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Grape Seed Extract Affects Body Weight, Liver Weight and Relative Tissue Weight Index in Aluminium Chloride Treated Rats

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ABSTRACT

Introduction: Grape seed extract (vitis vinifera) contains antioxidant compounds with ability to prevent aluminium chloride induced hepatotoxicity**.**

Aims & Objectives: To study the effects of grape seed extract on body weight, liver weight and relative tissue weight index in aluminium chloride treated rats.

Place and Duration of Study: This experimental study study was conducted at the Animal House of Post Graduate Medical Institute, Lahore. Duration of study was February 2017 to February 2018.

Material & Methods: 24 adult, healthy albino rats (male) were divided in to three groups. Group A was given distilled water by gavage (1ml/day), Group B was given aluminium chloride (34mg/kg body weight) and group C was given aluminium chloride plus grape seed extract (34mg/kg and 200 mg/kg body weight) by gavage. Whole treatment continued for 42 days. Statistical Package for Social Sciences (SPSS) version 21 was used for entering and analyzing data.p value of ≤ 0.05 was considered significant.

Results: A significant difference in body weights of Group B and C rats was noted at the end of the study. Mean liver weight was significantly reduced in aluminium treated group and near normal restoration of liver weight was seen in group C receiving grape seed extract.

Conclusion: Excessive aluminium chloride exposure led to a reduction in body weight, and liver weight. Grape seed extract being an antioxidant improved these effects.

Keywords: Grape seed extract**,** Aluminum Chloride, Vitis venifera, Body weight, Liver weight.

INTRODUCTION

Aluminum is the third most abundant element in the earth's crust. In urban areas water is usually treated with aluminum. Aluminum is found in spinach, potatoes, tea, antacids and baking powder[1,2]. In developing countries aluminum utensils are widely used[3]. Aluminum alloys are used in packaging, construction and electrical applications[4]. Aluminum salts bind with DNA and RNA. Reactive oxygen species produced by aluminium cause oxidative deterioration of lipid, proteins and DNA[5]. Plasma protein transferrin binds aluminium in blood[6].Aluminium chloride is rapidly excreted by kidneys in urine.[7] Human exposure to aluminium comes from food and drinking water[6]. Aluminium affects brain, bone, kidney and liver[8]. Aluminium toxicity causes normocytic, normochromic anemia and decreases life span of circulating RBCs[9]. Aluminium exposure leads to an increase in serum urea and creatinine and kidney failure may develop[10]. It can increase plasma glucose level[11].

Grapes are fruit of heaven, one of the world´s largest cultivated fruits[12]. Grape seeds contain poly phenols including phenolic acids, procyanidines and proanthocyanidines. 60-70 % poly phenol present in its seed and 28-35% in the skin[13]. European grapes (vitis vinifera) mostly cultivated for eating in Pakistan[14]. The composition of grape seed extract (GSE) are procyanidines (89%), flavonols (6.6%) and proteins(1.6%). Flavonoids are natural poly phenol present in skin and seeds of grapes. In grapes important flavonoids are resveratrol, quercetin and catechin.[16,17] GSE can decrease free radical from body, it also prevents increase in lipid peroxide in plasma[18].GSE has cardio-protective, hepato-

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protective and neuro-protective effects. GSE has antioxidant antimicrobial and anti-inflammatory effect[19]. Grape seed supplement may lower blood sugar and serum lipid in diabetic patient[20]. Their potential health benefits include protection against oxidative damage and anti-diabetic, anti-cholesterolemic and anti-platelet functions[21].Aluminium chloride reduces body weight by decreasing appetite and causing malabsorption of nutrients. The purpose of study was to observe the effects of grape seed extract on body weight, liver weight and relative tissue weight index in aluminium chloride treated adult albino rats.

MATERIAL AND METHODS

This experimental study study was conducted at the Animal House of Post Graduate Medical Institute, Lahore vide IRB consent No:1432. 24 adult healthy male albino rats, average body weight 180-220gm, were selected. Animals were kept at 23°C and light and dark cycle was maintained for 12 hours. They were provided with rat food and given water ad libitum. Before the start of experiment animals were acclimatized for 1 week. Aluminium chloride was purchased from local chemist. Grapes (*Vitis venifera*) were bought from fruit market Quetta. The ethanolic extract of seeds was prepared from PCSIR laboratory complex Lahore by following methods. Seeds were manually separated from grapes and dried. Powder was prepared from dried seed. Ethanolic extract was prepared by soaking 100gm of grape seed in 300ml of ethanol, with daily shaking and kept in refrigerator. The infusion was filtrated, and filtrate was centrifuged at 3000rmp for 10 minutes. Ethanol was evaporated by heating. 100gm of grape seed powder yielded 26.7gm ethanol extract[22].This ethanolic extract of grape seed contains phenolic content (160mg Gallic acid equivalent:GAE/g of extract) and flavonoids content (7.5mg (Quercetin equivalent: QE/g of extract). This quantification was done by the Chemistry Department PCSIR, Lahore.

Animals were divided randomly into three groups, 8 albino rats in each group. First group A(control) was given distilled water by gavage, second group (experimental group B) was given aluminium chloride 34mg/kg bw/day, and third group (experimental group C) given GSE 200mg/kgbw/day plus aluminium chloride 34mg/kg/day by gavage. The whole treatment continued for 42nd days. On 43rd day, these rats were weighted properly. Morphine 0.3-0.5 mg/kg and sodium phenol-barbital 45mg/kg body wt was administered intraperitoneally to anaesthetize the rats. Liver was dissected out, washed, weighed and kept in 10% neutral buffered formaldehyde solution for next 48 hours.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 21 was used for entering and analyzing data. Rats body weight and liver weight data was reported as Mean±S.D. ANOVA (one way) was used for comparison among groups. Tukey´s test was used for post hoc analysis. For all parameters p- value < 0.05 was considered significant.

RESULTS

Animals mean body weight of control group (A) was 203.4 ±6.9 (g) and experimental groups(B&C) was 203.8± 5.6 (g) and 200.1± 2.9 (g) at start of experiment. No significant difference was observed in mean body weight at start of experiment between all groups with p-value=0.357 by ANOVA (one way). Animals mean body weight of control group (A) was 235.0±4.10 (g) and experiment groups (B &C) was 191.5± 4.5(g) and 232.1± 6.5(g) at the end of experiment. Significant difference was observed in mean body weight at end of experiment with p-value <0.001by ANOVA (one way). Post hoc Tukey test was applied for comparison among groups. It was observed that group (B) rats' body weights were significantly lower as compared with group (A&C) as shown in Fig-1 and Table-1 & Table-2.

	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	63.6	2	31.792	1.08	0.357 +
Within Groups	616.3	21	29.345		
Total	679.83	23			

Table-1: Body weight (gm) Comparison among rats at start of experiment between control (A) and experimental (B&C) groups by using ANOVA (one way).

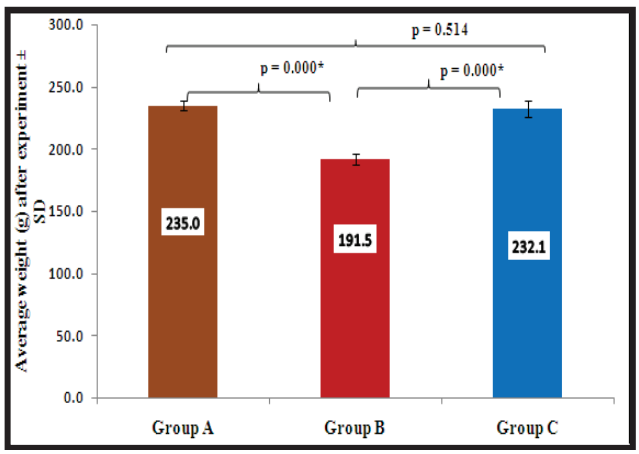

Fig-1: Body weight (gm) comparison among rats at end of experiment between control (A) and experimental (B&C) groups.

	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	9469.1	2	4734.5	179.2	< 0.001 *
Within Groups	554.9	21	26.423		
Total	10023.9	23			

Table-2: Body weight (gm)Comparison among rats at the end of experiment between control(A) and experimental(B&C) groups by usingone way ANOVA (* highly significant).

The mean weight of liver in all groups was observed. The mean liver weight of control (A) was 11.3 ± 0.6(g) and experimental (B & C) groups were 8.9 ± 0.8(g) and 10.6 ± 0.5(g) Significantly difference was observed in mean liver weight among the groups with p-value 0.000 by ANOVA (one way). Post hoc Tukey test was applied for comparison among groups. It was observed that group (B) rats' liver weight was significantly lower as compared to group (A&C). Group (A&C) showed no significant difference in liver weight as shown in Fig-2 and Table-3.

	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	24.790	2	12.395	30.9	0.000 *
Within Groups	8.436	21	0.402		
Total	33.226	23			

Table-3: Liver weight (gm) comparison among control(A) and experimental (B&C) groups by using one way ANOVA (*highly significant).

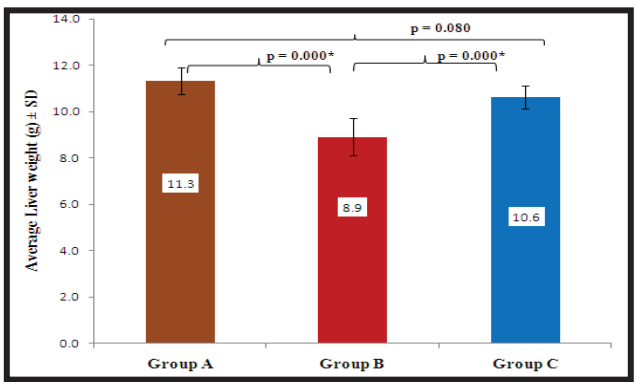

Fig-2: Liver weight (gm) comparison among rats of control(A) and experimental (B&C) groups.

The mean relative tissue weight index (RTWI) of liver in all groups was observed. Relative tissue weight index in group (A) was 5.16 ± 0.24 and in group (B&C) was 4.51 ± 0.32 and 4.90 ± 0.19 respectively. One way ANOVA test was used to compare the relative tissue weight index among groups. Statistically significant difference was observed in RTWI among groups with p- value <0.000 by ANOVA (one way). Post hoc tuckey test was applied for comparison among groups. It was observed that relative tissue weight index in group(B)was significantly lower as compared to group (A&C). No significant difference was observed between groups (A & C) with p-value 0.120 as shown in Table-4 and Fig-3.

	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	1.711	2	0.855	13.331	0.000 *
Within Groups	1.348	21	0.064		
Total	3.058	23			

Table-4: Relative tissue weight index comparison of liver in control(A) and experimental (B &C) group by using one way ANOVA (* highly significant).

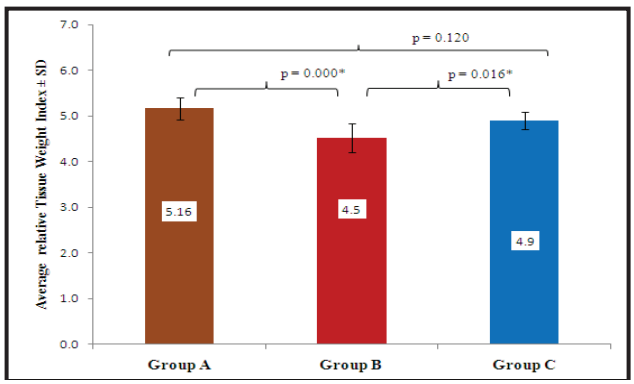

Fig-3: Relative tissue weight index comparison of rats among control (A) and experimental (B&C) group.

DISCUSSION

Increasing cases of liver diseases in Pakistan is a cause of genuine concern. This study was aimed to determine ameliorative effect of GSE(grape seed extract) against aluminium chloride induced hepatotoxicity in albino rats. Significant difference of mean body weight was observed in groups with p value <0.001 at end of experiment (Table-2). Weight loss in experimental (B) group can be explained based on loss of body fluids and adipose tissue after taking Aluminium chloride[23]. Reduction in weight was due to reduced food consumption and malabsorption of nutrients due to Aluminium chloride. Aluminium damaged hepatic and brain DNA by binding with it, which may lead to inhibition of protein synthesis and impaired functioning of both organs[24]. These results coincide with the findings of Buraimoh and Ojo who also observed statistically significant weight loss after

administration of Aluminium chloride in albino rats for eight weeks[23]. Similar findings were also observed by Dera and Amoudi, which showed significant weight loss in rats after administration of Aluminium chloride for six weeks[23,25]. Experimental (C) group weight was observed near to control after giving 200 mg GSE. The improvement in weight gain in experimental (C) group after grape seed extract was due to increase in sensitivity to insulin and as a result increase in glucose uptake[26]. These results coincide with Buloyan et al, who reported significant increase in weight gain after GSE[27]. Similar results were also observed on other organs, Shahari, reported decreased body weight in rats after monosodium glutamate and increase body weight after GSE while studying on rat testes[28].

Contradictory report was given by Hasona et al, who observed decrease in body weight and relative liver weight while using 200mg GSE against 0.1 mg Dexamethasone subcutaneously. Dexamethasone can cause insulin resistance, loss of appetite, weight loss and high level of blood glucose. These effects were reversed when 400mg GSE was used in the same study[26].

In the current study, mean liver weight was significantly different among the groups with p-value 0.000 (Table 3). Liver weight of rats of experimental (B) group was significantly lower in contrast with control (A) and experimental (C) group (Fig-2). Statistically significant contrast of Relative Tissue Weight Index(RTWI) was observed in groups with p-value< 0.000 (Table-4). The RTWI of experimental (B) group was significantly lower in contrast with control (A) and experimental (C) group (Fig-3). These effects, after taking Aluminium chloride, may be due to malabsorption of nutrients in gastrointestinal tract and less food and water consumption due to decreased appetite. While in some cases transient diarrhea was also reported causing this weight loss[24,29]. Almoudi, conducted a study that coincides with ours by finding a decrease in liver weight after Aluminium chloride[24]. Similar finding on liver weight and relative tissue weight after GSE administration was observed by Buloyan[27]. Hasona et al, also reported significant increase in relative liver weight after using GSE[24]. This study showed beneficial effect of grape seed extract after exposure of aluminium chloride induce hepatotoxicity in albino rats.

CONCLUSION

Significant destructive impact of aluminium chloride on liver is obvious. The present study results indicate that GSE, due to its high nutritional value phenolic contents and excellent antioxidant properties, improves aluminium chloride induced body weight and liver weight reduction in adult albino rats.

REFERENCES

1. Al-Amoudi WM. Effect of grapefruit juice on aluminum-induced hepatotoxicity in albino rats: histological, ultrastructural and histochemical assessment. Adv Bio Sci Biotechnol. 2017 Dec 6;8(12):463-77.
2. Lim JO, Jung TY, Lee SJ, Park SW, Kim WI, Park SH, Kim JH, Heo JD, Kim YB, Shin IS, Kim JC. Evaluation of 28-day repeated oral dose toxicity of aluminum chloride in rats. Drug ChemToxicol. 2022 May 4;45(3):1088-97.
3. Gupta M, Dey S, Marbaniang D, Pal P,Ray S, Mazumder B. Grape seed extract: Having a potential health benefits.J food sci technol. 2020 Apr;57:1205-15.
4. Fellows KM, Samy S, Rodriguez Y, Whittaker SG. Investigating aluminum cookpots as a source of lead exposure in Afghan refugee children resettled in the United States. J Expo Sci & Environ Epidemol. 2022 May;32(3):451-60.
5. Chiroma SM, Moklas MA, Taib CN, Baharuldin MT, Amon Z. D-galactose and aluminium chloride induced rat model with cognitive impairments. Biomed Pharmacother. 2018 Jul 1;103:1602-8.
6. Berihu BA, Afwerk M, Debeb YG, Gebreslassie A. Review on histological and functional effect of aluminium chloride on cerebral cortex of the brain. Int J Pharm Sci Res. 2015;6:1105-16.
7. Salah EI, Sabahelkhier MK, Adam SI. Effects of Deionization Water Treated with Different Dose of Aluminum Chloride (AlCl3) on Creatinine and Liver Enzymes of Wistar Rats. Annu Res Rev Biol. 2015 Aug 29:1-7.
8. Saad HA, Hassieb MM, Oda SS, Tohamy HG, Khafaga AF. Histopathologic Study on The Toxic Effect of Aluminium Chloride on the Heart, Liver and Kidneys of Rabbits. Alex J Vet Sci. 2018 Jan 1;56(1), pp, 102-109.
9. Shittu BO. Potentials of Massularia acuminata stem bark extracts on Serum enzymes and haematological parameters of aluminium chloride-induced toxicities. Intl J Chem Biol Sci. 2021 Jul 24;2(4):53-64.
10. Elkatry HO, Ahmed AR, El-Beltagi HS, Mohamed HI, Eshak NS. Biological activities of grape seed by-products and their potential use as natural sources of food additives in the production of Balady bread. Foods. 2022 Jun 30;11(13):1948.
11. Abdrabba S, Hussein S. Chemical composition of pulp, seed and peel of red grape from Libya. Glob J Sci Res. 2015 Apr;3(2):6-11.
12. Khan AS, Ahmad N, Malik AU, Saleem BA, Rajwana IA. Pheno-physiological revelation of

grapes germplasm grown in Faisalabad, Pakistan. Int. J. Agric. Biol. 2011 Oct 1;13(5):791-95.

13. Krasteva D, Ivanov Y, Chengolova Z, Godjevargova T. Antimicrobial potential, antioxidant activity, and phenolic content of grape seed extracts from four grape varieties. Microorganisms. 2023 Feb 3;11(2):395.

14. Xu J, Zheng T, Huang X, Wang Y, Yin G,Du W. Procyanidine resists the fibril formation of human islet amyloid polypeptide. Int J Biol Macromols. 2021 Jul 31;183:1067-78.

15. Tutino V, Gigante I, Milella RA, De Nunzio V, Flamini R, De Rosso M, Scavo MP, Depalo N, Fanizza E, Caruso MG, Notarnicola M. Flavonoid and non-flavonoid compounds of autumn royal and Egnatia grape skin extracts affect membrane PUFA's profile and cell morphology in human colon cancer cell lines. Molecules. 2020 Jul 23;25(15):3352.

16. Abd-Allah AA, El-Deen NA, Mohamed WA, Naguib FM. Mast cells and pro-inflammatory cytokines roles in assessment of grape seeds extract anti-inflammatory activity in rat model of carrageenan-induced paw edema. Iran j basic med sci. 2018 Jan;21(1):97.

17. Gupta M, Dey S, Marbaniang D, Pal P,Ray S, Mazumder B. Grape seed extract: Having a potential health benefits. J food sci technol. 2020 Apr;57:1205-15.

18. Kıvanç İR, Nihat ME, Handan ME, AYŞİN N. The effects of grape seed extract on the some enzymes and metabolites in diabetic rats. Van vet j. 2018 Dec 27;29(3):147-52.

19. Kwatra B. A review on potential properties and therapeutic applications of grape seed extract. World J. Pharm. Res. 2020 Mar 19;9:2519-40.

20. Chuemere AN, Akangbou PC, Ilochi O. Evaluation and predictor ratio of toxicity of aluminium-tainted water impact male rats: Oxidative stress in heart and kidney. Sci Technol. 2018; 4:183-8.

21. Konda VR, Eerike M, Chary RP, Arunachalam R, Yeddula VR, Meti V, Devi TS. Effect of aluminum chloride on blood glucose level and lipid profile in normal, diabetic and treated diabetic rats. Indian J. Pharmacol. 2017 Sep;49(5):357.

22. Al-Shahari EA, El-Kott AF. Potential effect of grape seeds extract against monosodium glutamate induced infertility in rats. Int. J. Pharmacol. 2019;15(2):287-94.

23. Buraimoh, Ojo SA. Effects of Aluminium chloride exposure on the body weight of Wistar rats. Ann Bio sci. 2014.

24. Al-Amoudi WM. Effect of grapefruit juice on aluminum-induced hepatotoxicity in albino rats: histological, ultrastructural and histochemical assessment. Ad BiosciBiotechnol. 2017 Dec 6;8(12):463-77.

25. Al Dera HS. Protective effect of resveratrol against aluminum chloride induced nephrotoxicity in rats. Saudi med j. 2016 Apr;37(4):369.

26. Hasona NA, Alrashidi AA, Aldugieman TZ, Alshdokhi AM, Ahmed MQ. Vitis vinifera extract ameliorate hepatic and renal dysfunction induced by dexamethasone in albino rats. Toxics. 2017 Apr 11;5(2):11.

27. Buloyan S, Mamikonyan V, Hakobyan H, Harutyunyan H, Gasparyan H. Grape Seed Extract in Prevention and Treatment of Liver Toxic Cirrhosis in Rats. Int J pharm Sci. 2014 Nov 4;8(12):1362-6.

28. Al-Shahari EA, El-Kott AF. Potential effect of grape seeds extract against monosodium glutamate induced infertility in rats. Int J Pharmacol. 2019; 15(2):287-94.

29. Rashno M, Gholipour P, Salehi I, Komaki A, Rashidi K, Khoshnam SE, Ghaderi S. p-Coumaric acid mitigates passive avoidance memory and hippocampal synaptic plasticity impairments in aluminum chloride-induced Alzheimer's disease rat model. J Funct Foods. 2022 Jul 1; 94:105-117.

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