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# Academia Open



*By Universitas Muhammadiyah Sidoarjo*

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## Protection Effect of Soy Isoflavones (Genistein and Daidzein) on Hematologic Parameters in Acute Kidney Injury

*Efek Perlindungan Isoflavon Kedelai (Genistein dan Daidzein) terhadap Parameter Hematologi pada Cedera Ginjal Akut*

**Hawraa Mohammed, hawraa@gmail.com, (0)**

*Department of Pharmacology and Toxicology, Institute of Pharmacy, Basra, Iraq, Iraq*

**Asia S. Abdullah, asiaselman2016@gmail.com, (1)**

*Department of Pharmacology and Toxicology, Institute of Pharmacy, Basra, Iraq, Iraq*

**Muhsin S.G. AL-Mozie'l, Muhsin@gmail.com, (0)**

*Department of Pharmacology and Toxicology, Institute of Pharmacy, Basra, Iraq, Iraq*

<sup>(1)</sup> Corresponding author

### Abstract

**Background:** Acute kidney injury (AKI) is a severe, high-morbidity condition with limited effective preventative and therapeutic strategies despite advancements in understanding and treatment. **Specific Background:** Rhabdomyolysis-induced acute kidney injury (AKI) presents significant challenges in renal research, but soy isoflavones, particularly GN and DZ, have shown potential in mitigating oxidative stress and inflammation. **Knowledge Gap:** Soy isoflavones, while potentially providing renal protection, their impact on renal and hematologic parameters in glycerol-induced AKI models has not been thoroughly studied. **Aims:** The study evaluated the effectiveness of soy isoflavones in regulating renal and hematologic parameters in a glycerol-induced AKI rat model, assessing their potential as therapeutic agents. **Results:** The study involving adult female Wistar rats showed that pretreatment with glycerol or dihydroxystilbene significantly reduced urinary  $\beta$ 2-microglobulin, albumin, BUN, and serum creatinine levels in the AKI-induced group, reversing hematological changes. **Novelty:** The study explores the protective effects of soy isoflavones on renal function and hematologic parameters in AKI, highlighting GN's superior efficacy over DZ. **Implications:** Soy isoflavones, particularly GN, may be potential preventive or therapeutic strategies for AKI caused by rhabdomyolysis, warranting further research for clinical applications.

### Highlights:

GN and DZ reduce kidney damage in glycerol-induced AKI.  
GN is more effective than DZ in kidney and blood parameters.  
Soy isoflavones could treat or prevent AKI.

**Keywords:** Acute kidney injury, soy isoflavones, glycerol-induced AKI, renal protection, hematologic parameters

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## Introduction

A clinical case called rhabdomyolysis produced from an acute skeletal muscle disturbance that caused by chemical or physical damaging due to comprehensive exercise, injury, extreme temperature or due to toxins [1]. Acute kidney injury (AKI) increases the risk of the chronic renal diseases and causes an adverse effect on the other organs, such as heart [2]. Treatment with glycerol causes skeletal muscle trouble that can be used as a test model like the case of rhabdomyolysis induces AKI in the human [3]. Pathophysiology of AKI induced by glycerol exposed different mechanisms, definitely inflammatory factors, oxidative stress and apoptosis. Damage of skeletal muscle causes cellular proteins and myoglobin leakage from the cells to the circulation triggering myoglobin concentration in the urinary tubules followed by the leak of ROS that causes tubules injury [4]. Additionally, rise of the macrophages number [5] and cytokines release for example tumour necrosis- factor- $\alpha$  (TNF- $\alpha$ ) [6] and then, TNF- $\alpha$  causes IL-6 release in plentiful quantities by endothelium cells [7].

Genestein (GN) and Daidzein (Dz) are isoflavone phytoestrogens in soy products, their structures are analogous to 17- $\beta$ -oestradiol structure and produce oestrogenic action [8-10]. Soybean, is a legume species, which is a natural isoflavonoid type and the benefits of soy clinically considered, with indication linked to the decreased incidences of type II DM, coronary- heart disease and atherosclerosis [11]. Moreover, soy isoflavones causes reduction of the risk of many chronic diseases, such as some cancers, particularly BC, and prostate adenocarcinoma [12, 13].

Previous study revealed that soybean intake meaningfully increases Fe levels in the spleen. Furthermore, propensity for enhanced levels of iron after soybean consumption was detected in liver, kidneys and femur. This may be attributed to the presence of ferritin molecules in the soybean [14]. Iron (Fe) is an important playing essential role in hemoglobin, immune system and oxidative stress. However, an excess Fe concentrations is harmful to cells and tissues. Excess Fe concentrations causes suppression of transferrin binding capacity and free Fe increases in tissue causes pathological state called iron overload [14].

The present study aimed at approximating the effect of soy isoflavones (GN and DZ) on renal and hematologic parameters against the rhabdomyolysis induced by glycerol, mimicking to AKI, in female rats.

## Methods

Hypertonic Glycerol solution (50% v/v in normal saline), GN (5,7- dihydroxy-8-[4-hydroxyphenyl]chromen-4- one) and DZ (4,7 Dihydroxyisoflavone) powders both from axenic research and formulation materials, China. GN and DZ were dissolved in distilled water. Rats obtained from college of veterinary medicine\university of basrah and acclimated for a period of one week before starting experiment. Each three animals caged in a plastic cage with standard bedding. Standard food pellets and water were supplied ad libitum unless otherwise stated. All the animal experiments were carried out in accordance with guidelines evaluated and approved by the National Institute of Health (86/609/EEC) Guidelines for using Laboratory Animals. Furthermore, College of Pharmacy /University of Basrah/ Ethical Committee approval number 3/5/414, October 2022 were obtained.

### Experiment

Twenty four adult female rats equally divided into four groups of six rats each. Animal's weights measured at the beginning and the end of experiment. Group 1 rats represent the control group, administered distilled water (8 ml /kg body weight)/ day orally by gavage for 20 days. Group 2 rats represent the glycerol-induced ARI model, Groups 2, 3 and 4 rats administered 8 ml/kg body weight water, 21.7mg/kg body weight GN and 17.4mg/kg body weight DZ respectively, daily for 14 days orally by gavage and after that animals of the last three groups deprived from water for 24h then administered hypertonic solution (8ml/kg body weight/day) IM for 3 days to induce AKI. The injected volume was divided equally between the two hind limbs.

Urine samples from the control and treatments groups were collected in sterile Petri dishes, preserved in Eppendorf tubes and frozen until analysis for the selected biomarker. After that, the animals were sacrificed on day 20 (at the end of experiment and 24h after last dose) and the blood samples were taken by intracardiac puncture and blood kept in tube with anticoagulant for hematology test and the sera collected by centrifugation at a speed of 1000 rpm for 10 min. Sera were kept for biochemical tests at - 20°C.

### Biochemical analysis

Urine samples were centrifuged for 20 min at 2000 rev/min. The supernatant was carefully collected according to the manufacturer's instructions in order to test for  $\beta$ 2-microglobulin using a rat ELISA kit (Shanghai YL Biotech Company, China). The serum urea and creatinine concentrations were determined using a diagnostic automated laboratory analyzer per the manufacturer's instructions (Abbott Architect 4000c, USA). Spectrophotometric analysis was used for kidney function tests (serum urea and creatinine) Renal function tests, includes urinary  $\beta$ 2 myoglobin, urine albumin, blood urea nitrogen (BUN) and serum creatinine. were determined by colorimetric methods using kits supplied by Spinreact (Girona, Spain) and spectrum (Cairo, Egypt), respectively, according to



the manufacturer's instructions.

Hematological parameters done in the University of Basrah by using hematological autoanalyzer (Count 60) made in Genex Company. White and red blood cell counts, hemoglobin (Hb) and hematocrit (Ht) concentrations, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet count (PLT), Lymphocyte count, neutrophil count, and MID (the combined value of the other types of white blood cells not classified as lymphocyte or granulocyte) also estimated.

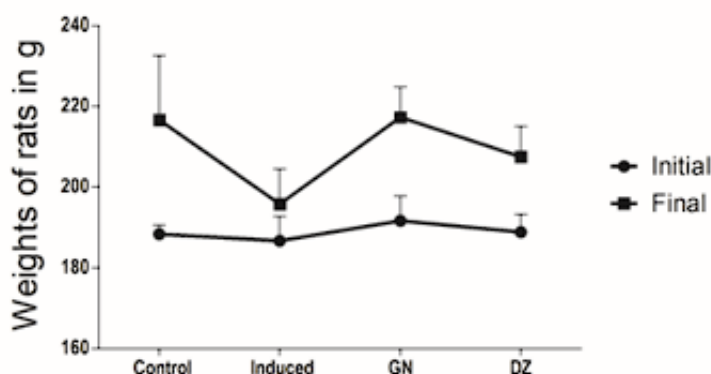
Statistical analysis

Statistics achieved using Graph Pad Prism software (version 7.0, Inc., San Diego, CA). Descriptive data presented statistically as mean  $\pm$  SEM for wholly estimated parameters. One-way analysis of variance (ANOVA) and Tuckey's Multiple Comparison tests used for comparison between groups. p values less than 0.05 considered as significantly different.

## Result and Discussion

### Result

This study showing no deaths in all groups during the experiment period and no stated change in the appearance of animals. The rat weights documented at the beginning and the end of test. Increases in body weight appears in all groups, except the AKI induced group as demonstrated in figure 1.



**Figure 1.**

Figure 1: Variations in rats body weight in different study groups. All data showed as mean  $\pm$  SEM. Initial indicate weights of rats at the begening of experiment while final represent weights of rats at the end. Pretreatment with 21.7mg/kg/day Genestein indicated as (GN) and 17.4mg/kg/day Daidzein (DZ). P<0.05 is significant difference.

This study exposed that AKI induction by a single dose of glycerol significantly enhances urinary  $\beta$  myoglobin, urine albumin, BUN and serum creatinine levels compared to control. Furthermore, pretreatment of rats with 21.7mg/kg/day GN or 17.4mg/kg/day DZ conserved the normal levels of urinary  $\beta$  myoglobin, urine albumin, BUN and serum creatinine, as demonstrated in table 1.

Variables	Groups (n=6 in each group)			
	Control	Induced	GN	DZ
Urinary $\beta$ -Microglobulin (ng/ml)	30.4 $\pm$ 4.1	86.98 $\pm$ 7.1a	35.6 $\pm$ 4.6 b	44.176 $\pm$ 8.2 a, b, c
Urine albumin	33.2 $\pm$ 6.5	160.7 $\pm$ 18.9 a	50.5 $\pm$ 4.0 b	70.3 $\pm$ 6.5 a, b, c
Blood urea nitrogen (BUN)	20.0 $\pm$ 5.1	51.4 $\pm$ 6.8 a	23.3 $\pm$ 8.6 b	30.9 $\pm$ 6.2 b
Serum creatinine	0.3 $\pm$ 0.04	3.6 $\pm$ 0.35 a	0.9 $\pm$ 0.19 a, b	1.0 $\pm$ 0.09 a, b

**Table 1.** Evaluation of different renal parameters among different study groups.

Data presented as mean  $\pm$  standard deviation, a P < 0.005 compared with control, b P < 0.005 compared with induced group, c P < 0.005 compared with GN group.

Results discovered a significant reduction in hemoglobin percentage and RBCs count after exposure to hypertonic glycerol solution ( $p < 0.005$ ), While there was an increase in WBCs, platelet count, Lymphocyte, and Monocyte in this group compared to the control group. Furthermore, pretreatment with GN (21.7mg/kg body weight)/ day or DZ (17.4mg/kg body weight) per day for 14 days orally causes a decrease in WBCs, PLT, Lymphocyte, and Monocyte in these groups compared to the induced group, as well as a decrease in hemoglobin percentage and RBCs compared to the control group. As demonstrated in table 2.

Variables	Groups (n=6 in each group)			
	Control	Induced	GN	DZ
Hb (%)	7.4 ± 0.6 a	7.2 ± 1.0 a	7.5 ± 2.2 a	
Haematocrit (%)	40.2 ± 3.6	23.5 ± 2.3 a	28.6 ± 2.6 a, b	22.3 ± 2.4 a, c
MCV (fL)	57.4 ± 3.5	60.6 ± 2.9	53.6 ± 1.5 b	54.6 ± 3.5 b
MCH (pg.)	18.9 ± 1.5	19.0 ± 1.0	21.5 ± 0.5 a, b	18.3 ± 1.4 c
RBC (×10 <sup>12</sup> /L)	7.0 ± 0.6	3.9 ± 0.6 a	3.7 ± 0.5 a	4.2 ± 0.4 a
Platelets (×10 <sup>9</sup> /L)	265.6 ± 10.7	317.1 ± 30.5 a	181.9 ± 15.2 a, b	251.1 ± 8.8 b, c
WBC (×10 <sup>9</sup> /L)	9.9 ± 2.1	20.7 ± 2.2 a	13.9 ± 1.2 a, b	10.6 ± 1.7 b, c
Lymphocyte (×10 <sup>9</sup> /L)	7.0 ± 1.7	13.6 ± 1.4 a	7.6 ± 1.6 b	6.9 ± 1.9 b, c
Monocyte (×10 <sup>9</sup> /L)	0.6 ± 0.3	3.3 ± 0.4 a	3.0 ± 0.3 a	2.2 ± 0.3 a, b, c

**Table 2.** Evaluation of hematological parameters among different study groups.

Data presented as mean ± standard deviation, a  $P < 0.005$  compared with control,

b  $P < 0.005$  compared with induced group, c  $P < 0.005$  compared with GN group.

## Discussion

The present research illustrates that acute exposure to hypertonic glycerol solution is associated with a general lack of well-being characterized by muscle spasms at the site of injections, significant tiredness, low energy, lethargy, changes in eating habits with low appetite, and decrease in body weight; these changes were especially noticeable in the last days of the experiment. These findings could result from glycerol-induced AKI that results from a mismatch between the oxygen and nutrients delivered to the kidney cells and their needs, leading to cellular stress and increased energy demands [15]. Pretreatment with soy isoflavones (GN and DZ) results in a significant increase in the body weights of rats; this study is in line with a survey on fat Zucker rats [16].

Kidneys are the chief organ in the human body, it is essential to maintain homeostasis, regulate extracellular fluid and to discharge the medications and toxic substances [17], a vast number of external toxicants can damage the kidneys, affecting their function causes acute harm of tubules, acute nephritis or chronic intoxication [18].

This study aimed at approximating the effect of soy isoflavones (GN and DZ) on renal and hematologic parameters against the rhabdomyolysis induced by glycerol, mimicking to AKI, in female rats.

In the current study, induction of AKI in female rats using a single dose of glycerol distinctly increases urinary  $\beta$ 2-microglobulin level, and this increase may be related to renal vessel constriction, obstruction of renal tubules, or oxidative stress [19]. Moreover, urine albumin, BUN, and SCr are also elevated in the induced group compared to the control, this elevation indicates renal injury by glycerol.

In addition, this study revealed a decrease of tested renal parameters in the groups pretreated with GN and DZ, these results are in cope with previous studies stated that soy milk causes Reno protective effect in animal models with renal disease [20, 21]. These effects may be attributed to the impact of soy phytochemicals on the enzymes affects gene expression, that can enhances antioxidant effects [22].

The current study revealed that administration of hypertonic glycerol solution caused a significant increase in PLT and WBCs count, and a significant decrease in RBCs and Hb levels compared with the control group. An identical outcome was observed in the study conducted by Hakami et al., on glycerol-induced AKI rats [23].

The specific influence of GN and DZ on RBCs and Hb levels necessitates additional research to identify its exact role in hematological parameters. The study of Pahari et al., highlights the potential of flavonoids like GN to penetrate erythrocyte membranes and interact with Hb, which could be significant for their pharmacological actions in treating oxidative stress-related disorders [24]. Furthermore, GN can potentially decrease the production and activity of WBCs by inhibiting various signaling pathways involved in inflammation, such as nuclear factor kappa-B (NF- $\kappa$ B) and proinflammatory cytokines that are involved in the inflammatory response, thereby helping to control inflammation [25].

## Conclusion

The current study indicates that burning waste contributes significantly to air pollution with fine particles and toxic gases, which pose health and environmental risks to humans and living organisms near waste dump sites, including respiratory problems. Burning waste also leads to the release of other air pollutants such as nitrogen oxide (nitrogen oxide). NO<sub>x</sub> and sulfur dioxide (SO<sub>2</sub>), which contribute to acid rain and smog formation and release greenhouse gases such as carbon dioxide and methane, which drive climate change. Pollution of soil and surface water with heavy metals poses a threat to human health and the ecosystem, as they can leak into the food chain through absorption by plants, and then transmit to humans when ingested, which requires continuous monitoring and evaluation of these sites. Therefore, this current study emphasizes the management of soil and surface water with heavy metals. Waste properly and take preventive measures to reduce the negative effects of landfills on human health and the environment and work to re-encourage waste sorting and recycling to reduce the amount of waste that is thrown into landfills.

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