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AN *IN-VIVO* ASSESSMENT ON THE HEPATOPROTECTIVE ACTIVITY OF ETHANOL EXTRACT OF *COCCINIA GRANDIS*

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ABSTRACT

Background: The liver is a crucial detoxifying organ in the mammalian body that also performs a number of metabolic functions. A wide range of toxic substances and drugs have been linked to liver damage. *Coccinia grandis* have been used to treat a wide range of diseases. In this study, an *in vivo* assay was conducted to assess the hepatoprotective activity of *C. grandis*. **Method:** Fresh *C. grandis* was collected and dried. Ethanol extract of *C. grandis* was obtained by mixing 400g of the powder with 2400 ml of 100% ethanol, filtering and then evaporating the filtrate. Test animals were categorized into 14 groups for measuring different parameters to assess the hepatoprotective activity of *C. grandis*. **Result:** C. grandis significantly normalized body weight, SGOT, SGPT, ALP, creatinine, total cholesterol, LDL, triglycerides level in CCl4-induced hepatic toxicity containing rodents ($p < 0.05$). The plant extract also reversed the impaired level of proteins, such as ϒ-GT, SOD, MDA, CAT, LDH which are closely related to hepatic function. **Conclusion:** This study shows that different doses of C. grandis extracts give significant hepatoprotective activity compared with established marketed drugs. Therefore, more study should be conducted to detect the responsible mechanism to exert desired activity.

KEYWORD: Hepatotoxicity, SGOT, SGPT, rodent, *Coccnia grandis*, MDA, CCL4.

INTRODUCTION

The liver is a fundamental detoxifying organ in the mammalian body, and it performs a variety of metabolic functions.[1] It performs a multitude of functions in the detoxification and elimination of a wide range of endogenous and extraneous compounds.[2] Numerous toxic substances and drugs have been shown to cause liver damage, which is a known toxicological issue. Notwithstanding its common phenomenon, high morbidity, and mortality rates, its diagnosis and management is currently insufficient; to date, no therapy has managed to prevent the growth of fibrosis and cirrhosis; and, while recently designed drugs were being used to treat chronic liver abnormalities, such substances frequently have adverse effects.[3] It is responsible for more than half of all acute liver failure cases in the United States today. Idiosyncratic medication responses result in liver transplantation or death in more than 75% of cases.[4] Chemical substances can cause liver damage, which can develop to fibrosis and eventually cirrhosis and liver failure. In 2008, there were an estimated 748 300 new liver cancer cases and 695 900 cancer deaths globally.

For the experimental production of liver injury, carbon tetrachloride (CCl4) is commonly employed.[6] Induced liver damage in lipid peroxidation, reduced antioxidant enzyme activities, and the formation of free radicals are the main causes of carbon tetrachloride (CCl4) poisoning.[7,8] The metabolic activation of CC14 to short-lived reactive intermediates is intimately connected to its hepatotoxic effect.[9] Cytochrome P450, the terminal oxidase of the hepatic mixed function oxidase system, catalyzes the reductive dehalogenation of CC14.[10] This causes lipids to be destroyed, notably unsaturated phospholipids, leading in intracellular and cellular membranes damage.[11] The breakdown products, predominantly reactive aldehydes, are present all around

the cell and might cause further injury, such as increased membrane permeability, which is one sign of approaching cell death. The partial pressure of oxygen in tissues influences the course of CCl4-induced hepatotoxicity to some extent: low partial pressure leads in the generation of CC13* and CHC12* radicals and covalently metabolites binding.[12,13] This mostly impacts lipid metabolism (increased production, reduced transport out of the hepatocyte) and leads to steatosis, or fatty liver, which leads to cell death.[14]

Silymarin is an antioxidant that protects the liver from the harmful effects of free radicals.[15] Aescin and diosmin, either separately or in a low-dose combination, reduce liver damage.[16] Also different diuretics i.e. furosemide, bumetanide, hydrochlorothiazide (microzide), Antibiotics i.e. ofloxacin, amoxicillin-clavulanate may be administered to avoid bacterial infection in persons with cirrhosis and gastrointestinal bleeding.[17] Nonetheless, these synthetic drugs have negative side effects, and they are frequently blamed for the ineffectiveness in chronic liver disease.[18]

Ayurveda, India's ancient discipline of traditional medicine recognized liver disease early on and prescribed a variety of herbal therapies to treat it. Cucurbitaceae species have been found to have strong hepatoprotective effects in recent investigations.[19,20] Coccinia grandis belongs to the Cucurbitaceous family, which is known as "Telakucha" (Bengali), "Kanduri-ki-bel" (Hindi) and "Bimba" (Sanskrit).[21] There are 29 more species of Coccinia, all of which are only found in tropical Africa. People often use Coccinia grandis as a cultivated plant in Australia, Asia, the Caribbean, and the southern United States, as well as the Pacific Islands.[22] Hepatoprotective, Anti-diabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antibacterial, cathartic, and expectorant properties are found in the plant's leaves. Jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, eye infections, allergies, syphilis, gonorrhoea, and other conditions are treated using the roots, stems, leaves, and complete plant of C.grandis.[23] Secondary metabolites found in the plant include β-amyrine, lupeol, cucubbitacin, cephalandrol, cephalandrine, and tannins. Alkaloids, triterpenoids, and reducing sugars have been tested as preliminary phytoconstituents from the plant's leaves.[24] We, therefore, aimed to assess the hepatoprotective activities of Coccinia grandis. Satisfactory therapeutic activity findings may give persuasive rationale for the scientist to pursue more research in order to extract therapeutic ingredients and apply more comprehensive and reliable components to minimize the ailment.

MATERIALS AND METHODS

Plant Material Collection

The *Coccinia grandis* (Family: Cucurbitaceae) leaves were collected from a closest plant nursery of Dhaka, Bangladesh. The specimen was identified by the Department of Pharmacy, University of Dhaka.

Preparation of Extract

The *Coccinia grandis* plants were cleaned and thoroughly washed with distilled water, air-dried and powdered coarsely. In all steps, components were protected from direct sunlight. Then 400 g of the powder was mixed with 2400 ml of 100% ethanol (1:6) in a metabolic shaker for continuous shaking. After 7days this mixture was filtered with the help of a filter paper and this process was repeated for three times with remaining filtrate. The solvent was completely removed from the extract solution by rotary vacuum evaporator at 50-60°C temperature to obtain the desired extract and frozen to prolong the quality until use.

Botanical Authentication

According to the standards of our National Herbarium, we submitted a sample of each portion of the plant species under study, and the herbarium authorities took the necessary steps. The authorities, however, obliged the institute to restrict outsiders for a lengthy time due to the pandemic's abrupt and catastrophic onslaught. For the reasons stated above, we have yet to acquire botanical authentication (accession number).

Biochemical Kits Collection

Commonly known hepatotoxicity generating agent carbon tetrachloride, CCl_4 was bought from the sigma company, USA. The standard anti–oxidant drug silymarin is used, was bought from Incepta Pharmaceuticals Ltd as Livasil 140 mg. SGPT (Serum glutamic pyruvic transaminase), SGOT (Serum glutamic oxaloacetic transaminase), ALP (Alkaline phosphatase) was purchased from Plasmatec Laboratories, UK. As Biochemical analyzer, Humalyzer 3000 is used which is provided by the laboratory of University of Dhaka. SOD (superoxide dismutase), CAT (Catalase), DNA fragmentation enzyme was measured at PG hospital, Dhaka.

Experimental Animal Procurement, Nursing, and Grouping

Total One hundred and forty male rats each weighing between (120-150) gm were purchased from Jahangirnagar University, Savar. Each of them was kept in the Institute of Nutrition & Food Science in a well-controlled environment (temperature 25±3°C, relative humidity 55±5%, and 12 hours light/dark cycle) at the University of Dhaka. They were treated with a standard eating regimen and permitted admittance to cleaned water. All of the animals were kept in this environment for at least one week prior to the experiment. All of the experimental methods were performed according to the Institutional Animals Ethics Committee (IEAC).

A sample of 140 rats were allocated at random into 14 groups, each with ten rats. The rats for each group were chosen at random in all of the studies. We took ten rats in each group to increase the investigation's validity. Some random concerns relating to the pandemic condition may have an impact on the elevated sample size. Because our

rats were housed in the animal house throughout the pandemic lockdown, and we, the researchers, visited the lab twice a week, the lab curator was the sole person accountable for their care. During the reproductive season, we, on the other hand, maintained a close eye on the rat every day. We included both positive and negative control groups in our study.

Group 1: Animals served as negative control and received normal saline.
Group 2: Animals received only CCl4 treatment to induce hepatotoxicity.
Group (3-5): Animals were constituted the hepatotoxic group, which received CCl4 to induce hepatotoxicity and then a marketed medicine was administered at low (100mg/kg), medium (250mg/kg) and high (500mg/kg) dose.
Group (6-8): Animals were constituted the hepatotoxic group, which received CCl4 to induce hepatotoxicity and then *Coccinia grandis* leaf extract was administered at low (500mg/kg), medium (750mg/kg) and high (1000mg/kg) dose.
Group (9-11): Animals were administered only with marketed medicine.
Group (12-14): Animals were administered only with *Coccinia grandis* leaf extract.

RESULT AND FINDINGS

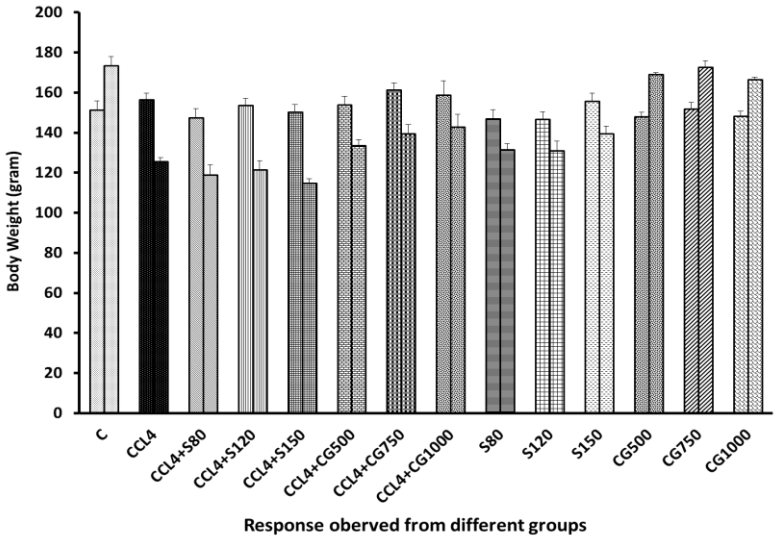

Figure 1. The body weight of rats (in grams) of 14 groups represented before and after completing the experiment.

As shown in the graph above, we established evidence of weight gain in *Coccinia grandis* and Silymarin treated groups following CCL_4 induced liver damage in group 1, 12, 13, and 14. The weight of the rats in all of the other groups decreases after treatment.

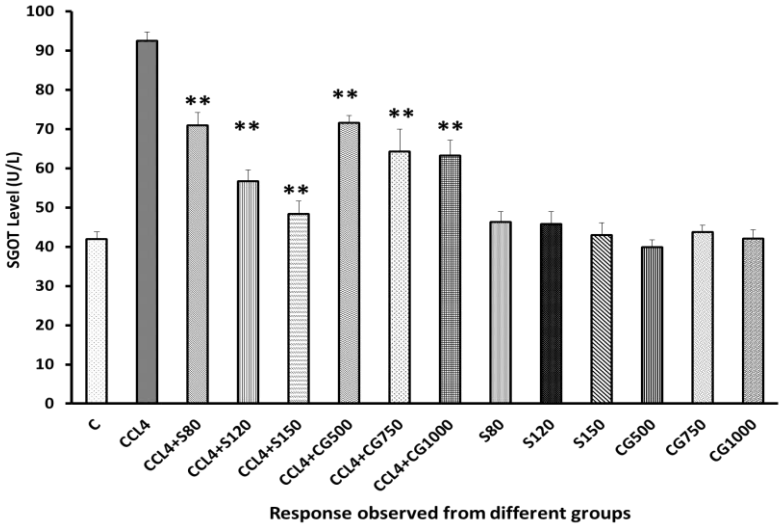

Figure 2. The SGOT (U/L) level of rats from 14 groups. The data were expressed as a mean ± standard deviation. (indicates statistically significant change)**

Serum glutamic–oxaloacetic transaminase (SGOT) level, is a hepatic marker which is reduced significantly after Silymarin treatment. Consecutive increased use of dose of Silymarin reduced SGOT level in a significant amount. *Coccinia grandis* administration in CCL_4 treated rats lowered SGOT level in groups 6, 7, 8, but not as much as Silymarin did in groups 3, 4, 5. The SGOT levels in other non–CCL_4 treated groups did not fluctuate in a significant level compared with negative control groups.

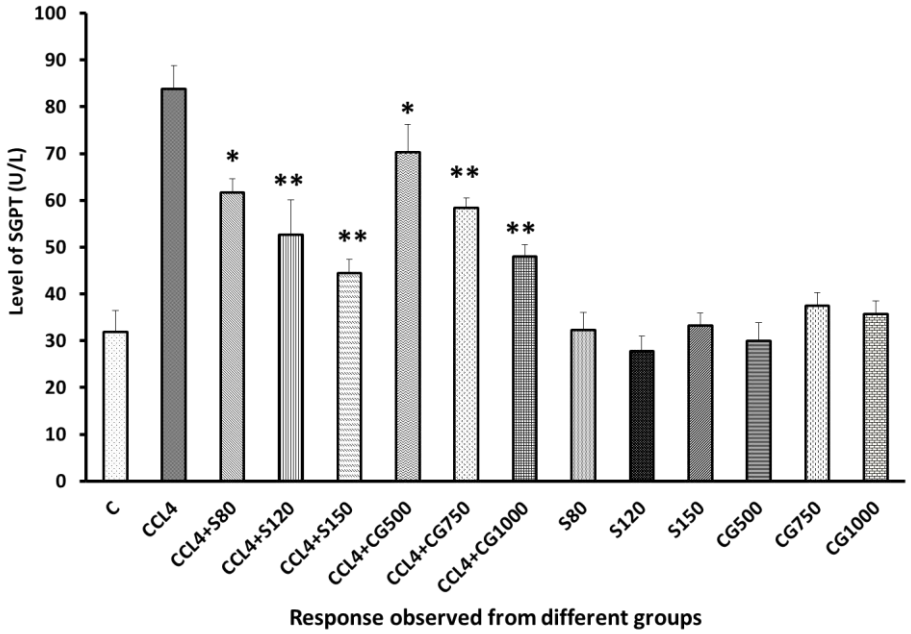

Figure 3. SGPT (U/L) level of rats from 14 groups. The data were expressed as mean ± standard deviation (*indicates statistically significant change)

As shown in the graph above, In the CCL4-treated group considerable drop in SGPT levels after administration of both Silymarin and *Coccinia grandis* was observed, particularly in groups 3, 4, and 5. Although Silymarin lowered SGPT levels slightly more than *Coccinia grandis*. In non–CCL4 treated groups, SGPT level was not deviated much after Silymarin or plant extract administration.

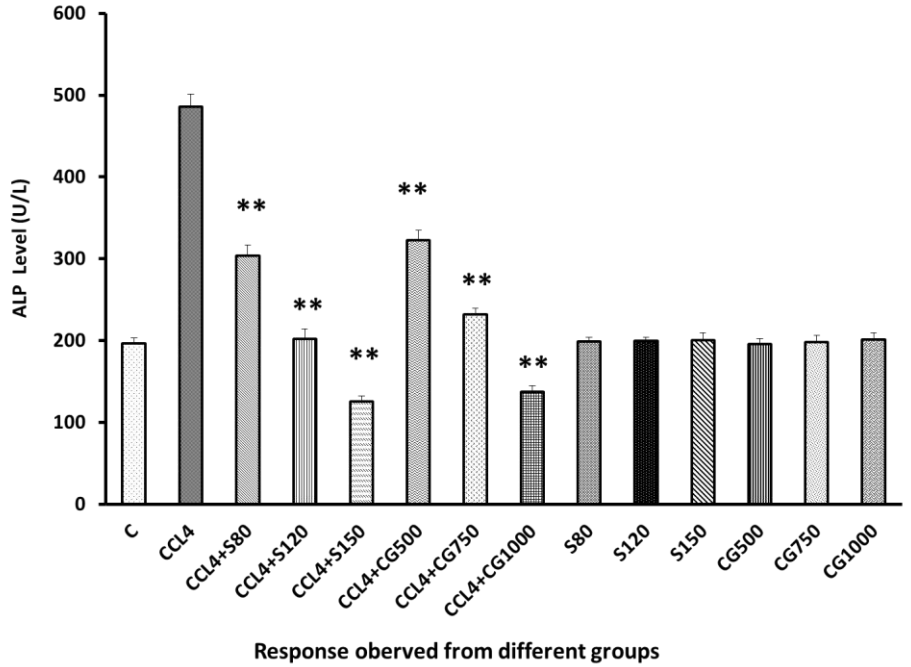

Figure 4. ALP (U/L) level of rats from 14 groups. The data were expressed as mean standard deviation. (*indicates statistically significant change)

As shown in figure 4, treatment of low, medium, and high dosages of *Coccinia grandis* resulted in a steady decrease in ALP levels. In CCL4-treated groups, the outcome is virtually identical to Silymarin. *Coccinia grandis* and Silymarin administration in no-CCL4 treated groups were unlikely to cause harm to rats because there was no significant statistical difference between groups 1, 4, 8, 9, 10, 11, 12, 13, and 14 when compared to the negative control.

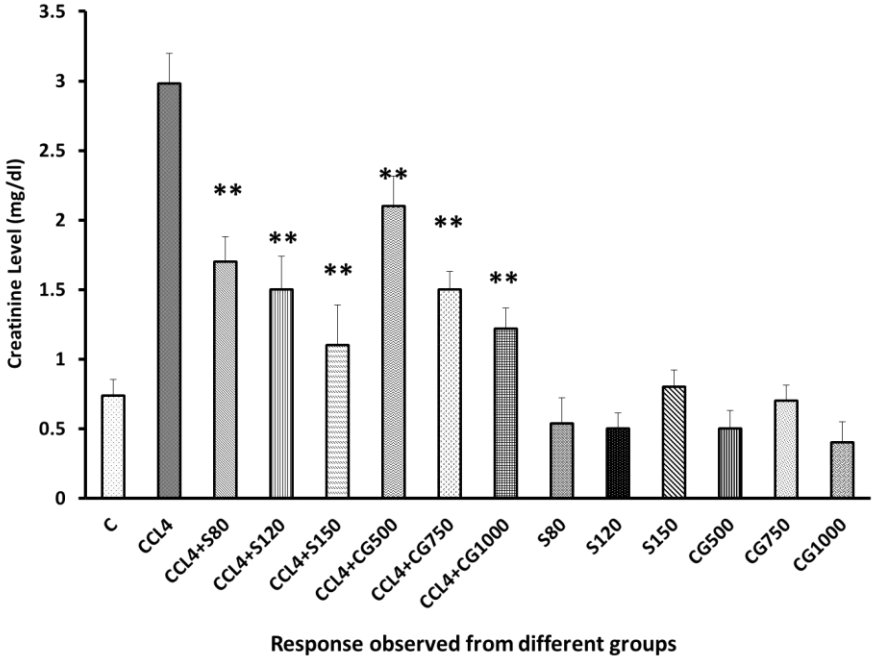

Figure 5. Creatinine (mg/dl) level of rats from 14 groups. The data were expressed as a mean standard deviation. (*indicates statistically significant change)

Higher dosages of *Coccinia grandis* decreased the creatinine level in CCL4 treated groups, as seen in the graph. However, when silymarin was given to groups 3, 4 and 5 the decrease in creatinine levels was substantial. In non–CCL4 treated groups, there were modest variations following administration of the medication and plant extract, but they were statistically insignificant.

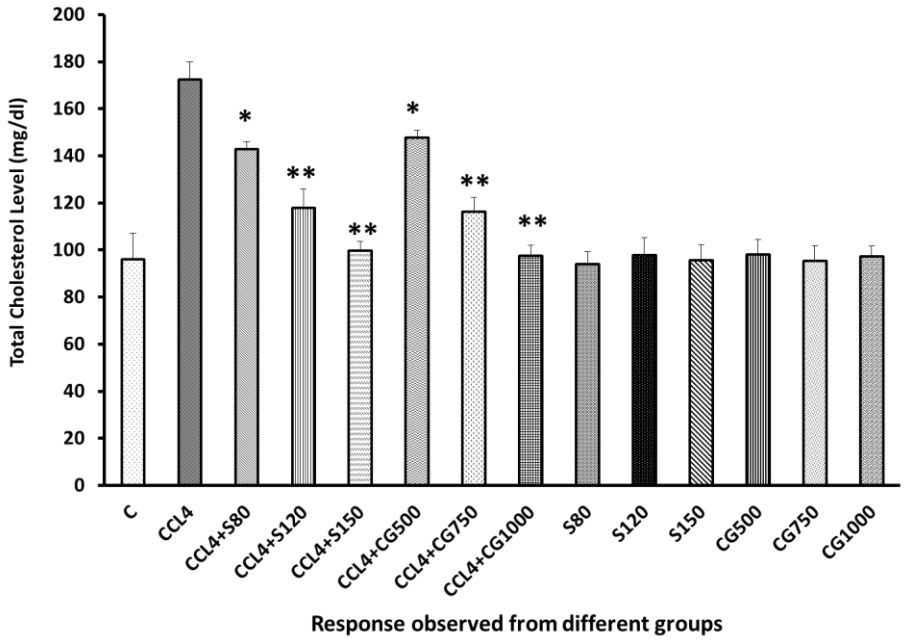

Figure 6. Total cholesterol (mg/dl) level of rats from 14 groups. The data were expressed as mean ± standard deviation. (*indicates statistically significant change)

When CCL4 therapy was given to those groups, total cholesterol levels increased; however, when *Coccinia grandis* plant extracts were given to those groups, total cholesterol levels decreased dramatically. Silymarin treatment in groups 9, 10, 11 and *Coccinia grandis* treatment in groups 12, 13, 14 likewise brought cholesterol down to that level.

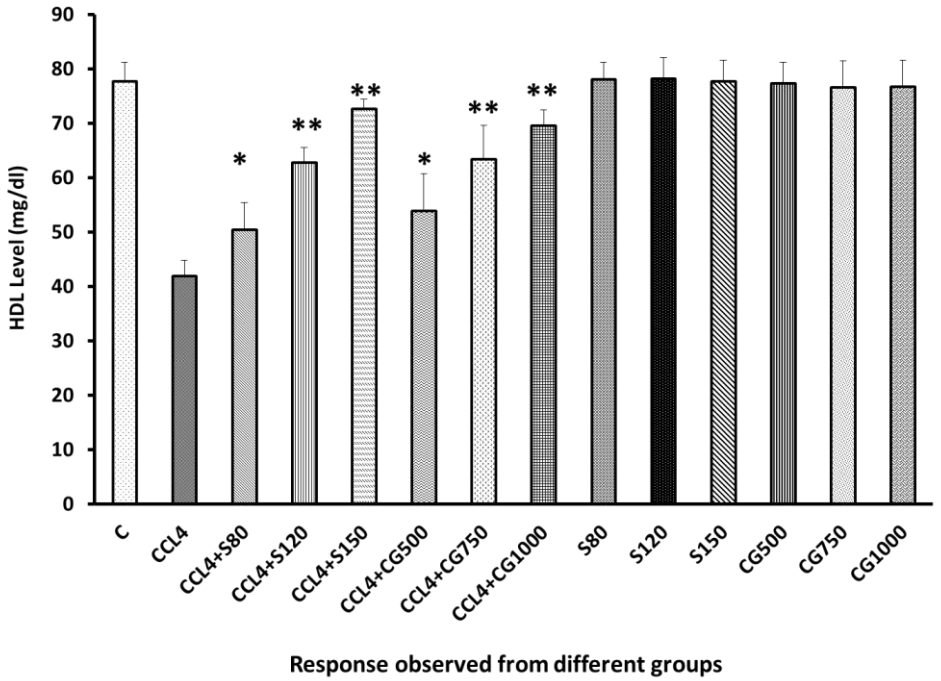

Figure 7. HDL (mg/dl) level of rats from 14 groups. The data were expressed as a mean ± standard deviation. (* indicates statistically significant change).

The considerable decline in HDL following injection of CCL4 was reversed by treatment with larger doses of the plant extract, however the lowering impact was more in the case of Silymarin than the plant extract. The non–CCL4 treated groups had no obvious adverse effects after either the medication or plant extract, indicating that the plant extract is safe.

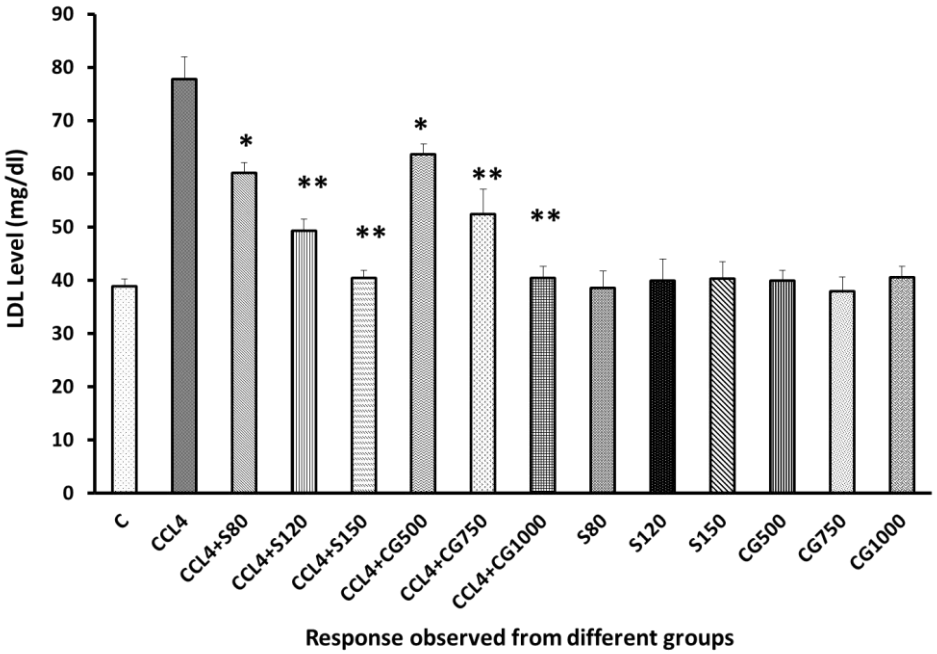

Figure 8. LDL (mg/dl) of rats from 14 groups. The data were expressed as a mean ± standard deviation. (* indicates statistically significant change).

As seen in graph, plant extracts and medications were continuously raised, while the amount of LDL was gradually lowered. Silymarin's lowering impact was somewhat stronger than that of *Coccinia grandis*. When compared to the negative control group, the other groups without CCL4 that were fed plant extract and medication exhibited no significant variations.

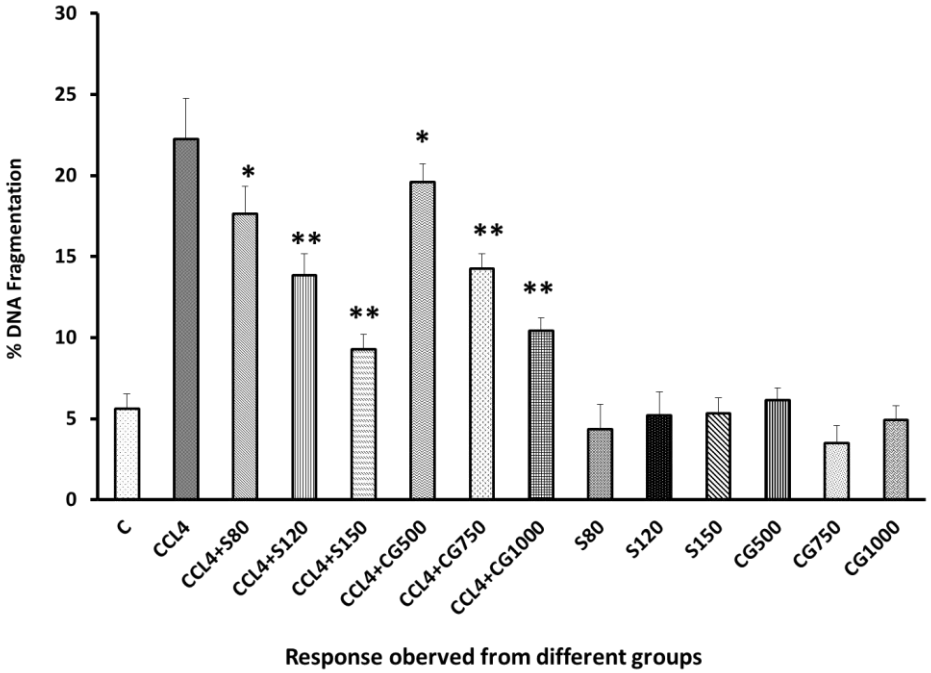

Figure 9. Comparison of percentage DNA fragmentation in 14 groups of rats after administering the drug or *Coccinia grandis* extract. The data were expressed as mean ± standard deviation (*indicates statistically significant change)

As shown in the graph, higher dosages of *Coccinia grandis* decreased the creatinine level in CCL4 treated groups. However, when silymarin was given to groups 3, 4 and 5 the percentage of DNA fragmentation levels was substantially decreased. In non–CCL4 treated groups, there were modest variations following administration of the medication and plant extract, but they were statistically insignificant.

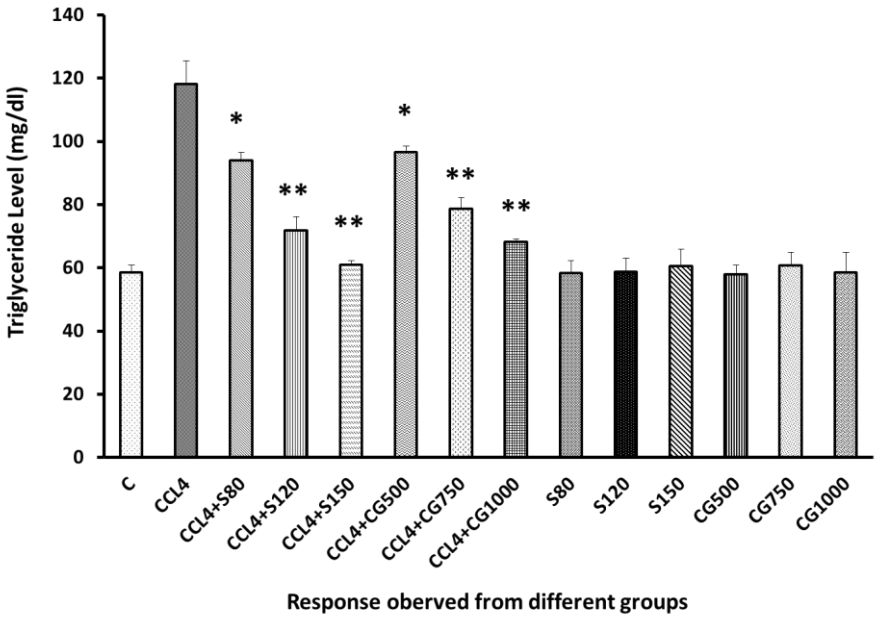

Figure 10. Triglyceride (mg/dl) level of rats from 14 groups after administering the drug or *Coccinia grandis* plant extract. The data were expressed as mean ± standard deviation (*indicates statistically significant change)

Plant extracts and medications were continuously raised, while the amount of Triglyceride levels was gradually lowered. Silymarin's lowering impact was somewhat stronger than that of *Coccinia grandis*. When compared to the negative control group, the other groups without CCL4 that were fed plant extract and medication exhibited no significant variations.

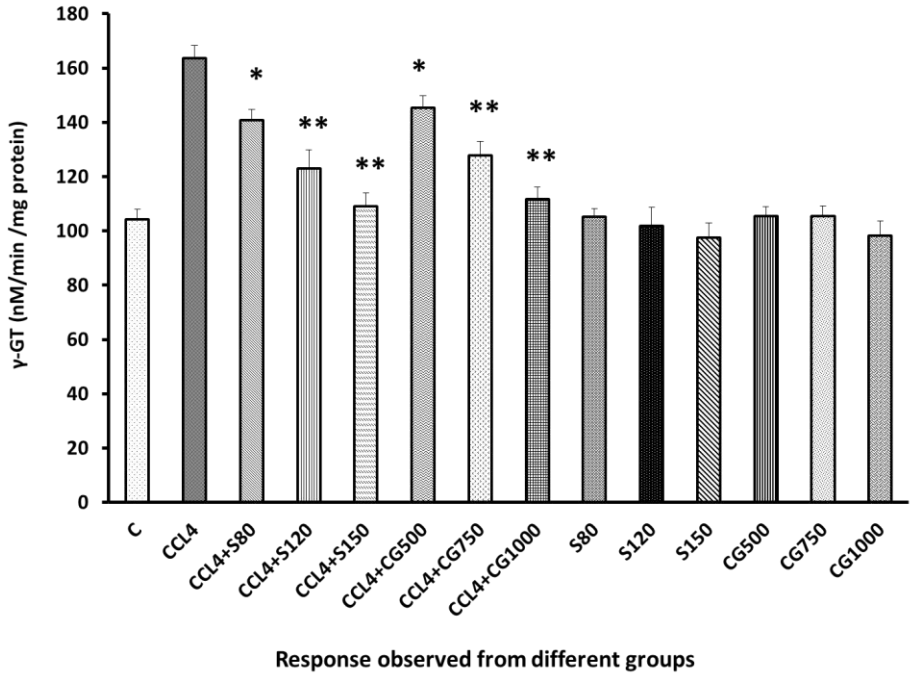

Figure 11. γ–GT (nM/min/mg protein) of rats from 14 groups after administering the drug or *Coccinia grandis* plant extract. The data were expressed as mean ± standard deviation (*indicates statistically significant change)

As stated above, consecutive increased use of dose of Silymarin reduced γ–GT level in a significant amount. *Coccinia grandis* administration in CCL_4 treated rats lowered γ–GT level in groups 6, 7, 8, but not as much as Silymarin did in groups 3, 4, 5. The γ–GT levels in other non–CCL_4 treated groups did not fluctuate in a significant level compared with negative control groups.

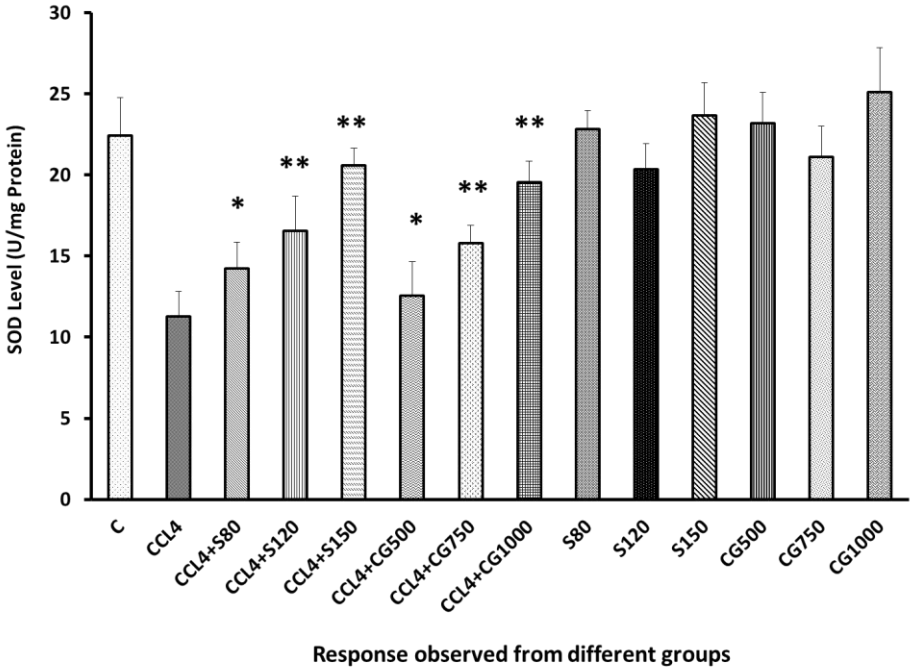

Figure 12. SOD (U/mg Protein) of rats from 14 groups after administering the drug or *Coccinia grandis* plant extract. The data were expressed as mean±standard deviation (*indicates statistically significant change)

Treatment with higher dosages of the plant extract reversed the significant drop in HDL caused by CCL4 injection, however the lowering effect was greater in the case of Silymarin than the plant extract.

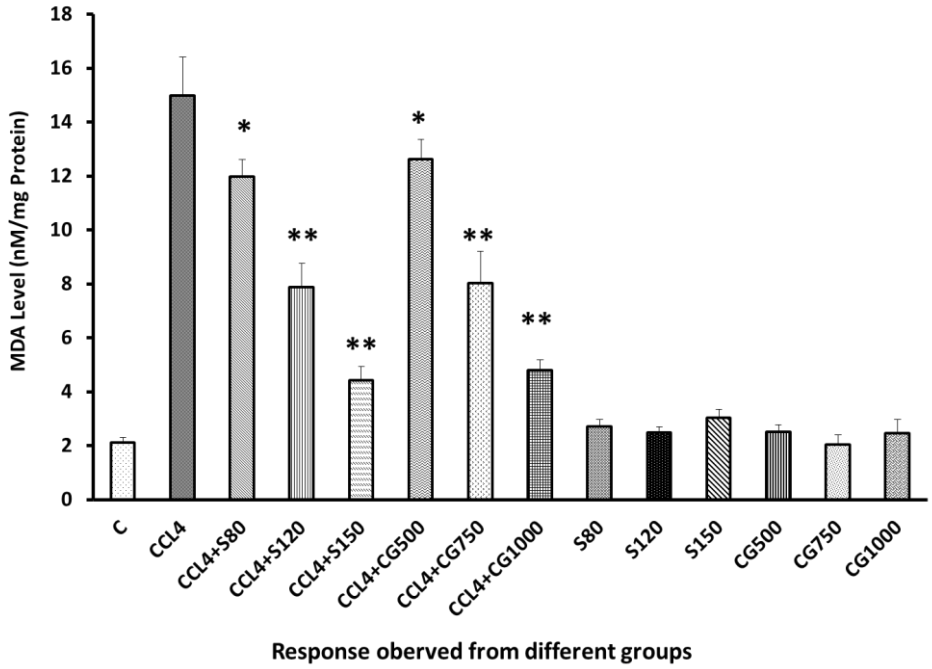

Figure 13. MDA (nM/mg protein) of rats from 14 groups after administering the drug or *Coccinia grandis* plant extract. The data were expressed as mean±standard deviation (*indicates statistically significant change)

As shown in the graph, higher dosages of *Coccinia grandis* decreased the MDA level in CCL4 treated groups. However, when silymarin was given to groups 3, 4 and 5 the MDA levels was substantially decreased. In non–CCL4 treated groups, there were modest variations following administration of the medication and plant extract, but they were statistically insignificant.

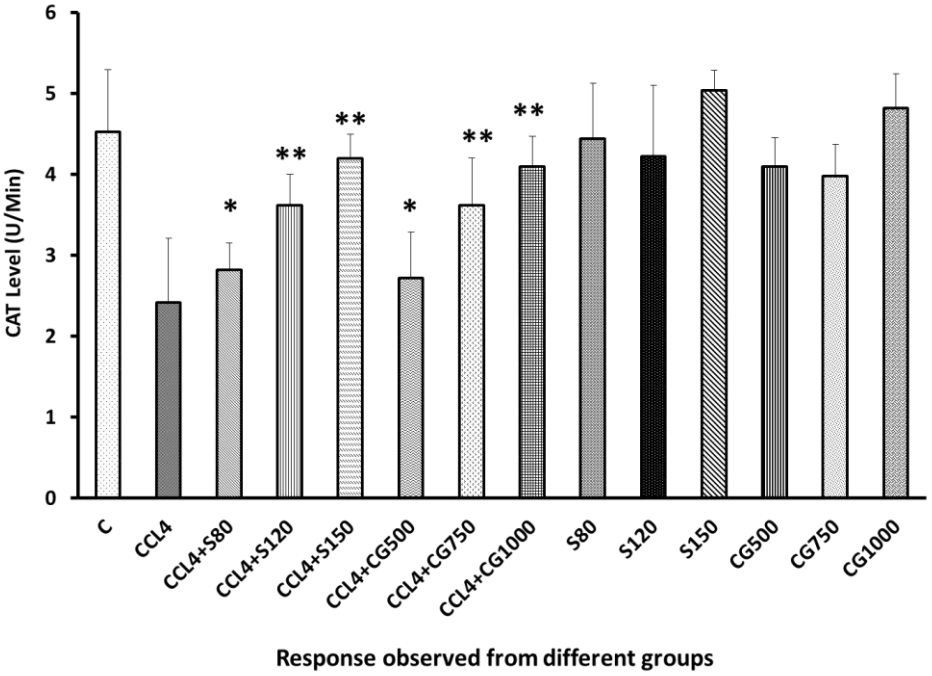

Figure 14. CAT (U/Min) of rats from 14 groups after administering the drug or *Coccinia grandis* plant extract. The data were expressed as mean ± standard deviation (*indicates statistically significant change)

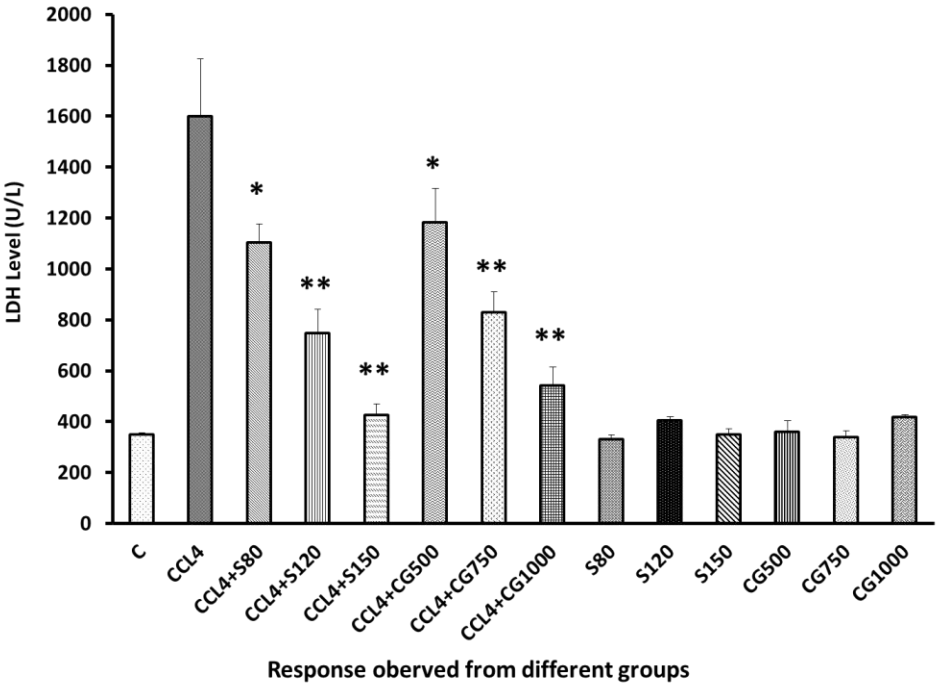

Figure 15. LDH (U/L) of rats from 14 groups after administering the drug or *Coccinia grandis* plant extract. The data were expressed as mean ± standard deviation (*indicates statistically significant change)

Higher doses of C. grandis reduced LDH levels in CCL4-treated groups, as demonstrated in the graph. LDH levels significantly decreased when silymarin was administered to groups 3, 4, and 5. There were minor differences in non–CCL4 treated groups when the drug and plant extract were administered, but they were statistically insignificant.

DISCUSSION

Liver diseases continue to be a significant public health concern. However, there are no adequate liver protective medications available in allopathic medicine for serious liver problems. Herbal medicines aid in the management of a variety of liver diseases, as well as other natural liver healing processes.[25] Carbon tetrachloride (CCl4) has previously been used to successfully produce hepatotoxicity in experimental animals.[26] In experimental hepatopathy, the toxin carbon tetrachloride is bio transformed by cytochrome P-450 to create the trichloromethyl free radical, which promotes peroxidative breakdown in adipose tissue, leading in hepatocyte fatty infiltration. In the presence of oxygen created by metabolic discharges from mitochondria, trichloromethyl free radicals cause lipid peroxidation of membrane lipids. All of these processes result in the breakdown of cell membrane integrity and hepatic tissue injury.[27]

In the CCl4-induced liver injury groups, a substantial drop in total body weight, absolute liver weight, and relative liver weight was found. Both C. grandis extract and the standard drug Silymarin resulted in a considerable increase in body weight. While Silymarin performed better than the test extract, activities of both groups increased with increase of dose. Rutin was also shown to have comparable effects in CCl4-induced rats.[28]

Treatment groups reversed the CCl4-mediated increase in SGPT levels, with a progressive drop observed at low, medium, and high dosages of Silymarin and plant extract, respectively. When compared to the negative control group, the SGPT levels of the other six non-CCl4 treated groups didn't differ considerably, ruling out the likelihood of serious consequences from consumption of Silymarin or the plant. The SGOT level followed a similar pattern among experimental groups. In another study, ethanol extract of C. grandis was observed to nomalize the levels of SGPT and SGOT in both paracetamol and CCl4-induced hepatoxicity treatment groups.[29]

The current investigation shown that inducing rats with CCl4 significantly enhanced the levels of ALP and ϒ-GT. CCl4 produces acute hepatocyte injury, alters membrane integrity, and results in the leakage of enzymes from hepatocytes.[30] However, both C. grandis extract and Silymarin effectively recovered the pathological elevations in ALP and ϒ-GT. These findings show that the ethanol extract of C. grandis is capable of protecting hepatocytes from CCl4-induced toxic effects. Aqueous extract of C. grandis leaves also exerted hepatoprotective activity by reducing ALT, AST and ALP level.[31] In another study, Rutin exerted similar pattern in normalizing ALP and ϒ-GT levels in CCl4-induced hepatotoxic groups.[28]

For creatinine and urea, the Silymarin and plant treatment groups elicited nearly identical responses. Nonetheless, when the two groups were compared, it was shown that the groups given Silymarin had a little superior outcome. Additionally, in both treatment groups, a better outcome was reported with a higher dose of medicines. The total creatinine and urea levels obtained from the remaining non- CCl4 induced groups were found to be comparable to those obtained from the negative control group, therefore ruling out the potential of significant side effects associated with either the medicine or the plant. Similar result was obtained in case of the antidiabetic activities of Terminalia arjuna, where these tests were conducted in the same way.[32]

Notably, the treatment of plant extract restored normal blood triglyceride, total cholesterol, and LDL levels, as well as a reduced amount of HDL. This may be explained by the presence of possible therapeutic compounds capable of chelating multivalent metal ions, including zinc, calcium, and iron, in the plant extract. Indeed, its capacity to chelate minerals has been shown to have some beneficial benefits in experimental animals, including a decrease in iron-mediated free radical production and a decrease in serum cholesterol, triglycerides, and lipid peroxides.[33] Similar result was obtained in case of the antidiabetic activities of Terminalia arjuna, where these tests were conducted in the same way.[32]

It is widely established that CCl4 causes liver damage by producing reactive oxygen species (ROS) and depleting both enzymic and non-enzymic antioxidants. CCl4 treatment resulted in significant decreases in SOD, CAT, and MDA levels. Antioxidant levels were increased following treatment of the ethanol extract of C. grandis in CCl4-induced hepatotoxicity. In another investigation, at 200 mg/ kg body weight, C. grandis fruit extract was shown to reduce SOD, CAT, MDA and Vitamin-C levels in DEN-induced hepatotoxic rats.[34]

Additionally, the ethanol extract of C. grandis was also found to be capable of reversing the enhanced DNA fragmentation process. Both the control and treatment groups demonstrated a dose-dependent reduction in DNA fragmentation, albeit Silymarin had a greater effect than the plant extract.

However, the method by which the plant exerts its hepatoprotective effects is unknown, although it may be connected to the plant's capacity to suppress lipid peroxidation in the liver. The CCl4-induced hepatotoxicity in rats results in the formation of harmful radicals, which can be mitigated by employing an appropriate antioxidant in sufficient quantity. Coccinia grandis contains flavonoids, tannins, saponins, and terpenoids, which contribute to its hepatoprotective properties by reducing free radical-mediated damage.[35,36] It was claimed that flavonoids, triterpenes, and tannin were antioxidant agents that may interfere with free radical generation and that particular flavonoids have hepatoprotective properties.[37] Triterpenoids may exert hepatoprotective effects by inhibiting the haemolysis of red blood cells produced by phenyl hydrazine.[38]

On the basis of enzymatic activity and histological findings in the Coccinia grandis treated groups, it may be inferred that the ethanol extract of C. grandis has strong hepatoprotective action.

CONCLUSION

Ethanolic extracts of the barks of Coccinia grandis have been shown to have the ability to reverse a number of abnormal pathophysiological states in rat models, as demonstrated by our results. Dose-dependent improvements in responses also suggested that the precise administration of exact dose of extract obtained by an appropriate extraction process may amplify the therapeutic action to a reasonable degree. As a result, new avenues for disease management may open up if phytochemical analyses as well as pharmacological response of C. grandis are carefully studied in the future.

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