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Effect of Morphine and Genistein Treatments on Some Blood Hematological and Biochemical Characteristics of Laboratory Mice Males

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Author Details

Habib J. Saheb*, Alaa S. Kathim and Murtadha F. Al-Hillo

Authors Affiliations

Biology Department, Qurna College of Education, University of Basrah, Iraq

Corresponding Author*
HABIB J. SAHEB

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Abstract: This study looked at the therapeutic impact of genistein as an antioxidant and anti-inflammatory in lowering the deleterious effects of morphine on blood parameters, liver enzymes, triglycerides, cholesterol, and concentrations in mice's blood.48 male laboratory mices were used, divided into four groups. Each group included 12 mices.;The control group was injected with 0.1 m of normal saline; The morphine group morphine was administered at a 20 mg/kg dosage, The genistein group genistein was injected at a concentration of 25 mg/kg; Lastly the morphine and genistein group that was injected with morphine 20 mg/kg for 15 days, followed by 15 days of receiving an injection of genistein at a 25 mg/kg dosage.; The mices were injected once daily under the peritoneum and were divided for three periods (five, ten, and fifteen days of injection).The levels of WBC were significantly elevated by morphine and genistein (p 0.05), whereas the levels of RBC and HGB were significantly decreased (p 0.05). During the fifteen-day injection period, rise in triglyceride concentration., cholesterol, and during the ten and fifteen-day injection periods, and genistein also significantly (p 0.05) reduced the levels of cholesterol and triglycerides. A significant improvement in lipid concentration.

Keywords: Morphine, genistein, doll standards, Triglycerides, cholesterol.

1 -INTRODUCTION

Opioids are widely used in many areas of life, especially in the relief of acute and chronic pain (Tsuno *et al.*, 2022), the most prominent opiates used for this purpose is morphine. Studies that supported the existence of significant adverse effects of morphine abuse revealed that it can lead to addiction and withdrawal when used excessively over an extended period of time, as well as hypogonadism and a defect in hormone production, shortness of breath, vomiting, decreased intestinal secretions, weight loss, ataxia, and immune diseases (Hemati *et al.*, 2021). It may cause cytotoxicity through the production of free radicals, reactive oxygen, lipid peroxidation, and a decrease in the level of antioxidant enzymes, which may cause their association with lipids in cell membranes, causing oxidative stress and cancer diseases and their impact on blood parameters and activation of programmed cell death (Jia *et al.*,2022).

As a result of the harmful effects shown by the chronic abuse of morphine, the researchers focused largely on finding appropriate strategies to reduce these effects. Among the proposals put forward is the use of materials with antioxidant properties. Genistein, which belongs to the class of isoflavones, is a natural plant estrogen extracted from soybeans, legumes, seeds, fruits, and vegetables such as alfalfa, broccoli, and cumin (Ebrahimisadr *et al.*, 2021). It is characterized by a structure and properties similar to estrogen and has been widely used in medical and academic fields due to its antioxidant properties, as it is characterized by its ability to deplete free radicals and raise the level of antioxidant enzymes, anti-inflammatory and cancerous tumors (Gan *et al.*, 2022) Endocrine disorders, non-alcoholic fatty liver, osteoporosis (Semeniuk *et al.*, 2021), liver and kidney disorders, anti-depression and obesity reduction (Zamani *et al.*, 2021).

This study aims to identify the possibility of using the hormone genistein as an antioxidant and anti-inflammatory in reducing the negative effects that the use of morphine can have on blood parameters, concentration of liver enzymes, and level of lipids in the blood.

2-MATERIALS AND METHODS

2-1: Experimental Animals

Forty-eight male laboratory mice, *Mus musculus L*, belonging to the L/BALB strain, aged the animal produced young between 20–25 g and approximately 10–12 weeks old. House of the Qurna College of Education-University of Basrah and kept in plastic cages covered with sawdust in controlled environmental conditions. Animals were freely access to feed and water (Rubio, 2022 and Aubaeed *et al.*, 2020).

2-2: Experimenta Design

The animals were divided into four groups, and each group included 12 mice, as follows: 1: The control group: received 0.1 m of normal saline solution as an injection.; 2: Morphine group: Morphine 20 mg/kg of an injection was administered (Kuthati *et al.*, 2021);3: Genistein group: injected at a concentration of 25 mg/kg in (Al-Salashur *et al.*, 2019); 4: A group of morphine and genistein that were injected with morphine at a concentration of 20 mg/kg for 15 days and then injected with genistein at a concentration of 25 mg/kg for 15 days. The mices were injected once daily in the subperitoneal membrane for a period of 15 days and divided into three periods (five, ten, and fifteen days of injection). At the end of each period, the mices were anesthetized by chloroform and blood was drawn from the heart directly through a sterile 1 mL syringe and part of it was used for blood analysis. On serum and its use in biochemical analysis.

2-3: Measuring (WBC, RBC, and HGB) in the Blood

Blood was withdrawn from the heart directly and placed in an EDTA treatment tube the blood used to measure the total the quantity of hemoglobin, red blood cells, and white blood cells (WBC) through the use of the Baker Hematology analyzer. Spanish Spinreact Company.

2-4: Measurement of Triglycerides, Cholesterol and Glucose Levels

The levels of triglycerides, cholesterol, were measured according to the instructions specified in the Biolabo kit (France) and according to its method (Belfield & Goldberg, 1971).

RESULTS

3- Morphine and Genistein Effect on Blood

3-1: Effect of Morphine and Genistein on the Level of White Blood Cells WBC

The present study's findings revealed that injecting males Morphine administration to rats resulted in a substantial ($p \leq 0.05$) rise in the level of WBC during a period of fifteen days from the injection compared with the control group, while genistein injection caused a significant ($p \leq 0.05$) increase in the level of WBC during the period of injection. F-ifteen during the experiment's days compared with the control group, despite the fact that there was no discernible change compared to the group of morphine. The injection of morphine and genistein demonstrated that the amount of WBC increased significantly ($p \leq 0.05$) during the course of the five injection times., ten, and fifteen days of the experiment compared with the control groups, morphine and genistein (Fig. 1).

3-2: Effect of Morphine and Genistein on Red Blood Cells (RBC) Hemoglobin, HGB Levels

The present study's findings revealed that injecting males mices RBC and HGB levels were significantly reduced ($p \leq 0.05$) in rats given morphine. During a period of fifteen days from the injection compares the experimental group and the results showed that genistein injection caused a significant decrease ($p \leq 0.05$) in the level of RBC. The injection of morphine and genistein revealed a substantial drop in the level ($p \leq 0.05$) of RBC and HGB during the fifteen-day injection Compared to the control group during the experiment, while there was no significant difference compared to the morphine group, and the injection of morphine and genistein pshowed a substantial decline in the level of ($p \leq 0.05$) RBC and HGB during the five-day injection as compared to the morphine group during the trial, compared to the control, morphine, and genistein groups on the day of the experiment (Figure 2 and Figure 3).

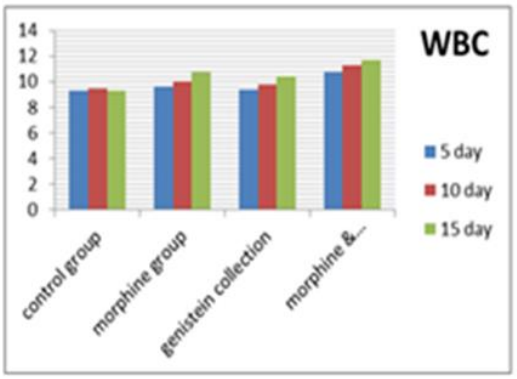

Figure 1: The effect of treatments on the level of white blood cells

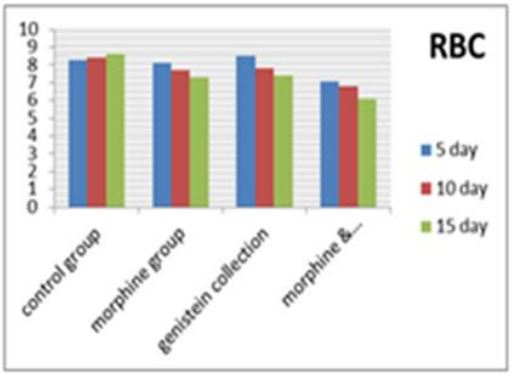

Figure 2: Effect of treatments on the level of red blood cells

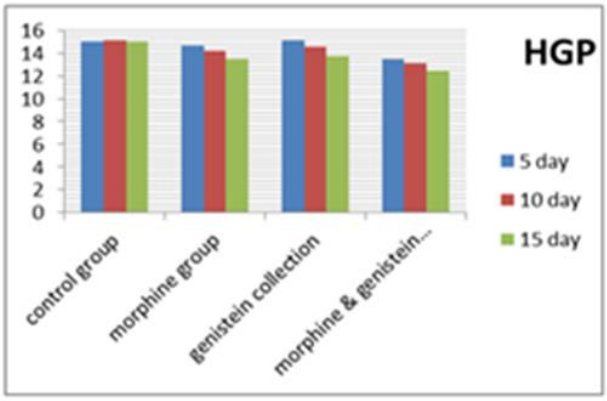

Figure 3 Effect of treatments on hemoglobin level

3-3: Effect of Morphine and Genistein on Lipids Level

The results of the study showed that injecting males mices Adding morphine to rats resulted in a substantial ($p \leq 0.05$) rise in triglyceride and cholesterol levels during the ten-and fifteen-day injection periods compared to the control and genistein groups;. The results showed that mice injected with genistein produced a considerable reduction in triglyceride and cholesterol levels ($p \leq 0.05$) and cholesterol during the fifteen-day injection period compared to the control group; While the group injected with morphine and genistein showed It showed a significant positive improvement in the levels of triglycerides and cholesterol, as there were no significant changes during a period of fifteen days compared with the control group.

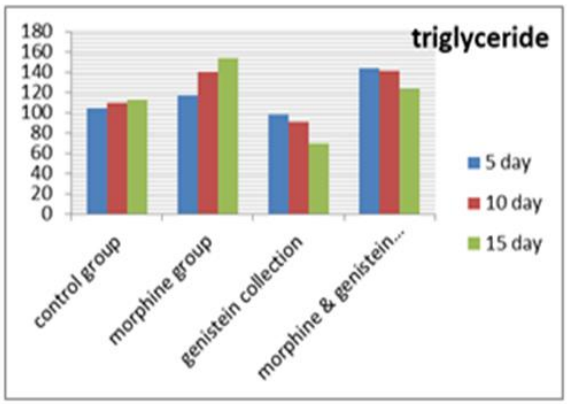

Figure 7 :Effect of treatments on the triglycerides

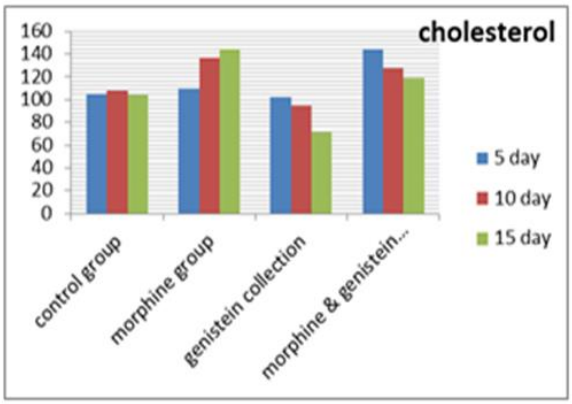

Figure 8: Effect of treatments on cholesterol levels

DISCUSSION

The outcomes demonstrated how morphine affected the immune status, as the injection of morphine caused a significant increase in the level of total white blood cells during the fifteen-day injection period, which may be due to the fact that the use of morphine stimulates the production of white blood cells by stimulating macrophages to release inflammatory cytokines such as interleukin-6, IL-6, as a result, IL-2, IL-4, IL-5, IL-10, and IFN- of bone marrow activate factors to increase WBC production in the bloodstream (Ebrahimisadr *et al.,* 2021). The use of morphine may also inhibits the production of the chemokine gene in the blood vessels, which plays a major role in allowing the migration of WBC from the blood vessels to the affected tissues through its permeation through the vascular-forming epithelial cells (Miao *et al.,* 2020).

The outcomes also revealed a notable rise in the level of WBC when genistein was injected into mice as well as in the group injected with morphine and genistein. Hematopoietic and reducing the time needed to rebuild hematopoietic cells, which helps to increase the production of WBC in the body and improve the immune status (Singh *et al.,* 2009); Genistein may inhibit the production of glucocorticoids, which causes an increase in WBC production and an increase in the body's immune ability (Whirledge *et al.,* 2015).

The results also showed that the injection of morphine causes a substantial drop in the amount of RBC and HGB in the blood serum, which may be due to the fact that morphine causes the body to produce free radicals, ROS, and lipid peroxidation, which cause the oxidation of the fats that make up the membranes of the renal cells, impeding their work and causing their death (Ghasemi *et al.,* 2022). Or it may be due to the fact that the use of morphine causes a marked decrease in the level of iron in the blood, which causes an impediment in the construction of red blood cells because iron participates in the construction of HGB, which is an essential part of RBC (Nash *et al.,* 2019).

Injecting mice with genistein caused a substantial drop in the concentration of RBC, HGB, and this may be due to the fact that genistein, a substance comparable to estrogen, inhibits the synthesis of the hormone erythropoietin, which is required to encourage the differentiation of red blood cell progenitors into mature RBC, causing a decrease in the ratio of mature RBC in the bloodstream (Horiguchi *et al.,* 2014), and estrogen, which is characterized by its ability to increase fat metabolism, increase red blood cell sedimentation, and reduce their number (Banik *et al.,* 2013). The results also showed a significant increase in the concentration of triglycerides and cholesterol after injecting mice with morphine, which could be due to the fact that morphine causes inhibition of the activity of the enzyme lipoprotein lipase, which performs the function of the breakdown of triglycerides into fatty acids that are absorbed by fat cells, and this enzyme is inhibited. When morphine causes elevated levels of triglycerides and cholesterol in the blood (Augustus *et al.,* 2004), or when morphine is metabolized in the liver, free radicals are released, and they interact with large molecules in cell membranes, causing oxidative stress and programmed cell death (Salahshoor *et al.,* 2018). (This reduces the liver's ability to break down fats and raises levels of triglycerides in the blood (Wicaksono *et al.,* 2019). The outcomes of our research also demonstrated a considerable decline in the concentration of triglycerides and cholesterol in mice injected with genistein.

The use of genistein stimulates the enzyme lipoprotein lipase present in fat cells, which may stimulate the metabolism of triglycerides to fatty acids and glycerol and their storage within fat cells, thus reducing the concentration of triglycerides and cholesterol in the blood (Jiang *et al.,* 2021), or genistein may reduce the formation of triglycerides and their esterification, reduce their absorption from the digestive tract, and increase the production of bile, which causes an increase in the breakdown of triglycerides and prevents (Semeniuk *et al.,* 2020).

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