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MANAGEMENT OF PARACETAMOL-INDUCED HEPATOTOXICITY BY USING *GARDENIA GUMMIFERA* L. LEAVES EXTRACT AND ISOLATED COMPOUND

Manish Srivastava*¹, Dr. Lavkesh Kumar Omray, Dr. Naveen Gupta¹,
Dr. Dharmendra S Rajput¹, Dr. Kapil Malviya¹

¹Patel college of pharmacy, Madhyanchal professional university, Bhopal, M.P.

Abstract:

Gardenia gummifera Linn. f. belongs to the family Rubiaceae and is one among the red-listed plants native to peninsular India. This study aims to evaluate its hepatoprotective effect against paracetamol-induced liver toxicity in a rat model. groups of rats were orally treated (once daily) with normal saline, paracetamol 640 mg/kg (negative control), 10 mg/kg silymarin (positive control), extract (100 and 200 mg/kg, and isolated compound (Gardenin A) for 14 days, followed by the hepatotoxicity induction using paracetamol (PCM). The blood samples and livers of the animals were collected and subjected to biochemical and microscopical analysis. The histological findings suggest that paracetamol caused lymphocyte infiltration and marked necrosis, whereas maintenance of the normal hepatic structural was observed in group pre-treated with silymarin and Gardenin A. The rats pre-treated with methanol extract (200 mg/kg) and Gardenin A significantly and dose-dependently reduced the hepatotoxic markers in animals. methanol extract (200 mg/kg) and Gardenin A 10 mg/kg significantly reversed the paracetamol-induced altered situations, including the liver enzymes, antioxidant enzymes and serum bilirubin, levels in animals. Taken together, Gardenin A exerted significant hepatoprotective activity in rats in a dose-dependent manner. paracetamol induced toxicity and Gardenin A induced hepatoprotective effects based on expression of inflammatory and apoptosis factors will be future line of work for establishing the precise mechanism of action of Gardenin A in Wistar albino rats.

Keywords: *Gardenia gummifera*, Paracetamol, Hepatotoxicity, Hepatoprotective activity

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Introduction: Liver is a vital organ that plays a role in controlling critical biochemical and physiological activities including homeostasis, growth, energy and nutrient supply, detoxification of drugs and other xenobiotics, and also combating infections. Therefore, it is very susceptible to being damaged by hepatotoxic agents [1-3]. Many newly developed drugs (e.g., rimonabant, propylthiouracil, or corticosteroids) have been used for treatment of liver diseases; however, these drugs possess harmful side effects such as insomnia, vomiting, constipation, and depression. For that reason, further research on plants and herbs that could potentially substitute the chemical-based drugs is very crucial as many medicinal plants have been found to possess hepatoprotective properties [4-7]. *Gardenia gummifera* Linn. belongs to the family Rubiaceae and is one among the red-listed plants native to peninsular India. It is commonly known as gummy gardenia. It is distributed in the forests of Tamil Nadu, Andhra Pradesh, Kerala and Karnataka, This plant is well known for its application in folk medicine. In the indigenous system of medicine Ayurveda, it is called Nadihingu (Sanskrit), and Dikamali (Hindi). This plant is claimed to have numerous medicinal properties possessing astringent and carminative properties that are used in the management of dyspepsia and hemorrhoid. It is also useful in the treatment of flatulence for cleaning foul ulcers and wounds, anticonvulsants. Ancient scriptures of Ayurveda have cited the medicinal use of the plant and have also mentioned its various synonyms. *Gardenia gummifera* L. is used in indigenous system of medicine to cure many diseases [8-11]. To authenticate the traditional medicinal claim of investigation has been under taken to evaluate the hepatoprotective activities of *Gardenia gummifera*. Chemical compounds from plant extract have been isolated and evaluated for hepatoprotective activity.

Materials and Methods

Collection of Plant Material: The leaves of *Gardenia gummifera* were collected around Bhopal (Madhya Pradesh) India, and authenticated by APS University Rewa. The leaves were dried under shade for 7 days at room temperature, separated, and pulverized by mechanical grinder to form coarse powder.

Preparation of Plant Extract: The coarse powder of the air-dried leaves of *Gardenia gummifera* was defatted with petroleum ether using soxhlet extractor. Petroleum ether exhausted leaves were extracted with chloroform, methanol and water sequentially. For getting aqueous extract plant material macerated with water. The solvent was then evaporated under reduced pressure (204 mbar) and controlled temperature (40°C) using a vacuum rotary evaporator (Buchi Rotavapor R210/215, Switzerland) [11].

Isolation and characterization of compounds from *Gardenia gummifera* leaves extract: Methanol extract (500 g) fractions were subjected to open column chromatography. The mobile phase was composed of CHCl₃ and MeOH and the subfractions were eluted with a step gradient of 0–100% MeOH. Eleven sub-fractions were obtained, out of which sub-fraction no five resulted in the isolation of compound I. Subfraction four was further separated by size exclusion chromatography (Sephadex LH20 column; GE) with an isocratic solution of 80% MeOH and 20% water, resulting in the isolation of compounds I. Purity of compound determined by TLC. By interpreting ¹H NMR, Mass and IR spectra of isolated compound obtained value matched with literature value and compound I was identified as Gardenin A (5-hydroxy-6,7,8,3',4',5'-Hexamethoxyflaone)

Animals: Healthy albino rats at 8-9 weeks of age weighing 180–220 g were used throughout the study. Animals were housed at room temperature of 27–30°C and allowed free access to food and tap water *ad libitum*. The animals were acclimatized to laboratory conditions for 7 days before the commencement of experiments. The rats were handled in accordance with current CPCSEA guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC Approval No.: IIP/CPCSEA/IEAC/2023/02) and ethical norms were strictly followed during all experimental procedures.

Pharmacological Studies

Hepatoprotective Assay: The in vivo hepatoprotective activity of Methanol and aqueous extract of *Gardenia gummifera* leaves was determined using the paracetamol induced hepatotoxicity test in rats. The animals (n = 6) were randomly divided into 6 experimental groups and administered with test solutions as follows.

Normal control: serving as normal control received Normal saline

Disease control received Paracetamol 640 mg/kg p.o. for 7 days

Positive control standard (silymarin 10 mg/kg treated) + Paracetamol (640 mg/kg p.o.)

MEGG 100 Methanol extract of *Gardenia gummifera* leaves (100 mg/kg)

MEGG 200 Methanol extract of *Gardenia gummifera* leaves (200 mg/kg)

AQGG 100 Aqueous extract of *Gardenia gummifera* leaves (100 mg/kg)

AQGG 200 Aqueous extract of *Gardenia gummifera* leaves (200 mg/kg)

Gardenin A Gardenin A (10 mg/kg)

These doses of extract (100 and 200 mg/kg) were used in the present study based on previous report on the acute toxicity study performed using three doses (50, 100, and a maximum dose of 2000 mg/kg Methanol and aqueous extract of *Gardenia gummifera* leaves) administered orally, which showed no signs of toxicity in rats [13]. The animals were fasted for 48 hours prior to the experiment under standard laboratory conditions but allowed free access to distilled water (dH₂O) ad libitum. After 48 hours, each group received the respective dose of test solution orally once daily for 7 consecutive days. The oral administration of paracetamol was performed 3 hours after the last extract administration on the 7th day except for group I, which received only normal saline. After 48 hours of hepatic injury induction, the animals were lightly anesthetized using diethyl ether and the blood was collected by cardiac puncture in sterilized centrifuged tubes which was then centrifuged at 3000 rpm for 10 minutes to get serum for biochemical parameters study. The animals were then sacrificed by cervical dislocation and the liver was removed for histopathological studies.

Clinical signs and mortality: Administration of paracetamol, *Gardenia gummifera* and *Origanum majorana* extracts and isolated compounds showed no mortality or morbidity in the animals during the period of study. Cage side observations did not show any observable clinical signs related to the compound toxicity. No tremors, convulsions, salivation, diarrhoea, lethargy, or unusual behaviors were observed in extract treated

animals throughout the study period.

Effect on body weight: The recorded body weights of the animals at day 0 and day 8 of the experiment are displayed in Table 6.23. The change in the body weights of the experimental animals from day 0 to day 8. Treatment with paracetamol induce hepatotoxicity caused significant reduction ($P < 0.01$) in body weight

Liver Enzymes estimation: Serum collected was assayed according to the standard liver enzymes estimation methods. Alanine aminotransferase (ALT), alkaline phosphate (ALP), and aspartate aminotransferase (AST) levels were measured using the Hitachi 902 Automatic Chemical Analyser.

Results and discussion

In-vivo hepatoprotective study

Effect on body weight: The initial (day 0) to day 8 body weight of animals (Mean \pm SD) from various experimental groups of animal were recorded. The gain in body weight has been calculated. The graph of change in body weight was plotted. Treatment with Methanol extract of *Gardenia gummifera* apparently took the animals towards normalcy since the weight gain in animals that of control animals. Treatment of animal groups with isolated compound (Gardenin A) was showed results comparable to standard treated group.

Effect on liver weight: In group received methanol extract of *Gardenia gummifera* leaves (200mg/kg) the absolute weight of liver remained close to the absolute weight of liver in control group. Disease control group increased in liver weight comparatively to control group. The liver weight of treated group with extract was restored with treatment of plant extract. Methanol extract of *Gardenia gummifera* leaves (200mg/kg) was significantly restored the liver weight of treated group.

Effect of plants extract on the activity levels of AST in blood plasma: The levels of AST activity in the blood plasma of animals in different experimental groups were performed. Treatment with *Gardenia gummifera* leaves extracts control AST level in dose dependent manner. Methanol extract of *Gardenia gummifera* was control the AST level more effective than aqueous extract. Treatment with isolated compound (Gardenin A and Hesperetin) control the AST level similar to standard treated group.

Effect on ALT levels in blood plasma of animals: ALT is present in heart, brain, skeletal muscle and liver; however, it is present in higher amounts in liver than any other organs. On the other hand, AST is considered to have lower specificity for liver

damage due its presence in other organs. The reactive species (NAPQI) produced by paracetamol overdose harm the hepatic cells by lipid peroxidation and thereby damage the cellular permeability resulting higher serum levels of ALT and AST. The levels of ALT activity in the blood plasma of animals in different experimental groups were performed. Treatment with *Gardenia gummifera* leaves extracts control ALT level in dose dependent manner. Methanol extract of *Gardenia gummifera* control the ALT level more effective than aqueous extract. Treatment with isolated compound (Gardenin A) control the ALT level similar to standard treated group.

Effect on ALP levels in blood plasma: ALP, a hydrolysable enzyme excreted through the bile, is present in biliary cells as well as other organs such as bone, placenta, intestine, and kidney. Hepatotoxicity causes the biliary congestion leading to the inability of excretion of the ALP from the body that leads to elevation of the ALP level as seen in the vehicle group. Disease control animal level of ALP was much higher; the difference in normal control and disease group was quite significant. ALP level significantly and dose dependent manner comparable to the control group. The methanol extracts of *Gardenia gummifera* leaves extracts showed reduced levels of ALP activity. Administration of isolated compound (Gardenin A) control the ALP level similar to standard treated group. This result indicates that isolated compound (Gardenin A) can reduce the obstruction of bile duct induced by paracetamol overdose.

Effect on bilirubin level: The methanol extracts of *Gardenia gummifera* leaves extracts showed reduced Bilirubin level in blood plasma of animals. Administration of isolated compound (Gardenin A) control the Bilirubin level similar to standard treated group. This too suggests that the methanol extract of *Gardenia gummifera* leaves at the dose of 200 mg/kg and isolated compounds are also able to relieve the symptoms of hepatotoxicity.

Natural products include herbals, extracts, formulations, etc., which have been used for the cure of many liver sicknesses in traditional systems of medicine. In the absence of reliable hepatoprotective drugs in modern medicine, a large number of medicinal plants with well established traditional use have been recommended for the treatment of liver disorders. In this study, the hepatoprotective effectiveness of *Gardenia gummifera* leaves extracts has been evaluated. This plant is known for its medicinal properties in indigenous medicine in India. It is very rich in phytochemicals, which gives a very strong purpose to select this plant for pharmacological assessment. Earlier reports on medicinal plants suggest that alkaloids and phenolic compounds have wide potential

biological properties. Numerous studies propose that the plant is having phenolic and alkaloids which has attracted an extreme deal of scientific and public interest due to the health- promoting effects exhibited through a large array of biological activities as well as antioxidant properties

Conclusion

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. The findings of this study showed that methanolic extract of *Gardenia gummifera* L. (200mg/kg) exhibited potential protective action against the hepatotoxicity induced by paracetamol. The methanol, aqueous extracts of *Gardenia gummifera* leaves and isolated compound (Gardenin A) were evaluated for hepatoprotective activity. Results showed that isolated compound (Gardenin A) treated group showed similar hepato-protective action as standard treated group. Gardenin A is a highly oxygenated flavone with high medicinal and commercial importance. The hepatoprotective role of methanolic extract of *Gardenia gummifera* might be due to the presence of chemical constituents like phenolic compounds and alkaloids. These phytochemicals may offer antioxidant activity and thus preventing the oxidative stress-induced in the liver. Hence, methanolic extract of *Gardenia gummifera* may act as a prophylactic as well as a curative drug in treating hepatotoxic conditions.

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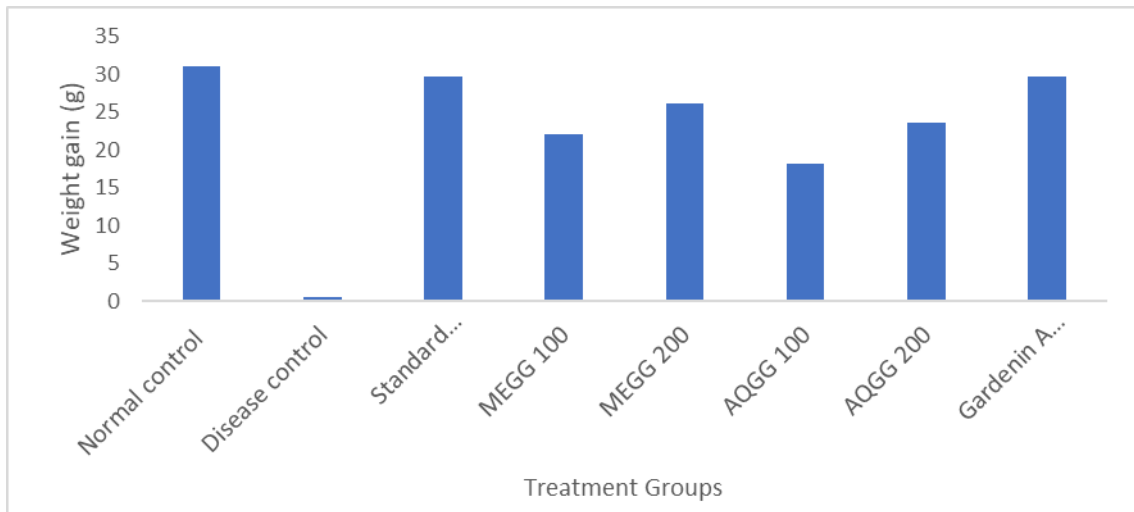


Figure 1: Effect of *Gardenia gummifera* leaves extract on body weight

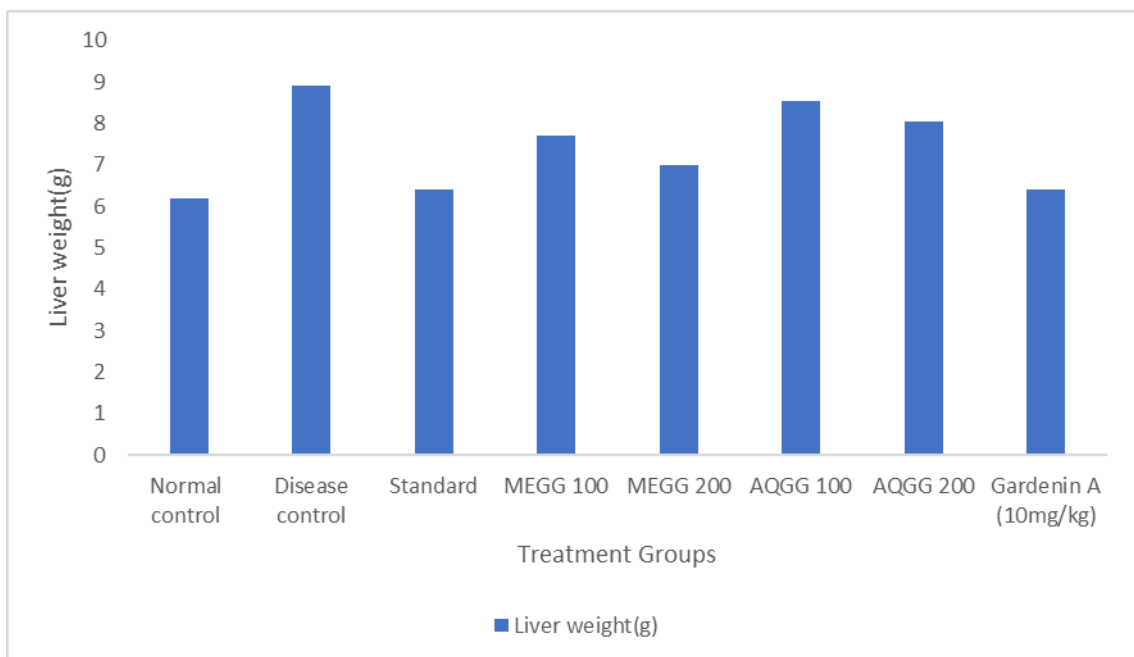


Figure 2: Effect of *Gardenia gummifera* leaves extract on absolute weight of Liver

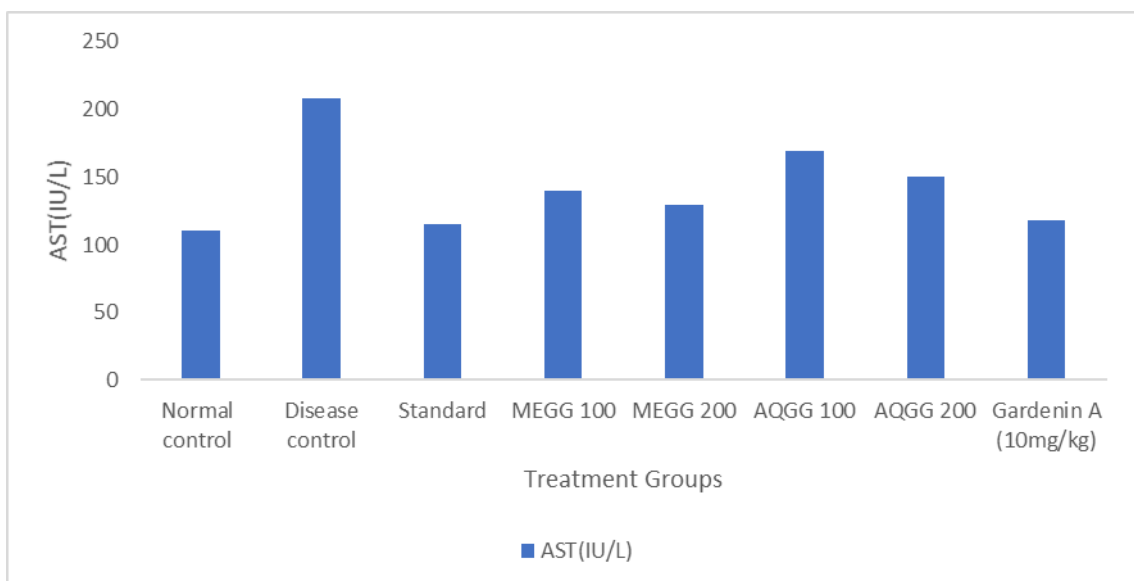


Figure 3: Effect of *Gardenia gummifera* leaves extract on AST levels in blood plasma of animals

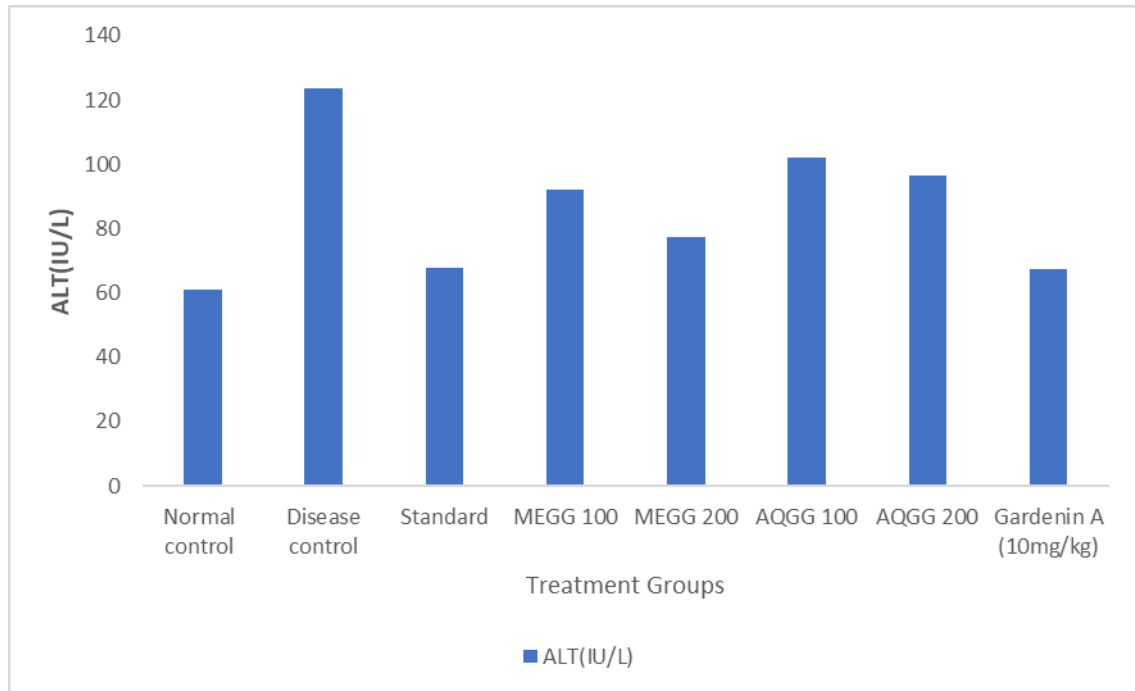


Figure 4: Effect of *Gardenia gummifera* leaves extract on ALT levels in blood plasma of animals

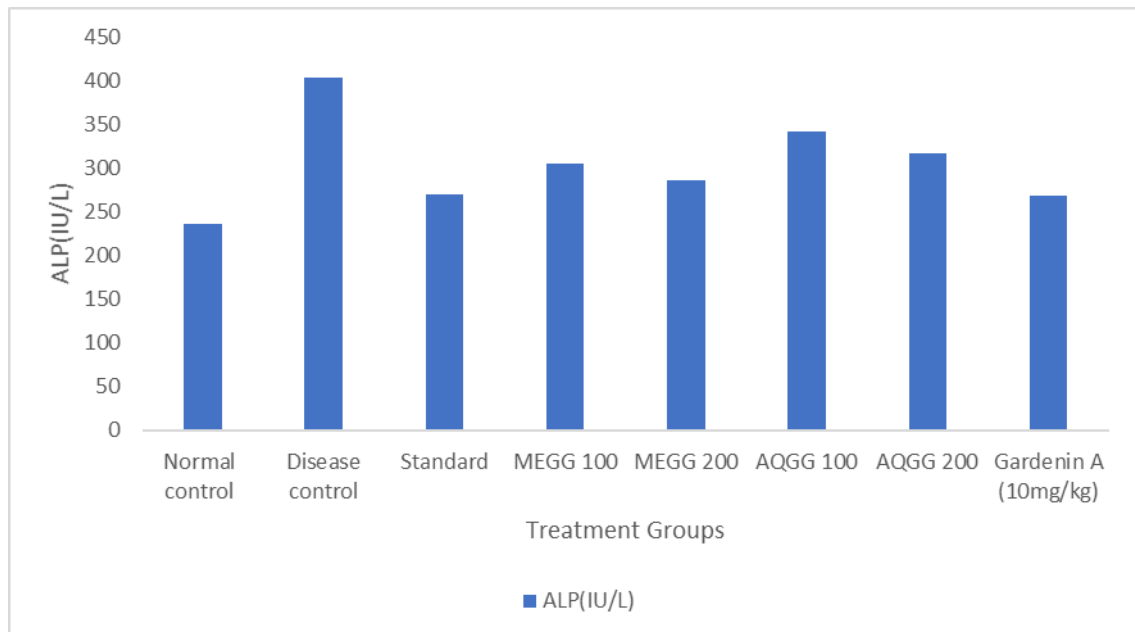


Figure 5: Effect of *Gardenia gummifera* leaves extract on ALP levels in blood plasma of animals

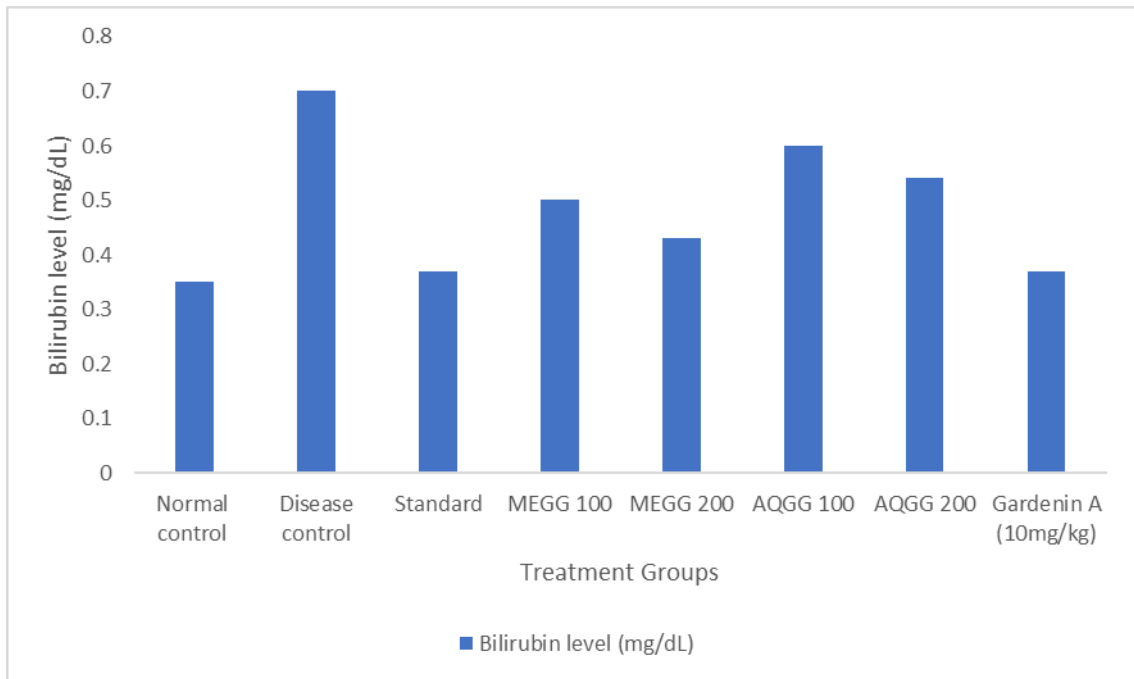


Figure 6: Effect of *Gardenia gummifera* leaves extract on Bilirubin level in blood plasma of animals