3.95	**100.13±4.15	$^{++}$66.78±7.70	$^{++}$74.46±4.83	**84.90±3.53	**91.52±6.39
CAT (mg/dl)	6.28±0.37	6.84±0.73	7.40±0.55	**8.50±0.54	$^{++}$3.68±0.68	$^{++}$4.21±0.69	$^{*++}$5.27±0.56	$^{**++}$5.99±0.77
GPx (µmol/gm/min)	0.50±0.08	0.55±0.07	0.60±0.08	**0.68±0.07	$^{++}$0.28±0.03	$^{++}$0.35±0.04	$^{*++}$0.43±0.06	$^{**++}$0.51±0.07

Table 2: Effect of LiCl on D-gal induced oxidative stress marker and antioxidant enzymes in kidney of rat (n=5, for each group).

	Saline LiCl				D-Gal LiCl			
	Saline (ml/kg)	10	20 (mg/ml/kg)	40	Saline (ml/kg)	10	20 (mg/ml/kg)	40
MDA (mM/gm)	6.36±0.80	5.69±0.62	5.13±0.74	5.24±0.97	$^{++}$12.03±1.10	$^{**++}$9.97±0.95	$^{**++}$8.21±1.37	**5.74±1.37
SOD (%)	67.22±5.59	72.44±3.13	78.26±6.22	**83.12±8.62	$^{++}$44.19±7.24	$^{++}$50.62±3.56	$^{**++}$58.24±3.59	$^{**++}$68.10±4.85
CAT (mg/dl)	6.27±0.77	6.98±0.69	7.71±0.71	**8.69±0.72	$^{++}$3.73±0.91	$^{++}$4.61±0.92	$^{++}$5.34±0.71	$^{**++}$6.40±0.64
GPx (µmol/gm/min)	0.45±0.08	0.50±0.08	0.57±0.06	**0.63±0.07	$^{++}$0.28±0.05	$^{*++}$0.34±0.03	$^{*++}$0.41±0.01	**0.53±0.06

Table 3: Effect of LiCl on D-gal induced oxidative stress marker and antioxidant enzymes in heart of rat (n=5, for each group).

	Saline LiCl				D-Gal LiCl			
	Saline (ml/kg)	10	20 (mg/ml/kg)	40	Saline (ml/kg)	10	20 (mg/ml/kg)	40
MDA (mM/gm)	5.46±0.65	4.91±0.56	4.30±0.59	3.70±0.52	$^{++}$8.80±1.40	$^{++}$7.29±1.22	$^{**+}$6.14±0.85	**4.47±0.69
SOD (%)	66.42±6.0	70.25±2.98	73.60±3.48	**77.88±6.48	$^{++}$46.09±6.38	$^{++}$53.88±4.41	$^{**++}$61.40±2.36	**71.26±2.14
CAT (mg/dl)	6.21±0.62	6.48±0.58	6.86±0.51	*7.43±0.50	$^{++}$3.91±0.68	$^{++}$4.63±0.52	**5.72±0.66	**6.40±0.55
GPx (µmol/gm/min)	0.46±0.06	0.48±0.05	0.52±0.07	0.55±0.06	$^{++}$0.20±0.01	$^{++}$0.26±0.03	$^{++}$0.31±0.04	$^{**++}$0.36±0.05

Table 4: Effect of LiCl on serum lipid profile of D-gal and saline treated rats (n=5, for each group).

	Saline LiCl				D-Gal LiCl			
	Saline (ml/kg)	10	20 (mg/ml/kg)	40	Saline (ml/kg)	10	20 (mg/ml/kg)	40
Cholesterol (mg/dl)	129.60±6.69	135±4.12	*139.80±4.43	**144±5.40	$^{++}$166±4.63	$^{++}$158.60±4.39	$^{**+}$150.60±2.70	$^{**++}$140±1.58
Triglycerides (mg/dl)	43±4.18	38.60±3.36	**32±2.23	**25.60±1.81	$^{++}$138.80±4.49	$^{**++}$122.20±4.96	$^{**++}$102.60±4.39	$^{++**}$84.20±6.05
HDL (mg/dl)	40.60±4.21	37.20±4.38	38.0±3.08	39.80±3.56	$^{++}$22.80±2.38	$^{++}$25.40±1.67	*27.60±1.51	**31.40±2.07
LDL (mg/dl)	35.20±4.76	33.20±3.19	31.20±3.11	29.20±5.16	$^{++}$52.40±8.47	$^{++}$46.40±6.46	**37.60±6.46	**37.60±5.72

Statistical difference is represented as*$p < 0.05$, *$p < 0.01$ versus respective control and +$p < 0.05$, ++$p < 0.01$ versus saline + LiCl treated rats.

Attenuation by Li [40mg/ml/kg] increased SOD activity than their control animals. Activity of SOD also increased in Li [20 & 40mg/ml/kg]+D-gal compared to D-gal treated animals. Action potential of SOD decreased in D-gal+Li [10mg/kg] than Li [10mg/kg] rats.

Data of CAT shown substantial effect of D-gal {$f_{(1,32)}$=136.83, *p<0.01*} and Li {$f_{(3,32)}$=21.04, *p<0.01*}, while insignificant interface of between D-gal x Li {$f_{(3,32)}$=0.309, *p>0.05*}. Post hoc test showed that D-gal decreased CAT activity than control animals. Administration of Li increased CAT activity at 40mg/kg in saline treated rats and at 20 & 40 mg/kg in gal treated rats when compared to control animals. Administration of D-gal+Li [10, 20 & 40mg/kg] decreased CAT activity than their respective control.

Data for activity of GPx gave substantial effect of D-gal {$f_{(1,32)}$=74.80, *p<0.01*} and Li {$f_{(3,32)}$=15.07, *p<0.01*}, while insignificant interactivity between D-gal x LiCl {$f_{(3,32)}$=0.426, *p>0.05*}. Tukey's test showed that administration of D-gal decreased GPx activity than saline injected rats. Administration of LiCl increased GPx activity at 40 mg/kg in saline treated rats and at 20 and 40mg/kg in D-gal treated rats compared to control animals. Administration of D-gal*Li [10, 20 & 40mg/kg] decreased GPx activity than their respective control.

Table 2 displays the outcome of LiCl on oxidative markers and antioxidant enzymes in kidneys of D-gal and saline given rats. All the data evaluated by *2-way-anova.* Data for lipid peroxidation showed substantial effect of D-gal {$f_{(1,32)}$=108.10, *p<0.01*}, Li {$f_{(3,32)}$=23.81, *p<0.01*}, and interface of D-galxLi {$f_{(3,32)}$=11.37, p<0.01}. Post hoc test showed that D-gal substantially enhanced MDA levels than control one. Li [10, 20 & 40mg/ml/kg)+D-gal decreased lipid peroxidation than saline+D-gal animals. Levels of MDA increased in D-gal+Li[10 and 20mg/kg] than saline+Li [10 and 20mg/kg].

Data for activity of SOD revealed substantial effect of D-gal {$f_{(1,32)}$=124.76, *p<0.01*} and Li{$F_{(3,32)}$=23.07, *p<0.01*}, while insignificant interaction between D-galxLi {$f_{(3,32)}$=0.972, *p > 0.05*}. Post hoc test showed that D-gal reduced SOD potential than control one. Administration of D-gal+Li [20 & 40mg/ml/kg] increased SOD activity than D-gal animals. D-gal+Li (all doses) decreased CAT activity than their respective control.

Data for activity of CAT revealed substantial effect of D-gal {$f_{(1,32)}$=97.25, *p<0.01*} and Li {$f_{(3,32)}$=19.84, *p<0.01*}, whereas insignificant interface of D-galxLi {$f_{(3,32)}$=0.047, *p>0.05*}. Post hoc test showed D-gal decreased CAT activity than control one. Activity of CAT increased in saline+Li [40mg/kg] than saline+saline and D-gal+Li [20 & 40mg/kg] than D-gal treated rats. D-gal+Li (all doses) decreased CAT activity than their respective control.

Data for activity of GPx revealed substantial effect of D-gal [$f_{(1,32)}$=52.5, *p<0.01*} and Li {$f_{(3,32)}$=20.06, *p<0.01*}, while insignificant interface of D-galxLi {$f_{(3,32)}$=0.718, *p>0.05*}. Post hoc test showed that D-gal reduced GPx activity than control animals. Activity of GPx increased in saline+Li [40mg/kg] than saline+saline and D-gal+Li [10, 20 & 40 mg/kg] than D-gal treated rats. D-gal+Li [at 10 & 20mg/kg] decreased CAT activity than their respective control.

Table 3 displays the properties of LiCl on oxidative markers and antioxidant enzymes in heart of D-gal and saline injected rats. All the data evaluated by *2-way-anova*. Data for lipid peroxidation revealed substantial effect of D-gal {$f_{(1,32)}$=57.11, *p<0.01*}, Li {$f_{(3,32)}$=22.06, p<0.01} and interface of D-galxLi {$f_{(3,32)}$=3.812, *p<0.01*}. Post hoc test showed that D-gal enhanced MDA levels than control one. Li [20 & 40mg/ml/kg]+D-gal decreased lipid peroxidation than saline+D-gal animals. Lipid peroxidation increased in D-gal+Li [10 & 20mg/kg] than saline+Li [10 & 20mg/kg].

Data for activity of SOD revealed substantial effect of D-gal {$f_{(1,32)}$=90.84, *p<0.01*], Li {$f_{(3,32)}$=28.73, *p<0.01*} and interface of D-galxLi {$f_{(3,32)}$=4.05, p<0.05}. Post hoc showed that D-gal decreased SOD activity than control one. Administration of D-gal+Li [20 & 40mg/ml/kg] and saline+Li[40mg/kg] increased SOD activity than D-gal and saline injected rats respectively. SOD's activity decreased in D-gal+Li [10 & 20 mg/kg] than saline+Li [10 & 20mg/kg].

Data for activity of CAT revealed substantial effect of D-gal {$f_{(1,32)}$=72.33, *p<0.01*} and Li {$f_{(3,32)}$=19.14, *p< 0.01*}, while non-significant interface of D-galxLi {$f_{(3,32)}$=2.61, *p>0.05*}. Post hoc test showed that D-gal decreased CAT activity than control one. Activity of CAT increased in saline+Li [40 mg/kg] than saline+saline and D-gal+Li [20 & 40mg/kg] than D-gal treated rats. Activity of CAT decreased in D-gal+Li [10mg/kg] than Li[10mg/kg].

Data for activity of GPx revealed substantial effect of D-gal {$f_{(1,32)}$=156.30, *p<0.01*} and Li {$f_{(3,32)}$=9.75, *p< 0.01*}, while insignificant interface between D-galxLi {$f_{(3,32)}$=0.48, p>0.05}. Post hoc test showed that D-gal reduced GPx activity than control one. GPx increased in D-gal+Li [40mg/kg] than D-gal injected rats. Potential of GPx decreased in D-gal+Li [at all doses] than their respective control.

Table 4 displays the properties of LiCl on serum cholesterol, triglyceride, LDL and HDL levels saline and D-gal treated rats. All the data evaluated by *2-way-anova*.

Data for levels of cholesterol revealed insubstantial effect of Li {f $_{(3,32)}$=2.75, *p>0.0*}. Effect of D-gal {f $_{(1,32)}$=135.02, *p < 0.01*}, and interaction between D-galxLi {f $_{(3,32)}$=38.54, *p<0.01*} was significant. Post hoc test showed that D-gal increased serum cholesterol levels than respective control. Li at doses 10 & 20mg/kg significantly increased cholesterol levels than their respective control. D-gal+Li [at all doses] increased cholesterol than their counterparts. The levels of cholesterol were decreased in D-gal+Li [20 & 40mg/kg] than saline+D-gal treated rats.

Data for levels of triglyceride revealed substantial effects of LiCl {f $_{(3,32)}$=141.97, *p<0.01*}, D-gal {f $_{(1,32)}$=34.50, *p< 0.01*}, and interaction between D-gal* Li {f $_{(3,32)}$=37.50, *p<0.01*}. Post hoc test showed that D-gal increased serum triglyceride levels than saline treated animals. Li at doses 10 & 20mg/kg significantly decreased triglyceride levels than their respective control. D-gal+Li [at all doses] increased triglyceride than their counterparts. The levels of triglyceride were decreased in D-gal+Li [10, 20 & 40mg/kg] than saline+D-gal treated rats.

Data for levels of LDL revealed significant effects of Li {f $_{(3,32)}$=11.50, p<0.01}, D-gal {f $_{(1,32)}$=31.74, p< 0.01}, and interaction between D-galxLi{f $_{(3,32)}$=3.65, p<0.05}. Post hoc test showed that D-gal increased serum LDL levels than saline treated animals. D-gal+Li [10 mg/kg] increased LDL than their counterparts. The levels of LDL were decreased in D-gal+Li [20 & 40mg/kg] than saline+D-gal treated rats.

Data for levels of HDL revealed non-significant effects of Li {f $_{(3,32)}$=0.51, *p>0.05*}. Effect of D-gal {f $_{(1,32)}$=75.07, *p<0.01*} and interactivity between D-galxLi{f $_{(3,32)}$=20.18, *p<0.01*} was significant. Post Hoc test showed that D-gal decreased serum HDL levels than saline treated animals. D-gal+Li [10 & 20mg/kg] decreased HDL than their counterparts. The levels of HDL were increased in D-gal +Li [40mg/kg] than saline*D-gal treated rats.

Fig. 1 displays the properties of LiCl on activity of ALT and AST (Liver function test) in saline and D-gal rats. All the data evaluated by *2-way-anova.* Data for serum levels of ALT revealed substantial effect of D-gal {f $_{(1,32)}$=61.47, *p<0.01*}, Li{f $_{(3,32)}$=0.038, *p<0.01*}, and interactivity between D-gal*Li {f $_{(3,32)}$=17.60, *p<0.01*}. Post hoc test showed that activity of ALT increased in D-gal+saline than saline+saline treated animals. Li [40mg/ml/kg]+D-gal decreased ALT activity than D-gal rats.

Data for serum levels of AST revealed substantial effect of D-gal {f $_{(1,32)}$=435.83, *p<0.01*}, Li{f $_{(3,32)}$=6.292, *p<0.01*} and interface of D-gal*Li {f $_{(3,32)}$=22.73, *p<0.01*}. Post hoc test showed that activity of AST increased in D-gal+saline than saline+saline treated animals. D-gal+Li [20 & 40 mg/ml/kg] decreased the activity of AST than D-gal+saline treated rats. Activity of AST increased in D-gal+Li [at all doses] than their respective control.

Fig. 2 displays the effects of LiCl on serum urea and creatinine (kidney function test) in saline and galactose treated rats. All data evaluated by *2-way-anova*. Data for serum urea levels revealed substantial effect of D-gal {f $_{(1,32)}$=19.99, *p<0.01*}, Li{f $_{(3,32)}$=25.17, 1*p<0.01*}, and interface of D-gal*Li {f $_{(3,32)}$=91.32, *p<0.01*}. Post hoc test showed that levels of serum urea increased in D-gal+saline than saline+saline treated animals. Li [20 & 40mg/ml/kg]+D-gal decreased urea than saline+D-gal animals. Levels of urea also decreased in Li [40mg/ml/kg]+D-gal than Li [20 & 40mg/ml/kg] saline treated animals.

Data for creatinine shown significant effect of D-gal {f $_{(1,32)}$ =518.72, *p<0.01*}, Li{f$_{(3,32)}$=3.97, p<0.01} and interactivity between D-gal*Li {f $_{(3,32)}$=7.81, p<0.01}. Post hoc test showed that administration of D-gal+saline substantially increased creatinine levels than saline+saline treated animals. D-gal+Li [all doses] increased creatinine than Li [all doses] +saline treated rats. Levels of serum creatinine decreased in D-gal+Li [40mg/ml/kg] than saline+Li [40mg/ml/kg] injected rats.

Fig. 3 displays the properties of LiCl on serum CK-MB and troponin-I in saline and D-gal treated rats . All the data evaluated by *2-way-anova*. Data for serum CK-MB levels revealed non-significant effect of Li {f $_{(3,32)}$=0.420, *p>0.05*}. Effect of D-gal {f $_{(1,32)}$=155.29, *p<0.01*}, and interactivity between D-galxLi {f $_{(3,32)}$=10.21, 3*p<0.01*} was significant. Post hoc test showed that D-gal+saline increased CK-MB levels than saline+saline treated animals. Li [40mg/ml/kg)+D-gal decreased CK-MB than saline+D-gal animals. Levels of serum CK-MB increased in Li [20 & 40 mg/ml/kg]+D-gal than Li [20 & 40 mg/ml/kg]+saline treated animals.

Data for levels of troponin-I revealed non-significant effect of Li {f $_{(3,32)}$=0.17, *p>0.05*]. Effect of D-gal {f $_{(1,32)}$=28.36, *p<0.01*}, and interactivity between D-gal x Li {f $_{(3,32)}$=4.45, *p<0.05*} was significant. Post hoc test showed that D-gal+saline increased troponin-I levels than saline+saline injected rats. D-gal*Li [10mg/ml/kg] increased troponin-I than Li [10mg/ml/kg]*saline treated rats.

DISCUSSION

Chronic administration of D-gal accelerates the aging process due to increased ROS production; therefore D-gal treated rats have been used as aging models in gerontology and pathophysiological investigational studies (Hoffman *et al.* 1997). It has been confirmed by several studies that oxidative insult induced by D-gal

leads to organ injury (Rathod *et al.* 2016). In the current study frequent administration of D-gal (300/mg/ml/kg/day) induced age-mimicking effects in various organs (liver, kidney, and heart) by increasing oxidative stress and reducing the natural antioxidant potential of defense system (table 1-3). Lipid profile (table 4) and serum levels of ALT and AST (fig. 1), creatinine and urea (fig. 2), and CK-MB and troponin-I (fig. 3) were also increased by treatment of D-gal. Various doses of LiCl i.e. (10, 20 and 40 mg/ml/kg/day) inhibited D-gal induced oxidative damage in various organs and altered blood parameters.

Several studies have confirmed that aging involves changes in organ indices and these physiological changes are accelerated by D-gal administration (James *et al.* 2015). It is now generally known that oxidative damage is mainly responsible for organ injuries and is determined by the overproduction of oxidative stress marker MDA and altered activities of antioxidant enzymes SOD, CAT, and GPx (Wang *et al*. 2020). The finding of the current study reveals that repeated administration of D-gal enhancing the lipid peroxidation in all tested organs with reducing antioxidant defense mechanism which is exhibited by decreased levels of SOD, CAT, and GPx enzymes activity (Table 1, 2 & 3). It is indicating that D-gal administration is not only involved in the oxidative damage of liver, kidney, and heart, it is also involved in the impairment of organs functions and increased serum levels of liver function enzymes i.e ALT, AST (fig. 1); kidney markers i.e. creatinine, urea (fig. 2) and cardiac markers CK-MB, troponin-I (fig. 3) and serum lipid profile (Table 4). Interestingly, our results showed that various doses of LiCl decreased lipid peroxidation and increased antioxidant enzyme activity in organs, indicating the antioxidant potential of LiCl mitigating the oxidative stress in heart, liver, and kidney while decreasing D-gal induced increased serum levels of ALT, AST, creatinine, urea, CK-MB and troponin-I and lipid profile in rats.

It is extensively reported that antioxidants include SOD, CAT, and GPx may lessen oxidative deterioration and potentially reverse oxidative related diseases (Fan *et al.* 2009). LiCl protected the antioxidant defense mechanism by increasing the activity of the enzyme (SOD, CAT, GPx) and suppressing lipid peroxidation (MDA) in the kidney, heart, and liver of D-gal treated rats. LiCl may correct the pro-oxidant antioxidant disequilibrium, contributing to the protection of liver, kidney and heart deterioration.

All vital organs are vulnerable to oxidative damage induced by D-galactose including kidneys, liver, and heart. Previous studies showed that the administration of D-gal causes liver and kidney injury and abnormalized functions by elevation of serum analytes which are essential for normal functioning (Yu *et al*. 2015; Park *et al.* 2015). In the present study administration of D-gal increased serum levels of ALT, AST in the liver, creatinine, and urea in the kidney and troponin-I and CK-MB in the heart. Furthermore, the administration of various doses of LiCl dramatically alleviated the D-gal induced dysfunction of organs.

The lipid profile is altered due to the aging process and caused various ailments. Several experimental studies reported that the administration of D-gal disturbed the lipid profile which enhancing cholesterol, triglycerides, and LDL and decreasing HDL, the main risk factor to cardiovascular disease development (Chapman *et al*. 2011). The results of the present study are consistent with the previous report. The effect of administration of LiCl on lipid profile is not constant in various studies; some studies exhibited an increase (Kanzariya 2011) while in some no effect was observed (Kiełczykowska *et al.* 2014). In the present study, various doses (10, 20 and 40 mg/ml/kg) of LiCl produced differential effects. It was observed that at doses 20 and 40mg/kg of LiCl, serum levels of HDL and triglycerides were decreased while cholesterol levels were increased, but no effect on LDL level was observed. Conversely, dyslipidemic effects of D-gal were inhibited by co-treatment with LiCl with decreasing plasma levels of cholesterol, LDL (at 20 and 40 mg/kg), triglyceride (at all doses) and increasing HDL (at 40 mg/kg). Although all doses (10, 20 and 40 mg/kg) of LiCl were effective to improve dyslipidemia at high dose effects were more potentiated. It is reported earlier that Li exerted the divergent effect when used in various dose ranges (i.e. low, medium, and high) (Li, 2001). So, it is suggested that this drug could improve dyslipidemia by decreasing cholesterol, LDL and triglyceride and reduce harmful effects lipid factors and increasing the serum levels of HDL in D-gal induced aging rats possibly via its antioxidant potential.

The relationship between oxidative stress and inflammation is reported extensively (Dandekar *et al.* 2015). IL-1, IL-6, TNFs are key pro-inflammatory cytokines which trigger inflammatory response involving massive cell death of hepatocytes (Niederreiter and Tilg 2018), nephrocytes (Kim *et al.* 2018) (and cardiomyocytes (Ueland *et al.* 2015). These pro-inflammatory cytokines are critical players for the damage of these organs. The inflammatory cascade is involved in the generation of free radicals including ROS which causes oxidative damage and dysfunction organs. Although in this study we didn't evaluate inflammatory markers but elevated oxidative stress by increasing MDA and decreasing antioxidant enzyme showing elevated inflammatory cascade (Chen *et al*. 2008; Shi *et al*. 2004). It is reported earlier that the body defense system of antioxidant enzymes including CAT, GPx, and SOD fight against free radicals' production and reduce oxidative stress (Rehman *et al.* 2016). It is recognized that LiCl inhibits pro-

inflammatory cytokines (Nassar and Azab 2014). It is also well reported that LiCl has great potential to alleviate oxidative stress via its antioxidant capability (Samad *et al.* 2019). In the present study, LiCl improved the antioxidant mechanism by increasing the activity of antioxidant enzymes (SOD, CAT, GPx) and inhibiting lipid peroxidation (MDA) in the liver, heart, and kidney of D-gal injected rats. LiCl may normalize the unevenness of pro-oxidant-antioxidant, contributing to the improvement of liver, kidney, and heart functions. Accompanied with the effects of D-gal induced oxidative stress on liver, kidney, and heart, the activity of ALT, AST, serum level of creatinine, urea CK-MB and troponin-I were also higher than those of normal rats. However, LiCl not only exhibited antioxidant properties by increasing antioxidant enzymes and decreasing lipid peroxidation, showing the contrasting effects on D-gal induced oxidative harms to rats liver, kidney and heart but also palliated these increases of ALT and AST enzyme activity, serum creatinine, urea CK-MB and troponin-I levels to an extent lower than the saline+D-gal treated group, suggesting an important role in protecting the normal function of the liver, kidney, and heart.

CONCLUSION

In conclusion, LiCl at various doses (10, 20, and 40 mg/kg) improved antioxidant mechanisms and improve the functioning of the liver, kidney and heart, and dyslipidemia. Although all doses used in this study exhibited their effects, a high dose (40 mg/kg) of LiCl was more effective against D-gal induced adverse effects. Hence, LiCl alleviates damages in the liver, kidney, and heart from D-gal rats and LiCl might be used as a therapeutic agent.

REFERENCES

Acosta PB and Gross KC (1995). Hidden sources of galactose in the environment. *Eur. J. Pediatr.,* **154**(7): 87-92.

Aherns B and Muller-Oerlinghausen B (2001). Does lithium exert an independent antisuicidal effect? *Pharmacopsychiat.,* **34**(4): 132-136.

Allagui MS, Nciri R, Rouhaud MF, Murat JC, El Feki A, Croute F and Vincent C (2009). Long-term exposure to low lithium concentration stimulates proliferation, modifies stress protein expression pattern and enhances resistance to oxidative stress in SH SY5Y cells. *Neurochem Res*., **34**(3): 453-462.

Barjasteh-Askari F, Davoudi M, Amini H, Ghorbani M, Yaseri M, Yunesian M, Mahvi AH and Lester D (2020). Relationship between suicide mortality and lithium in drinking water: A systematic review and meta-analysis. *J. Affect Disord.,* **264**(4): 234-241.

Baranov VS and Baranova EV (2017) Aging and ambiguous ROS System genetics analysis. *Curr Aging Sci.,* **10**(1): 6-1.

Boss GR and Seegmiller JE (1981) Age-related physiological changes and their clinical significance. *West J Med*., **135**(6): 434-440.

Chen P, Chen F and Zhou B (2018) Antioxidative, anti-inflammatory and anti-apoptotic effects of ellagic acid in liver and brain of rats treated by D-galactose. *Sci Rep.,* **8**(2):1465.

Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Boren J, Catapano AL, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA and Lesnik P (2011). Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J.,* **32**(11): 1345-136.

Chen S, Ge Y, Si J, Rifai A, Dworkin LD and Gong R (2008). Candesartan suppresses chronic renal inflammation by a novel antioxidant action independent of AT1R blockade. *Kidney Int.,* **74**(9): 1128-1138.

Chow CK and Tapple AL (1973). An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. *Lipids.,* **7**(8): 518-24.

Ciacka K, Tymiński M, Gniazdowska A, Krasuska U (2020) Carbonylation of proteins-an element of plant ageing. *Planta.,* **252**(1): 12.

Doan VM, Chen C, Lin X Nguyen VP, Nong Z, Li W, Chen Q, Ming J, XIe Q and Huang R (2015) Yulangsan polysaccharide improves redox homeostasis and immune impairment in D-galactose-induced mimetic aging. *Food Funct.,* **6**(5): 1712-1720.

Dietrich-Muszalska A, Chauhan V and Grignon S (2015) Studies on Psychiatric Disorders, Oxidative Stress in Applied Basic Research and Clinical Practice, doi: 10.1007/978-1-4939-0440-2.

Dandekar A, Mendez R and Zhang K (2015). Cross talk between ER stress, oxidative stress, and inflammation in health and disease. *Methods Mol Biol.,* **1292**(2): 205-14.

Ettenberg A, Ayala K, Krug JT, Collins L, Mayes MS, Fisher MPA (2020) Differential effects of lithium isotopes in a ketamine-induced hyperactivity model of mania. *Pharmacol Biochem Behav.,* **190**(3): 172875.

Flohe L and Gunzler WA (1984) Assays of glutathione peroxidase. *Methods Enzymol.,* **105**: 114-121.

Fan SH, Zhang ZF, Zheng YL, Lu J, Wu DM, Shan Q, Hu B and Wang YY (2009). Troxerutin protects the mouse kidney from d-galactose-caused injury through anti-inflammation and anti-oxidation. *Int. Immunopharmacol.,* **9**(1): 91-96.

Fernandes MS, Barbisan F, Azzolin VF, do Prado-Lima PAS, Teixeira CF, da Cruz Jung IE, Assmann CE, Riffel RT, Duarte MMMF, Aguiare-Ribeiro EM and da Cruz IBM (2019). Lithium is able to minimize olanzapine oxidative-inflammatory induction on macrophage cells. *PLoS One*., **14**(1): 209-223.

Hoffman A, Afargan M, Backon J and Perlstein I (1997). The anticonvulsant effect of deprenyl on pentylenetetrazol-induced seizures in Lewis rats. *Int. J. Neurosci.,* **90**(3-4): 223-232.

James EL, Michalek RD, Pitiyage GN, de Castro AM, Vignola KS, Jones J, Mohney RP, Karoly ED, Prime SS and Parkinson EK (2015). Senescent human fibroblasts show increased glycolysis and redox homeostasis with extracellular metabolomes that overlap with those of irreparable DNA damage, ageing and disease. *J. Proteome. Res.,* **14**(4): 1854-1871.

Kanzariya NR, Patel RK and Patel NJ (2011) Antidiabetic and vasoprotective activity of lithium: Role of glycogen synthase kinase-3. *Indian J. Pharmacol.,* **43**(4): 433-436.

Kim DJ, Park JW, Jee IG and Kim SH (2018). Effect of monitored anesthesia care using dexmedetomidine on stress hormones and interleukin-6 in patients undergoing arteriovenous fistula formation. *Medical Biol. Sci. Engg.,* **1**(1): 51-57.

Knight J and Nigam Y (2012). Physiological Changes Associated with Aging and Immobility. *J Aging Res.,* 2012:468469. doi: 10.1155/2012/468469. Epub 2012 May 3.

Knight J and Nigam Y (2008). The anatomy and physiology of ageing. *Nursing Times.,* **104**(31): 26-27.

Kielczykowska M, Kocot J, Kurzepa J, Lewandowska A, Żelazowska R and Musik I (2014). Could selenium administration alleviate the disturbances of blood para meters caused by lithium administration in rats? *Biol. Trace Elem Res.,* **158**(3): 359-64.

Lazarus NR, Lord JM and Harridge SDR (2019). The relationships and interactions between age, exercise and physiological function. *J. Physiol.,* **597**(5): 1299-1309.

Lis V and Long S (2001). Effects of manganese, zircon and lithium alone on rat liver lipid peroxidation: *Wei Sheng Yan Jiu J Hygiene Res.,* **30**(3): 142-143.

Niiederreiter L and Tilg H (2018). Cytokines and fatty liver diseases. *Liver Res.,* **2**(2): 14-20.

Nassar A and Azab AN (2014). Effects of Lithium on Inflammation. *ACS Chem Neurosci.,* **5**(6): 451-458.

Park YMM, Sui X, Liu J, Zhou H, Kokkinos PF, Laive CJ, Hardin JW and Blair SN (2015). The Impact of Cardiorespiratory Fitness on Age-Related Lipids and Lipoproteins. *J Am Coll Cardiol.,* **65**(19): 2091-2100.

Pari L and Latha M (2004). Protective role of *Scorparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. *BMC Complement Alternat Med.,* **6**(1):16.

Rathod R, Kale A and Joshi S (2016). Novel insights into the effect of vitamin B12 and omega-3 fatty acids on brain function. *J Biomed Sci.,* **23**(5): 1-7.

Rehman SU, Shah SA, Ali T, Chung JI and Kim MO (2017) Anthocyanins Reversed D-Galactose-Induced Oxidative Stress and Neuro inflammation Mediated Cognitive Impairment in Adult Rats. *Mol Neurobiol.,* **54**(1): 255-271.

Samad N, Saleem A, Yasmin F and Shehzad MA (2018). Quercetin protects against stress-induced anxiety- and depression-like behaviour and improves memory in male mice. *Physiol Res.,* **67**(5): 795-808.

Samad N, Imran I, Zulfiqar I and Bilal K (2019). Ameliorative effect of lithium chloride against d-galactose induced behavioral and memory impairment, oxidative stress and alteration in serotonin function in rats. *Pharmacol Rep.,* **71**(5): 909-916.

Shi M, Xu B, Wang X, Aoyama K, Michio SA and Takeuchi T (2004). Oxidative damages in chronic inflammation of a mouse autoimmune disease model. *Immunol Lett.,* **95**(2): 233-236.

Sikora E, Arendt T, Bennette M and Narita M (2011). Impact of cellular senescence signature on ageing research. *Ageing Res Rev.,* **10**(1): 146-152.

Siervo M, Lara J, Celis-Morales C, Vacca M, Oggioni C, Battezzati A and Leone A (2016). Age-related changes in basal substrate oxidation and visceral adiposity and their association with metabolic syndrome. *Eur J Nutrition.,* **55**(4): 1755-1767.

Thoppil H and Riabowol K (2020). Senolytics: A Translational Bridge Between Cellular Senescence and Organismal Aging. *Front Cell Dev Biol.,* **7**(3): 367.

Ueland T, Gullestad L, Nymo SH, Yndestad A, Aukrust P and Askevold ET (2015) Inflammatory cytokines as biomarkers in heart failure. *Clin. Chim. Acta.,* **443**(3): 71-77.

Wang C, Shen Z, Yu J, Yang J, Meng F, Jiang X, Zhu C (2020). Protective effects of enzyme degradation extract from Porphyra yezoensis against oxidative stress and brain injury in D-galactose-induced ageing mice. *Br. J. Nutr.,* **123**(9): 975-986.

Yu Y, Bai F, Liu Y, Yang Y, Yuan Q, Zou D, Qu S, Tian G, Song L, Zhang T, Li S, Liu Y, Wang W, Ren G and Li D (2015). Fibroblast growth factor (FGF21) protects mouse liver against d-galactose-induced oxidative stress and apoptosis via activating Nrf2 and PI3K/ Akt pathways. *Mol. Cell Biochem.,* **403**(1-2): 287-299.

Zhang W, Qu J, Liu GH and Belmonte JCI (2020). The ageing epigenome and its rejuvenation. *Nat. Rev. Mol. Cell. Biol.,* **21**(3): 137-150.

Zhen YZ, Lin YJ, Li KJ, Zhang GL, Zhao YF, Wang MM, Wei JB, Wei J and Hu G (2016). Effects of rhein lysinate on D-galactose-induced aging mice. *Exp. Ther. Med.,* **11**(1): 303-308.