Acute Toxicity and Antioxidant Ability of *Senegalia greggii* Seed Extract

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ABSTRACT

Senegalia greggii plant used as medicinal plant, it has many chemical components not known the toxicity effect. This study aimed to investigate the acute toxicity of *Senegalia greggii* seed Extract. Four groups of 10 rats in each group orally administration of various doses of *S. greggi* seed extract at single dose. After 14 days of dose administration all rats were given high dose of enthused, kidney and liver for histopathological examination and collect blood for antioxidant marker. The results revealed all the rats were increased in the weight at dose depended manner, the histopathological examination for liver and kidney was shown normal hepatic structure but minimal inflammatory cell infiltration. And the administration of extract was lead to increase in the level of antioxidant enzyme GSH but, decreased in the level of MDA in the treated rats. The concluded that the *S. greggi* seeds ethanol extract was safe at a dose of 1000 mg /kg and has antioxidant effect to be used for medicinal purposes.

Keywords: *Sanegalia greggi*, Acute toxicity, GSH, MDA

Introduction

Plants have historically given humans a supply of nutrition, scent, colour, gum, fibre, resin, and many other valuable goods. In order to cure a wide range of disorders, ethnopharmacologists are now focusing more on examining the bioactive characteristics and phytochemical analyses of medicinal plants. A number of medicinal plants are potential sources of natural medicine again for treatment of a wide range of diseases (1). Many of these plants have important therapeutic properties. The use of medicinal herbs to treat a number of illnesses, including stomach ulcers, has recently gained the support of numerous researchers. Numerous investigations have revealed that plants provide a range of important bioactivities (2, 3, 4).

Senegalia greggii one of these medicinal plants used as traditional medicine and investigated for antimicrobial and isolated biomolecule responsible for anti-HIV properties. *S. graggii* used in traditional using the pods to make eyewash to treat conjunctivitis, also grounding leaves and pods into powder that prevents bleeding and soothes sore skin. As a tea it may treat diarrhoea and dysentery, with the addition of its flowers it may also treat nausea and vomiting(5). Acacia entered into farming in California by Theodore Payne. From California Native Plants, Theodore Payne's 1941 index: "An impressive fallen shrub from the Colorado Desert. Instead of sprawling in habit, investigation height of 4 to 7 boldness and from time to time more, its branches being armed

with short curved prickles (6). An ocean of trees in the Senegalia genus, formerly known as Acacia greggii, are confined to the southwest United States and northwestern Mexico, from the max south of Moab southward through southern Nevada, southeastern California, Arizona, New Mexico, & western Tex to Baja California(7,8).

Medicinal plants are widely used in traditional medicine, and now in the recent years were the focused and involved by researchers to discover the biological activity and the chemical contents. Many previous studies were investigated the biological activities of medicinal plants as antimicrobial activity (9). The rise of antibiotic resistance attracted the scientists to investigate natural products for novel antimicrobial activity. Plants are widely used as an ethnomedicine and were used therapeutically since the middle paleolithic age (10).

The assessment of the safety dose and the toxicity of medications and chemicals that composed of the plant were very important to detect the safe dose. This study aimed to investigate the acute toxicity of *Senegalia greggii* seed Extract and detect the safe dose.

Methods

Extraction method

The seeds of *Senegalia greggii*, (voucher No: SEGR4) were collected from trees that distributed in Kalar region at Diyala provinces, Iraq from September to October, 2021. The seeds were cleaned washed, dried and grinded with an electrical grinder. For the extraction used solid liquid method, its most basic form, the extract material is combined with a suitable quantity of solvent to enable the solvent to become entirely immiscible with the extracted material, ensuring thorough solvent penetration and the ability to dissolve the required components. The raw material was then separated from the solvent and the dissolving transition material, and the solvent was evaporated. A 100 g from seeds powder was soaking with 95 % of ethanol for 72 hours. The solution was shaking many times for enhanced the powder to dissolve completely in the ethanol till change the solution color. After that the mixture was filtered by filter paper and the collected filtration extract was distilled with rotary evaporator (Figure 1). The extract was kept in sterile container at -20oC till used (11).

Figure 1: Weight the S.gragii seeds powder and soaked with etahnol

Acute toxicity of S.gargii

A single dosage is administered orally to a group of animals. The dosage level is established based on an estimated dose that will cause some toxic symptoms without generating severe toxicity indications or resulting in death. Any clinical indicators observed, as well as any deaths, would be documented. The dose level's safety is determined by the findings, the amount of toxicity that occurs, and mortality if it occurs (12). Wister albino rats, male (5-7) weeks old and weight between (100-140) g, were used. The rats were obtained from Centre for animal breading, Health Ministry, Iraq. The study was done according ethical role for College of Vet. Medicine, University of Diyala. All rats were housed separately and kept under standard humidity (50-60%), temperature (22 3°C), and light (12h light: dark cycle) conditions, and were fed a chow diet and water ad libitum. Each rat was confined alone for the experiment and fasted before to treatment (food, but not water, was prevented overnight).

There were Four groups 10 rats/ group are divided as: control group orally administration single dose of Tween 20 (5% v/v), the another three groups are orally administration single dose of S. gregii extract at (250, 500 and 1000) mg/Kg. The dose was administration by oral gavage tube. After administration doses, food was prevent for (3- 4) h. Toxicity symptoms and mortality were detected following treatment once during the first 30 minutes and recurrently within the first 24 hours, with a particular concentration throughout the first 4 hours and afterwards daily for a total of 14 days. After the 14th day, each group's rate were anesthetized with 0.01 mL/kg xylazine and 0.09 mL/kg ketamine and killed. Blood sample was collected to determine the level of GSH and MDA in the treated rat by ELISA (Glutathione, GSH was measured by ELISA Kit,Sunlong Biotech. CAT.NO: SL09998Ra), and (Malondialdehyde, MDA was measured by ELISA Kit, Sunlong Biotech. CAT.NO: SL0475Ra) the method was performed as manufactured instructions.

Statistical Analysis

The data was analysis by using IMP SPSS statistics 20. T test and ANOVA test were used for analyzing the data. The data was presented as mean ± SD. Significant of variance was at P value < 0.05.

Result

The results of this study were showed the effect of oral administration of *S. graggii* seed ethanol extract on the weight of rat as presented in the (Table 1). The *S. graggii* seed extract no effected on the body weight which was decreased at the 4th day after administration of extract as presented by mean ±SD: (136 ± 13.84, 138.3±25.62, 123.5±21.8) at dose depended manner (250, 500 and 1000) mg/mL of *S. graggii* seed extract, results shown not significant differences (P < 0.05) compared with control group which was (126 ± 15.78). While, in the 7th, 10th and 14th days after administration of extract all the rats in the *S. graggii* groups increased in the weight of rat at dose depended manner, in the 7th day was (144.5±17.27,150.4±29.4,142.7±18.59), and in the 10th day was (187.5±21.6,175.9±31.76,165.6±21.95)and for the 14th day was (201.2±39,205.4±32.8,193.6±47.35).While for control group it was (147±14.94, 173±15.84, 201±15.05, 233±19.32) for 4, 7, 10 and 14 day.

Table 1: Showed the effect of oral administration of *S. graggii* seed ethanol extract on the body weight of rat at different doses (250,500 & 1000) mg/mL, (10 rats /groups).

Groups	1st day	4th day	7th day	10th day	14th day
Control	126 ± 15.78	147 ± 14.94	173 ± 15.84	201 ± 15.05	233 ± 19.32
Sg 250 mg/mL	124.1 ± 23.05	136.6 ± 13.847	144.5 ± 17.27	187.5 ± 21.6	201.2 ± 39
Sg 500 mg/mL	144.4 ± 22.78	138.3 ± 25.62	150.4 ± 29.4	175.9 ± 31.76	205.4 ± 32.8
Sg 1000 mg/mL	133 ± 13.612	123.5 ± 21.8	142.7 ± 18.59	165.6 ± 21.95	193.6 ± 47.35

Data was presented as (Mean ± SD).Significant differences (P value < 0.05)

For the period of 14th days of experimental from oral administration of *S. graggii* seed extract, there is no death happened, but the loss of the hairs rat in the first week for the rats administration *S. graggii* seed ethanol extract doses (500 & 1000) mg/mL. The histological examination of the liver (Figure 2) and kidney (Figure 3) was not appeared any poisonous symptoms, normal hepatic structure but minimal inflammatory cell infiltration was associated with mild congestion in the portal area was seen at dose 1000 mg/ml.

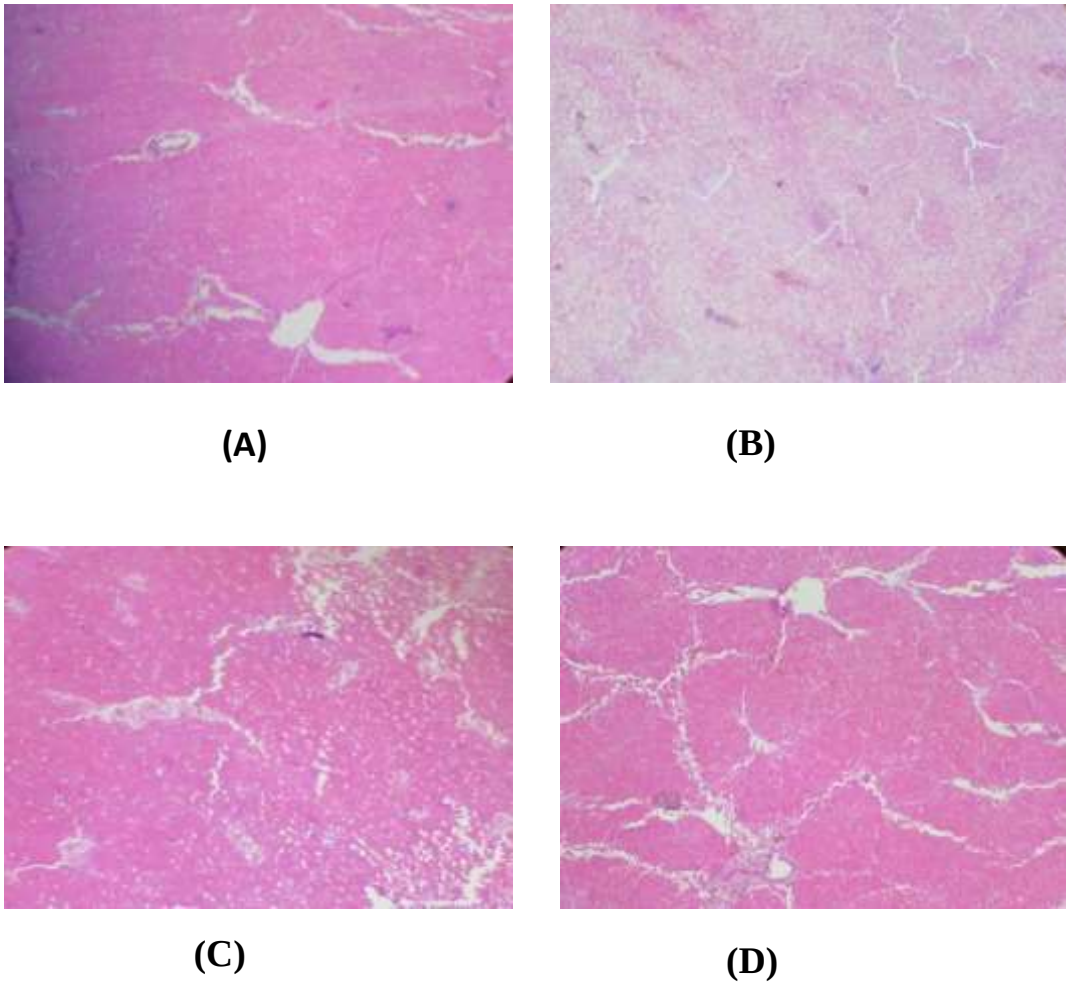

Figure 2: Effect of *S. graggii* on liver. Rat (A) as control group. Rat (B) treated with 250mg/kg. Rat (C) treated with 500mg/kg and rat (D) treated with 1000mg/kg) of *S. graggii*. Histological examination by (hematoxylin and eosin staining, 10x).

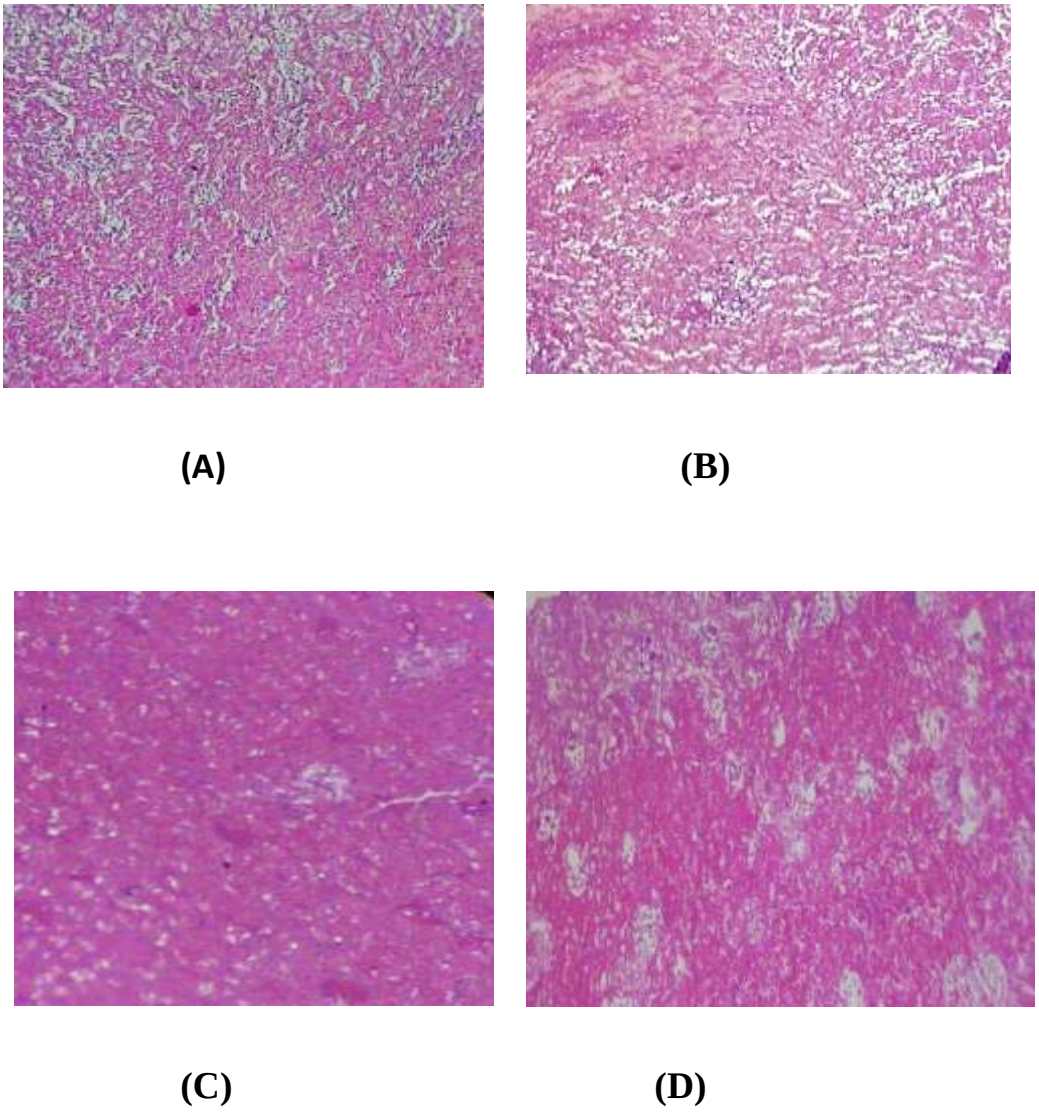

Figure 3: Effect of *S. graggii* on kidney. Rat (A) as control group. Rat (B) treated with 250mg/kg. Rat (C) treated with 500mg/kg and rat (D) treated with 1000mg/kg) of S.graggii, Histological examination by (hematoxylin and eosin staining, 10x).

To give an evidence for the importance of *S. greggii* in the medicinal purpose, investigated the antioxidant and antibacterial activity. The result showed that *S. greggii* seed extract affected the Glutathione (GSH) level which was increased in the rat at the 14th day after administration of extract while decreased in the Malondialdehyde (MDA) level.

The GSH level, as presented in the (Figure 4) was increased in the rat at the 14th day after administration of extract as presented by mean ±SD: (102.71±71.43,108.13±108.69, 118.17±114.43) for 250, 500 and 1000mg/mL shown no significant differences when compared with control group which was (69.47±49.3).

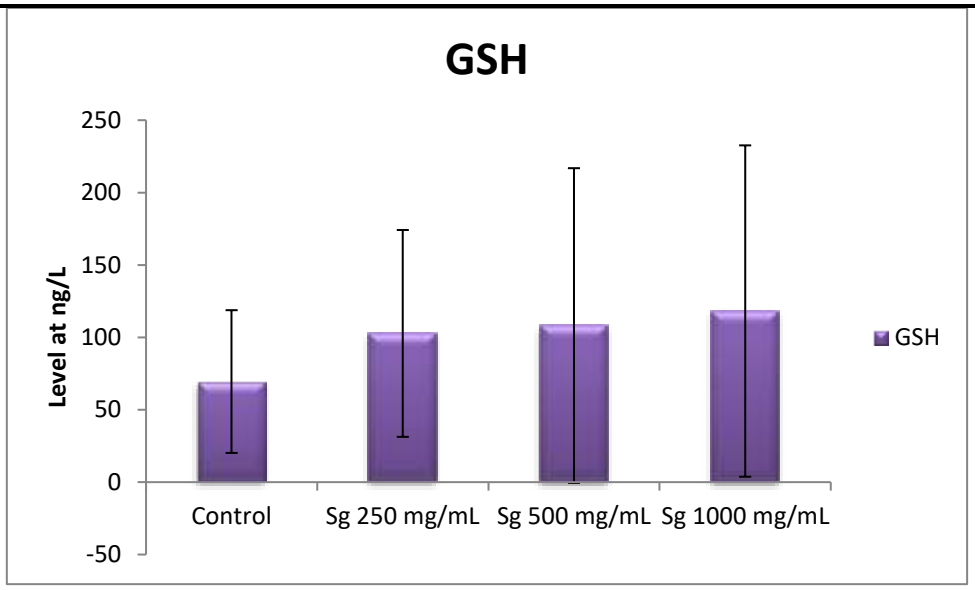

Figure 4: Shown the effect of oral administration of *S. graggii* seed ethanol extract on GSH level of rat at different doses (250,500 & 1000) mg/mL) at 14th day after administration.

While the level of MDA shown (Figure 5) was decreased in the rat at the 14th day after administration of extract as presented by mean ±SD: (73.47±54.05, 60.92±33.09, 52.7±41.39) for 250, 500 and 1000 mg/mL of *S. graggii* shown significant differences (P < 0.05) compared with control group was (84.66±67.58).

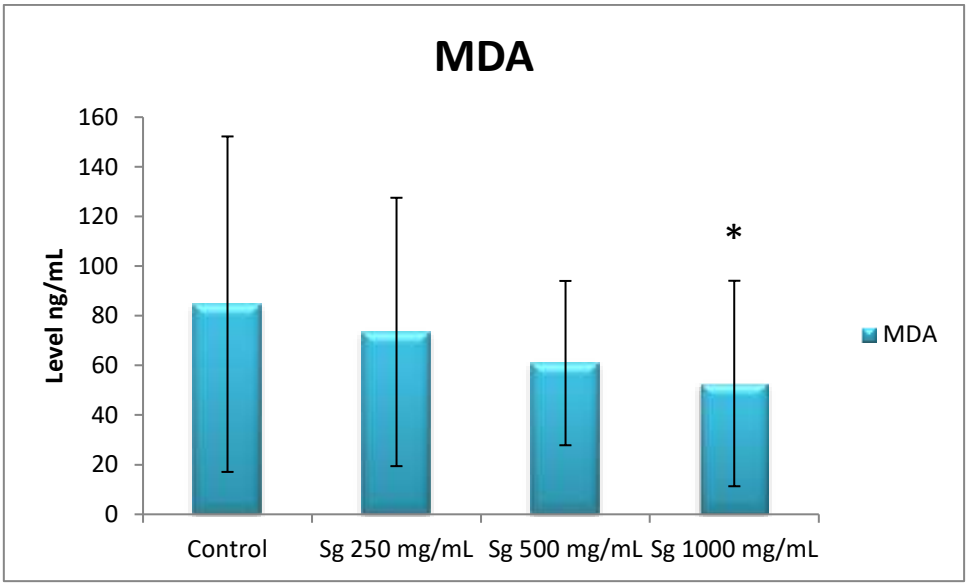

Figure 5: Show the effect of oral administration of *S. graggii* seed ethanol extract on MDA level of rat at different doses (250,500 & 1000) mg/mL) at 14th day after administration. *Significant differences (P value < 0.05).

Discussion

Oxidative stress is caused by a discrepancy between the body's capacity to eliminate reactive metabolites or to repair cell damage and the systemic expression of superoxide anion (13).Cells continuously create reactive oxygen species and free radicals as a result of metabolic processes (ROS). The antioxidant defence system, which is made up of both enzymatic and nonenzymatic antioxidants such

vitamins A, E, and C and glutathione, combats the latter (14) .Oxidative stress (OS) has been linked to the development of a number of illnesses, including diabetes, cancer, atherosclerosis, hypertension, renal disease, and cardiovascular disease (15).

S. graggi is distributed in different countries and have used as food and in medicinal properties so the knowledge of its toxicity is important. To investigate the acute toxicity, seed extract of *S. graggi* was orally administered to rats in different doses for 14 days. On the 14th day, there is no death with the ad administrated to rats and the histological examination of the kidney and liver revealed no toxic signs. The recent findings of phytochemistry of the *Senegalia* genus indicate have compounds less toxic compared with relatives others Acacia species like Faidherbia and Vachellia (16). It is important to give highlight on the toxicity and safety concern of *Senegalia* seeds because for their important uses as food or potential for phytochemical and medicinal properties. *Senegalia* plant species synthesize many compounds or metabolites to survive and protect from environmental Conditions these compounds differ in chemistry, concentration and nutritional contents. The consuming of these compounds as nutrition is diverse in their application in food as to promoting health and nutritional factors or toxins that may be dangerous to the health (17). Senegalia seeds are flatو size about 5 to 15 mm that have waxy hard coated and it is Resistance to hydration and hard to cook So that it is needs several steps for enhancing cooking like soaking, washing and boiling steps to obtain the solution of seeds (18). Senegalia seeds solution have many compounds and nutrition as carbohydrates and proteins so many countries use the solution to enhance their health problem or in promoting Foods in Burkiba Faso and Rajasthan North India State recommended seeds solution for indigenous people with cases of chronic gastrointestinal disorder, diabetes , hypertension and cardiovascular disease (19,20).

The effect of S.g. on the weight was thought for the relatively high nutritional vale of the seed extract as (21) revealed that *Senegalia greggii* seeds may have potential as an supplementary feed. According to the proximate composition, edible Senegalia seeds have the potential to be high in resistant starch, dietary fiber, proteins, and lipids and low in glycemic index foods (22). In addition,(23) demonstrated the modulation of cutaneous innate immunological responses by a high molecular weight arabinogalactan protein (411 kDa) isolated from acacia seeds (24).

Free radicals produced by the human body may raise the risk of chronic illnesses such as cancer and cardiovascular disease. Aerobic respiration is generally the source of these free radicals. Although the human body develops antioxidant enzymes to eliminate free radicals (25) a diet high in edible antioxidants is suggested to help the human body defend itself from free radicals found in food. The purported antioxidant protective benefits against this harmful oxidation-induced damage have attracted increased attention in recent years, particularly in the biological, medical, nutritional, and agrochemical fields. Among natural antioxidant, phenols, secondary metabolites from plants, are the most abundance of natural antioxidants, acting as reducing agents, hydrogen donors, scavengers of free radicals and singlet oxygen quenchers, therefore as cells saviors (26). The result of this study showed that *S. greggii* seed extract affected the Glutathione GSH level which was increased in the rat at the 14th day after administration of extract while decreased in the Malondialdehyde (MDA) level. This result indicated the antioxidant potential for *S. greggii* seed extract and its effect on lessening of inflammation process by enhancing the production scavenging enzyme to remove the effect of oxidative stress and decreased the cell damage. On the other hand, the extract was showed antibacterial activity for *S. aureus*, *E.coli*, and *K. pneumonia*.

The primary purpose of antioxidants is to neutralize free radicals, which are constantly created in the biological system. Reactive oxygen species (ROS) quickly react with oxidizing molecules like carbohydrate, proteins, and lipids, rendering them inactive

and causing damage to cells, tissues, and organs (27). Several defensive systems protect healthy persons from free radicals. Reduced (GSH) is one of the most essential intracellular free radical scavengers. In oxidation processes that result in the generation of GSSG, GSH acts as a reluctant. As a result, lower GSH levels and increasing GSSG levels may indicate that the antioxidant reserve has been depleted (28). MDA may be used to detect lipid peroxidation and oxidative stress. Increased oxidative stress has been linked to a variety of disease types. One of the principal secondary oxidation products of peroxidized polyunsaturated fatty acids, with mutagenic and cytotoxic properties and the potential to contribute in cell death. Since MDA levels have been revealed to be high in a variety of disorders considered to be related to free radical damage, measurement of this biomarker has become the most extensively used method for assessing lipoperoxidation in biological and medical sciences. MDA is also commonly used in the food sciences as a measure of lipid oxidation and rancidity in foods and food products (29).

In previous study investigated the antioxidant effect of many of Acacia species and found that all species have high antioxidant activity. This activity related with its biological activity as antibacterial, antifungal, antiparasitic and anti-inflammation effect. A. nilotica phenol extract increased cellular antioxidant markers (SOD, CAT, GPx, and GSH) while decreasing intrinsic ROS and lead to manage levels glucose in the blood (30).

OS plays a significant part in renal injury, making it a possible target for treatment approaches. Antioxidants may be administered orally or intravenously in a number of attempts to reduce or avoid the inflammatory condition, CVD, and the effects of these events on mortality that follow. Among the most often utilized antioxidants to combat OS are dominant male, vitamin C, and D e (31). Gum Arabs (GA) supplementation has long been thought to have positive effects on CKD patients (32). Chronic renal failure (CRF) has traditionally been treated with GA as part of traditional medicine. It appears to have the capacity to alter human physiological condition in a positive way (33). Its beneficial effects on oxidative stress and inflammatory state have only recently been discovered in clinical trials including CKD patients and animal models (34).

The considerable improvement in creatinine clearance that GA exhibits as part of its protective function against nephrotoxicity suggests favourable effects on renal. Based on research done on animals, the following mechanism of action has been proposed: GA causes CRF patients to excrete more nitrogen in their feces and have lower serum urea concentrations (35). As a result, patients only need two dialysis treatments per week instead of three, which has a significant favourable economically and psychologically impact (36).A prior research from Central Sudan found that supplementing patients on haemodialysis with 50 g of GA per day improved their metabolic profiles (36,37).

In rats with induced hepatotoxicity and CRF, GA dramatically reduced MDA and superoxide generation and elevated GSH and TAC levels .The amino acids in GA may be responsible for its antioxidant potential. Since GA raises TAC levels and lowers oxidative stress indicators in sickle cell anemia patients, Kaddam et al., (2017) revealed a unique effect of GA as an antioxidative agent among people in 2017 (38).

Conclusion: The concluded that the *Sanegalia greggi* seeds ethanol extract was safe at a dose of 1000 mg /kg to be used for medicinal purposes.

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