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### Original Paper

## Calories restriction modulates the enzymatic antioxidant status in D-galactose induced aging in male rats

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### ABSTRACT

In post-industrial and modern societies, overeating and high calories consumption has been emerged as new challenge in community health. Large effort is now being devoted to detect and evaluate the pathophysiologic consequences of aging and aging accompanying diseases. Several lines of evidence propose that caloric intake influences the speed of aging and the onset of linked diseases in animals and, possibly, humans. These are the clues of decreasing calories intake in this study to evaluate the effect on aging and to detect if there are ways to control it. Thirty adult male albino rats were used in this work, ten rats were used as control group. D-galactose was injected SC to induce aging, then rats were sub divided into equal two groups, one group subjected to calories reduction while the other took normal diet. Catalase activity, reduced glutathione and L-MDA concentration-were carried out to test the oxidative effect of D-galactose and possible protective effect of treatments. D-galactose induced impaired oxidativative functions. Calories reduction improved D-galactose induced deterioration to a great extent as evidenced biochemically.

### 1. INTRODUCTION

Aging has been targeted by dietary manipulation, drugs and antioxidants, to increase lifespan, and promotion of healthy aging. The National Institute of Aging has studied numerous treatments, including nutraceuticals and diets, with the ability to prolong life spans and postpone disease and dysfunction in animals. The beneficial effect of lifestyle on ageing meets these rigorous requirements from a pharmaceutical point of view: fasting regimens, calorie restriction and exercise (Omayma et al., 2021). Aging is marked by a progressive drop in metabolic processes and physiological functions which end to mortality and morbidity. Agreeing to the theory of aging; free radical, reactive oxygen species (ROS), created waste-products after biological oxidations, encourage casual and collective oxidative destruction to intracellular macromolecules prompting cellular changes that increase with age and lastly cell death (Fedarko, 2018). Accumulating proof in rat model of accelerated senescence demonstrates a rescuing accountability of calories restriction in early aging. Calories decreasing seems to arrest oxidative stress, cell growth, telomere attrition, disordering of chromatin, and extreme secretion of inflammatory factors, and therefore prolonging lifespan (Nam et al., 2019). The process how aging takes place distresses all kinds of organisms. Though presenting characters that are tissue-related, the feature of an advanced functional and structural corrosion with progressing age, finally resulting in death, is totally known. However, the negative relates of aging (cognitive and physical deterioration) make older people fewer independent on their self (Passarino et al., 2016). A huge

deal of study definite that aging doesn't occur due to unique reason, while numerous mechanisms control the entire aging procedure (Carmona et al., 2016). Appreciations to the leading study done on the *Caenorhabditiselegans* model by means of Kenyon et al. (2019), it come to be non-doubtable that aging progression is elastic and could be attenuated or accelerated by a variety of nutritional and genetic interferences (Kenyon 2019). According to Omayma et al. (2020), oxidative chemicals cause disturbance in nucleic acid repair mechanisms leading to excessive production of ROS. Based on the results of several scientists studies, Lopez-Lluch et al. (2016) reported that calories restriction has an intense effect in lengthening both maximal and median lifecycle in rats, and it delays or stops the beginning of numerous age-related illnesses like type-2 diabetes obesity, cardiomyopathy neuro-degeneration, and malignancy (Most et al., 2017). The united theory of CR and endurance by Sinclair suggested that CR may boost the endurance ability of the organism by inducing an extremely preserved stress reaction (Sinclair, 2005). Meanwhile the early investigational decisions on CR, a main argument was ascended about the nature of the reason of the comprehensive longevity namely if it was back to the decrease of protein intake or to that of calories. For a long period, the identification of decreased calorie consumption as a cause for improved endurance succeeded (Masoro 2003). It has been reasoned that the deceptive outcome of the declined energy intake was certainly due to a different ratio of protein to non-protein constituents of the diets (Solon-Biet et al 2014).

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This study was performed to evaluate the possible effect of calories restriction on D-galactose injected male rats.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals:

D-galactose was obtained in the form of powder from Medicinal Biochemistry Department, Faculty of pharmacy, Assuit University, Egypt.

### 2.2. Animals:

the present study was carried out using thirty adult male albino rats (*Sprague Dawley strain*) weighing 200- 250 g and of 8 weeks age and obtained from the Nile company for pharmaceutical and chemical industries (Cairo-Egypt). Rats were housed in metal cages, ten rats per cage, under natural environmental laboratory conditions and diurnal cycle with permitted access to water. All ethical protocols of animal treatment were supervised and followed by the animal house, Faculty of veterinary medicine, Benha University.

### 2.3. Experimental design:

Rats were classified to three equal groups, ten rats each group: 1- Control group (Gp Ct n=10) Untreated group. 2- Model group (GpM, n=20) injected subcutaneously by 50% D galactose (100 mg/kg/d) for 8 weeks (*Tao Tang and Bixiu He., 2013*). This group was divided into two subgroups: A) GpT1 (n=10): rats received basal diet for 6 weeks) GpT2 (n=10): rats will receives 30% less calories for six weeks. At the completion of the trial, rats fasted overnight. Samples were collected from the heart of the animal then sacrificed by cervical dislocation. Blood was allowed to coagulate and then centrifuged at 4000 rpm for 15 min.

### 2.4. Biochemical analysis

After 30 days, serum CAT activity, (Aebi, 1984) MDA (Sato, 1978) and glutathione levels (Ahmed, et al. 1991) in addition to cystatin C and P53 were measured using colorimetric method according to (Pfaffl 2001). The results were subjected to statistical analysis.

### 2.5. Histopathological findings

Sections from the livers were examined to evaluate the damage occurring due to D galactose and the possible modulatory effect of decreasing diet (*Ros, et al., 2020*) Specimens from liver of all examined groups were washed, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5-6  $\mu\text{m}$  in thickness were cut out, deparaffinized and stained with Hematoxylin and Eosin (H and E) for examination under the light microscope. (Banchroft, et al. 1966).

### 2.6. Statistical analysis.

The SPSS form 18 was used in study analysis. Data were analyzed using one way analysis of variance followed by Duncan's multiple range test. A value of  $p < .05$  was considered to indicate significance. The data were expressed as mean  $\pm$  standard error.

## 3. RESULTS

Table (1) demonstrate the (Mean  $\pm$  SE), significance for reduced glutathione ( $\mu\text{mol}/\text{mg}$  tissue), L-MDA ( $\mu\text{mol}/\text{mg}$  tissue), CAT (U/g tissue), Cystatin C (pg/ml) and P53 (pg/ml) in the studied groups (Control, D-galactose and-

calories-restriction). The results clearly showed statistically significant decrease in mean value of all groups but with variable percentage of decrease from control in case of reduced glutathione concentration and CAT activity and as well as increase in cystatin C, P53, and MDA levels after D-galactose injection.

Table 1 The mean value of concentrations of glutathione, L-MDA concentrations, CAT activity, cystatin C and P53 in normal, D-galactose and calories restriction groups.

Parameters	Control	D-galactose	Calories restriction
Glutathione GSH ( $\mu\text{mol}/\text{mg}$ tissue)	95.4 $\pm$ 2.9	39 $\pm$ 6.8 <sup>(a1)</sup>	73.3 $\pm$ 6.8 <sup>(a1,b1)</sup>
L-MDA ( $\mu\text{mol}/\text{mg}$ tissue)	44.95 $\pm$ 1.15	126 $\pm$ 6.6 <sup>(a1)</sup>	55.8 $\pm$ 7.1 <sup>(a1,b1)</sup>
CAT (U/g tissue)	212.05 $\pm$ 1.35	106.9 $\pm$ 5.6 <sup>(a1)</sup>	200.2 $\pm$ 8.1 <sup>(a1,b1)</sup>
Cystatin C (pg/ml)	0.55 $\pm$ 0.025	2.35 $\pm$ 0.45 <sup>(a1)</sup>	0.67 $\pm$ 0.11 <sup>(a1,b1)</sup>
P53 (pg/ml)	1.035 $\pm$ .025	5.25 $\pm$ .45 <sup>(a1)</sup>	2.155 $\pm$ 0.145 <sup>(a1,b1)</sup>

Data are presented as (Mean  $\pm$  S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ( $P \leq 0.05$ ).

(a1)= significant increase from control group

(a1)= significant decrease from control group

(b1)= significant increase from D-galactose group

(b1)= significant decrease from D-galactose group

Figure (1) liver section from normal control group clarify normal hepatic cells with regular pattern and good healthy condition, while figure (2) shows multifocal areas of necrosis with karyorrhectic debris, mononuclear inflammatory cells infiltration and severely congested hepatic sinusoids due to D galactose administration, on the other hand the group which fed less calories showed less congestion and slight necrosis as shown in figure (3)

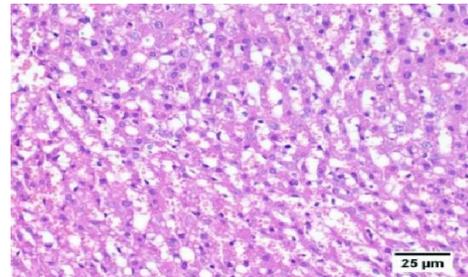


Figure 1: Liver of rat from group 1 showing histological arrangement of hepatic parenchyma with normal hepatic sinusoids (H&E)

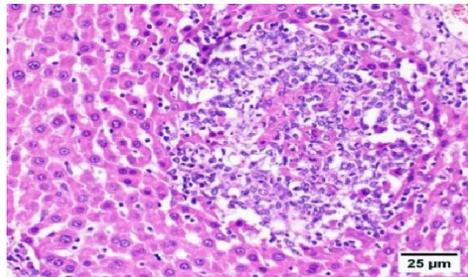


Figure 2: Liver of rat from group 2 showing focal area of lytic necrosis with marked mononuclear inflammatory cells infiltration (H&E).

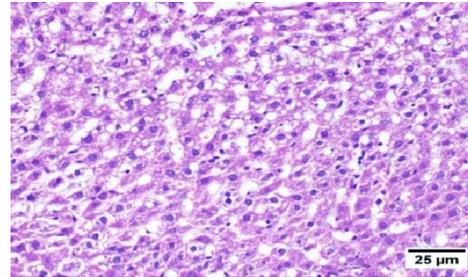


Figure 3 Liver of rat from group 3 showing mild vacuolar degenerated hepatocytes (H&E).

#### 4. DISCUSSION

Aging is the advanced increase of variations during time that are connected to increasing liability to disease which attends advancing age such changes are endorsed to the aging progression. The nature how aging procedure takes place has attracted attention many years ago. Large evidence now prove that the summation of the toxic free radical inter actions going throughout the tissues and cells establishing the aging progression is a main provider to it. (Golubev et al., 2018). Thus, in this trial, we examined the effect of calories restriction on D-galactose-induced adolescence in rats. The outcomes of this study displayed that the administration of D-galactose (100 mg/kg/d) for 8 weeks resulted in oxidative changes analyses of biochemical parameters, indicated significant decline antioxidant store of rat taking D-galactose, with D-galactose bringing oxidative stress through an upsurge in lipid peroxidation (MDA) and decreases in the reduced glutathione contents. The results of this study are in covenant to other experiments that publicized that, in aged animals, the concentration of MDA was significantly increased while the GSH level and the control levels of antioxidant enzymes, such as glutathione peroxidase, glutathione reductase, in addition to catalase, were diminished (Chen et al., 2017). In our experiment, decreasing calories to two thirds had real effects on aging; the GSH exhaustion was resisted and lipid peroxidation was inhibited, this is in agreement with (Granado et al., 2019). In the current work calories restriction caused reduction in the deterioration of glutathione content occurred in rats after injection of D galactose, this is agreed to (Mastaloudis et al., 2020) who posted that decrease calories intake by rats mimetic increases GSH concentrations and improves neuron protection in aged rats. We found that injecting rats with D galactose significantly dropped CAT activity, this is agreed to (Lopez et al., 2016) who found that such harmful effect of D galactose can be minimized by decreasing diet. In other study, (Zhao et al., 2019) found that D galactose resulted in no significant change in CAT activity concentration, this may be back to using another animal model rather than rats. Jové et al. (2020), explained that malondialdehyde increases through aging due to the high rate of lipid oxidation and such findings was agreed with our study that confirms the accumulation of MDA due to the use of D galactose while decreasing diet has been improved such harmful effect. Moreover, D-galactose administration causes contraction of the cell's nucleus and addition to apoptosis in the neurons hippocampus while dropping the calories taken by the rats in group calories restriction modulate the D galactose harmful effect, this is in agree with Wang et al., (2020) who proved that increase in the level of cystatin C protein have been found after exposure to D-galactose. We also found that the levels of P53 has been increased greatly after administration of D galactose, while decreasing diet to two thirds caused correction to the parameter level, such results are in agree with Krzysztoforska et al., (2019) who stated similar results. Morphologically, rats injected by D galactose showed severe changes in hepatic cells noticed as multifocal hemorrhagic areas as compared to control group. In similar study, Kalaz et al. (2014) reported that rats injected by D galactose showed similar effects on liver while decreasing calories consumption in group 3 rats corrected the appearance of hepatic cells and resisted the harmful effect of D galactose.

#### 5. CONCLUSION

From the results obtained from this study and discussed above, we can conclude that calories restriction could possibly delay aging and its accompanying diseases

#### 6. REFERENCES

1. Abozaid, O.A.R., Abdelmageed, A.D., Badawi, A.M., Eid, A.A.Y. 2020. Effect of Cetyltrimethylammonium bromide surfactant coated Curcumin copper nanocomposite on hepatocellular carcinoma in rats. *Benha Veterinary Medical Journal* 38, 79-81
2. Abozaid, O.A.R., Elshawi, O.E., Mohammad, M.A., Moawed, F.S.M. 2021. Caloric restriction combined with vitamin c and Metformin regulates inflammatory mediators through the JNK/ P53/FOXO3 pathway in aged rats *Journal of Biology and Today's World* 10(2), 1-7.
3. Aebi, H. 1984. Catalase in vitro. *Methods Enzymol* 105, 121-126
4. Ahmed, A.E., Hussein, G.I., Loh, J.P., Abdel-Rahman, S.Z. 1991. "Studies on the mechanism of haloacetonitrile induced gastrointestinal toxicity: Interaction of dibromoacetonitrile with glutathione and glutathione-S-transferase in rats. *J Biochem Toxicol* 6(2), 115-121.
5. Bancroft, J.D., et al. 1966 "Theory and practice of histological techniques."
6. Carmona, J.J. and Michan, S. 2016. Biology of healthy aging and longevity. *Rev Invest Clin* 68(1), 7-16.
7. Chen, Z., Tang, Y., Liu, A., Jin, X., Zhu, J., Lu, X. 2017. Oral administration of Grifolafrondosa polysaccharides improves memory impairment in aged rats via antioxidant action. *Molecular nutrition & food research* 61(11), 1700313
8. Fedarko, N. S. 2018. Theories and mechanisms of aging. In *Geriatric Anesthesiology*, 19-25. Springer, Cham.
9. Golubev, A., Hanson, A. D., Gladyshev, V. N. 2018. A tale of two concepts: harmonizing the free radical and antagonistic pleiotropy theories of aging. *Antioxidants & redox signaling* 29(10), 1003-101
10. Granado, M., Amor, S., Martín-Carro, B., Guerra-Menéndez, L., Tejera-Muñoz, A., González-Hedström, D., García-Villalón, Á.L. 2019. Caloric restriction attenuates aging-induced cardiac insulin resistance in male Wistar rats through activation of PI3K/Akt pathway. *Nutrition, Metabolism and Cardiovascular Diseases* 29(1), 97-105
11. Jové, M., Mota-Martorell, N., Pradas, I., Martín-Gari, M., Ayala, V., Pamplona, R. 2020. The Advanced Lipoxidation End-Product Malondialdehyde-Lysine in Aging and Longevity. *Antioxidants*, 9 (11), 1132.
12. Kalaz, E.B., Çoban, J., Aydın, A. F., Doğan-Ekici, I., Doğru-Abbasoğlu, S., Öztezcan, S., Uysal, M. 2014. Carnosine and taurine treatments decreased oxidative stress and tissue damage induced by D-galactose in rat liver. *Journal of physiology and biochemistry* 70(1), 15-25.
13. Kenyon, C.J. 2019. The genetics of ageing. *Nature* 464, 504-512.
14. Lopez-Lluch, G. and Navas, P. 2016. Caloric restriction as an intervention in aging. *J Physiol* 594(8), 2043-2060.
15. Masoro, E. 2003. Subfield history: caloric restriction, slowing aging, and extending life. *Sci Aging Knowledge Environ* (8), RE2.
16. Mastaloudis, A., Sheth, C., Hester, S. N., Wood, S. M., Prescott, A., McGlade, E., Yurgelun-Todd, D. A. 2020. Supplementation with a putative caloric restriction mimetic micronutrient blend increases glutathione concentrations and improves neuroenergetics in brain of healthy middle-aged men and women. *Free Radical Biology and Medicine* 152, 112-121
17. Most, J., Tosti, V., Redman, L.M., Fontana, L. 2017. Caloric restriction in humans: an update. *Ageing Res Rev* 39, 36-45.
18. Nam, S. M., Seo, M., Seo, J. S., Rhim, H., Nahm, S. S., Cho, I. H., Nah, S. Y. 2019. Ascorbic acid mitigates D-galactose-induced brain aging by increasing hippocampal neurogenesis and improving memory function. *Nutrients* 11(1), 176.

19. Passarino, G., De Rango, F., Montesanto, A. 2016. Human longevity: Genetics or Lifestyle? It takes two to tango. *Immunity & Ageing*, 13(1), 1-6.
20. Pfaffl, M.W. 2001. A new mathematical model for relative quantification in realtime RT-PCR. *Nucleic Acids Res* 29: e45
21. Ros, M., & Carrascosa, J.M. 2020. Current nutritional and pharmacological antiaging interventions." *Biochim Biophys Acta Mol Basis Dis* 1866, 165612.
22. Satoh, K. 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 90: 37-43
23. Sinclair, D.A. 2005. Toward a unified theory of caloric restriction and longevity regulation. *Mech Ageing Dev* 126, 987–1002.
24. Solon-Biet, S.M., McMahon, A.C., Ballard, J.W. 2014. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab* 19,418–430.