

Antioxidant and Ameliorative Effects of *Zingiber officinale* Against Aluminum Chloride Toxicity

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Abstract: The present study was performed to assess the antioxidant capacity of different doses of *Zingiber officinale* extract and its efficacy in alleviating the biochemical alterations induced by aluminum chloride in rabbits. Twenty-eight male rabbits were allocated into four groups (7 rabbits in each); Group I: served as normal control, Group II: treated with aluminum chloride (AlCl₃) (150 mg/kg body weight), Group III: treated with AlCl₃ and *Zingiber officinale* extract (100mg/kg. bw) and Group IV: treated with AlCl₃ and *Zingiber officinale* extract (200mg/kg. bw). Rabbits in groups III and IV were orally treated daily with *Zingiber officinale* extract for 4 weeks. Aluminum exposure caused a significant elevation of BUN, creatinine, lipid profile, ALT, ALP, TNF- α , and amylase activity. All these parameters showed the reverse trend following oral *Zingiber officinale* treatment. Aluminum exposure showed a significant decrease in hepatic GSH and catalase activity. Treatment with *Zingiber officinale* extract significantly reversed aluminum effects, in the level of GSH content and hepatic catalase activity. *Zingiber officinale* is effective in alleviating the oxidative stress and inflammation and is thus effective in improving lipid profile and hepatotoxicity and nephrotoxicity in AlCl₃ administration.

Keywords: Aluminum Chloride, *Zingiber officinale*, GSH, Antioxidant, Catalase, TNF- α , ALT

1. Introduction

Plant derived products have been used for medicinal purposes for centuries and also being used in our daily food intake. Drugs of plant source are known to play an important role in the controlling of many diseases. Ginger is one of the world's best-known spices. Ginger (*Zingiber officinale*, Family: *Zingiberaceae*), an herbal drug, produced in South-East Asia and then became prevalent in many ecological areas. Ginger (*Zingiber officinale*) is one of the most widely used spices for the seasoning of food worldwide [1]. The major chemical ingredients of the ginger rhizome are essential volatile oil and non-volatile pungent compounds, such as gingerols, shogaols, paradols and zingerone [2]. The pharmaceutical importance of ginger is due to the presence of alkaloids, glycosides, resins, volatile oils, gums, and tannins etc. The active ingredients usually remain concentrated in the storage organs of the plants [3].

Aluminum is a plentiful element in the earth's layer and is widely distributed throughout the environment. Currently, aluminum salts are included in greasepaints, food handling, and packing also used in various nonprescription drugs [4]. Several authors designate that an excessive and prolonged aluminum exposure directly affects hematological and biochemical parameters, interrupts lipid peroxidation and diminishes the activities of the antioxidant enzymes in plasma and tissues of animals models [5]. This impairment of the physiological prooxidant/antioxidant balance causes oxidative stress.

The serum biochemical profile is a key index that reveals the main organ functions. The liver and kidney are the main organs used for metabolism and excretion. Liver, the vital organ involved in numerous metabolic functions and detoxification of lethal substances, is a frequent target of a

number of toxicants. The disruption in the transport function of the hepatocytes as a result of hepatic damage causes the outflow of enzymes from the liver cytosol into the blood due to altered permeability of membrane [6].

The kidney is a complex organ for its role as an organ of excretion, reabsorption, and general homeostasis, has an extensive blood flow, receiving approximately 1.2 L/min and filtering on average 125ml plasma/min. The processes of reabsorption and secretion, particularly of organic acids and bases, may, however, lead to the accumulation of toxins within the tubules, making this vital organ more susceptible to toxic insults than other organs [7].

The aim of the present study is to appraise the phytochemical characterization of the ethanol extract of ginger and evaluate the antioxidant, anti-inflammatory, hepatoprotective, hypolipidemic and nephroprotective effects against $AlCl_3$ toxicity in rabbits.

2. Materials and Methods

2.1. Chemical

Aluminum chloride anhydrous ($AlCl_3$), M. W. 133.34 was purchased from Aldrich Chemical Company (Milwaukee, WI, USA). All other chemicals and reagents used were of analytical grade.

2.2. Plant Material

Ginger (*Zingiber officinale*) was purchased from a local market of the herbs in Hail city, KSA.

2.3. Preparation of the Extract

10g of ginger powder were placed in the round bottle flask; 100ml of ethanol (70%) were added to the flask. After soaking 12 hours the extract was filtered by using Whatman No. 31 filter paper the filtrate so obtained was placed in the oven to facilitate evaporation of ethanol content [8]. The crude extract was used for further investigation of antioxidant properties.

2.4. Phytochemical Examination

The Phytochemical screening for the presence of alkaloids, tannins, flavonoids, phlobatannins, anthraquinone, coumarins, carbohydrates and terpenoids were carried out according to the methods of [9, 10].

2.5. Determination of Antioxidant Activity of Ginger Extract

2.5.1. Determination of Total Antioxidant Capacity

Total antioxidant capacity of extract was assayed by the phospho- molybdenum method as described by Prieto *et al* [11]

2.5.2. Determination of Reducing Power

The reducing power of extract was determined by the method of Oyaizu [12]

2.6. Experimental Animals

Male white rabbits (Initial weight of 1.00 ± 0.27 kg) were used. All animals received humane care in compliance with the guidelines of the Ethics Committee of the Experimental Animal Care Society, College of Medicine, University of Hail, Saudi Arabia. Animals were individually kept in stainless steel cages. Feed and water were provided *ad libitum*. Rabbits were fed with pellets consisted of Alfalfa pellets 35%; maize broke 15%; barley 15%; white sorghum 10%; sunflower white 5%; sunflower black 5%; wheat 10% and safflower 5%.

2.7. Design of the Experiment

After one week of acclimatization period, 28 mature male rabbits were randomly divided into four equal groups of seven rabbits each. Group I: served as normal control. Group II: administrated with aluminum chloride ($AlCl_3$) (150 mg/kg body weight) were given by intraperitoneal injection [13]. Group III: administrated with $AlCl_3$ (150 mg/kg body weight) by intraperitoneal injection and ginger extract (100mg/kg. bw) by oral gavage. Group IV: administrated with $AlCl_3$ (150 mg/kg body weight) by intraperitoneal injection and ginger extract (200mg/kg. bw) by oral gavage. Rabbits in groups 3 and 4 were orally treated daily with ginger extract for 4 weeks. The doses of ginger and $AlCl_3$ were calculated according to the animal's body weight on the week before dosing.

By the end of the experimental periods (4 weeks), the rabbits were sacrificed at fasting state. The blood samples were collected and allowed to coagulate at room temperature and centrifuged at 3000 r. p. m. for 10 minutes. The clear, non-haemolysed supernatant sera were quickly separated and stored at $-20^\circ C$ for subsequent biochemical analysis.

Liver tissues were quickly excised, weighed and homogenized in a saline solution (0.9%), and centrifuged at 3000 r. p. m. for 15 minute and the supernatant were stored at $-20^\circ C$ for the assay of biochemical parameters related to oxidative stress.

The determination of hepatic catalase activity was assayed as described by Cohen *et al* [14] and hepatic content of reduced GSH was assayed by the spectrophotometric technique according to Sedlack and Lindsay [15].

The following biochemical tests were performed, serum ALT and ALP activities by using clinical test kits (UDI/KSA); serum BUN according to Patton and Crouch [16] and serum creatinine level as per the method of Henry [17]. Serum total cholesterol, triglyceride and HDL-cholesterol were assayed according to [18], [19], [20], respectively, by using clinical test kits (UDI/KSA). Serum LDL-cholesterol and VLDL-cholesterol levels were calculated according to the following formula: LDL-cholesterol = $TC - (TG/5) - HDL\text{-cholesterol}$ [21] and VLDL-C = $TG/5$, respectively [22]. Serum TNF- α was determined by using specific ELISA kit (R&D system) following the manufacturer's instructions.

2.8. Statistical Analysis

The SPSS for Windows, version 18.0 (SPSS Inc., Chicago), was used for the statistical analyses. Results were expressed as mean \pm SE. Statistical analyses were performed using t test. Values of $P < 0.01$ and $P < 0.001$ were considered highly and very highly significant, respectively.

3. Results

3.1. Preliminary Phytochemical Examination

The crude extract of ginger was examined for the most common phytochemical ingredients of medicinal plants for which hepatoprotective activity of other plants has been ascribed. These included; saponins, tannins, flavonols, glycosides, terpenoids, alkaloids, reducing sugars, steroids, proteins, fats, and polyphenols. The results showed that the most abundant phytochemicals were tannins, alkaloids, phenols, vitamins, flavonoids and terpenoids as shown in table 1:

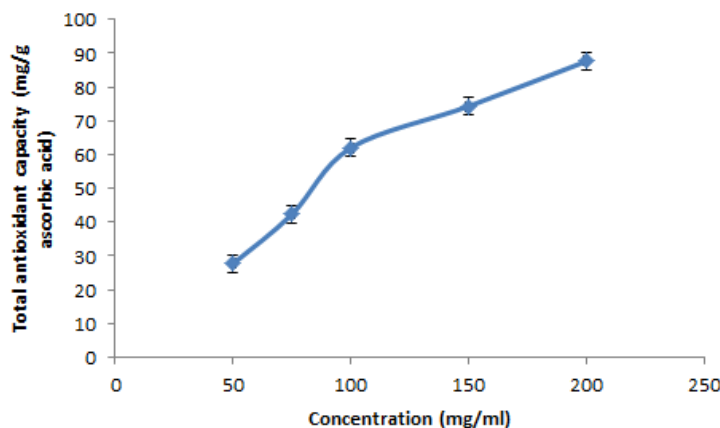
Table 1. Phytochemical screening of ethanolic ginger extract.

Chemical constituents	Ethanolic ginger extract (EGE)
Alkaloids	++
Tannins	+++
Flavonoids	+++
Phlobatannins	+
Anthraquinone	-
Coumarins	++
Carbohydrates	+
Terpenoids	++

+: Presence, +++: Presence in large quantity and -: Absence

3.2. Total Antioxidant Activity and Reducing Power

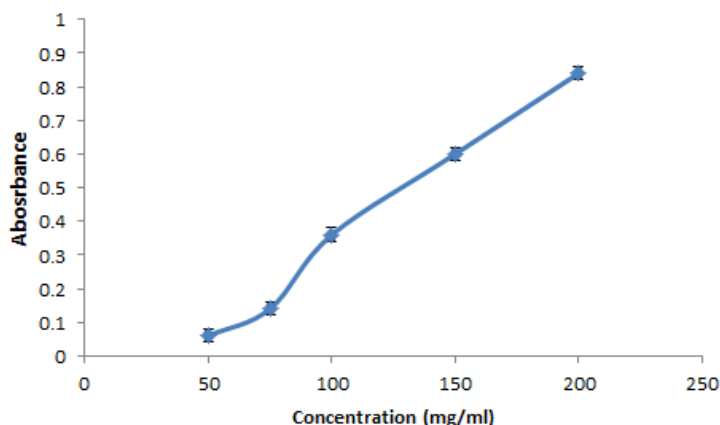
The total antioxidant activity is based on the reduction of molybdate [VI] to molybdate [V] at acid pH and formation of a green phosphate complex, which can be quantified spectrophotometrically at 695 nm. In the current study, as shown in figure 1, the total antioxidant capacity of ginger extracts was demonstrated. The results revealed that the antioxidant activity of the ginger extracts increased with increasing concentration of the ginger extract.



Data are expressed as Mean \pm SE.

Figure 1. Total antioxidant capacity of ginger extract.

On the other hand, the reducing power assays. Increased absorbance of the reaction mixture indicates increased reducing power. Figure 2 shows the dose response for the reducing power of the extract of ginger. The reducing power values were found to be correlated with the concentration of each extract.



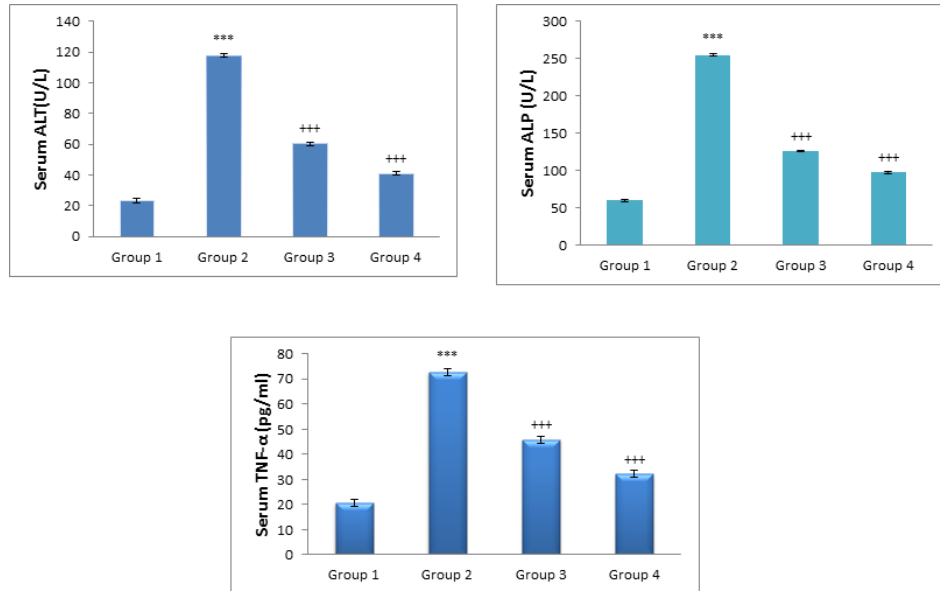
Data are expressed as Mean \pm SE.

Figure 2. Total reduction capacity of ginger extract.

3.3. Biochemical Analysis

As shown in figure 3 AlCl_3 -administered rabbits showed significantly ($P<0.001$) elevation in serum ALT and ALP activities as compared to normal control group. Whereas; a

significant decrease ($P<0.001$) in the serum ALT and ALP activities after treatment by either dose of ginger extract when compared with AlCl_3 treated control group.



Results are expressed as Mean \pm SE.

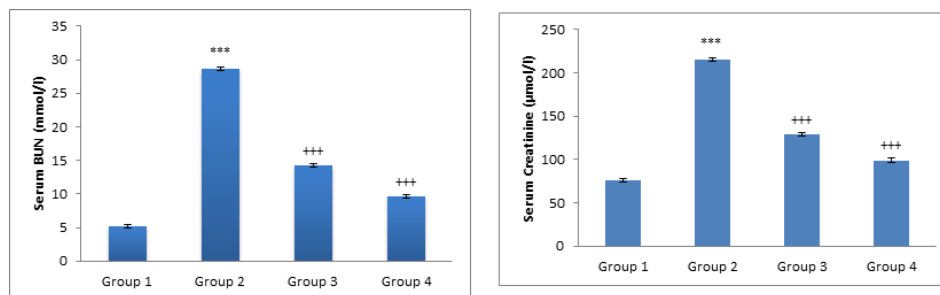
*** $P<0.001$ versus normal control. +++ $P<0.001$ versus AlCl_3 control

Figure 3. Serum ALT and ALP activities and TNF- α level in control and different treated groups.

Also, as shown in figure 3 AlCl_3 administered rabbits showed markedly ($P<0.001$) increased in the level of TNF- α when compared with control group. Treatment of AlCl_3 intoxicated rabbits with either dose of ginger extract (100mg/ml or 200 mg/ml) markedly ($P<0.001$) decrease the level of TNF- α as compared to AlCl_3 control group.

On the other hand, the serum creatinine and BUN levels in

the AlCl_3 treated control group showed a significant ($P<0.001$) increase when compared to the normal control one. The oral administration of ginger extract with either dose produced a marked ($P<0.001$) improvement in the altered serum creatinine and BUN levels of the AlCl_3 intoxicated group Figure 4.



Results are expressed as Mean \pm SE.

*** $P<0.001$ versus normal control. +++ $P<0.001$ versus AlCl_3 control.

Figure 4. Serum BUN and creatinine levels in control and different treated groups.

Data summarized in Table 2 show the effect of AlCl_3 administration and treatment with *ginger* extract on lipid profile. The administration of AlCl_3 produced a marked elevation of lipid profile as showed by the significant ($P<0.001$) elevation in serum total cholesterol, triglycerides, VLDL- cholesterol and LDL-cholesterol levels. Concurrent oral administration of either dose of ginger extract significantly decreased the elevated levels of serum total

cholesterol, triglycerides, VLDL- cholesterol and LDL-cholesterol levels when compared with the AlCl_3 control group. In contrast, a significant ($P<0.001$) decline in the level of HDL- cholesterol was observed in the AlCl_3 -treated group as compared to normal control one. On the other hand, there was significant ($P<0.001$) increase in HDL-cholesterol in ginger treated rabbits when compared to AlCl_3 - treated group Table 2.

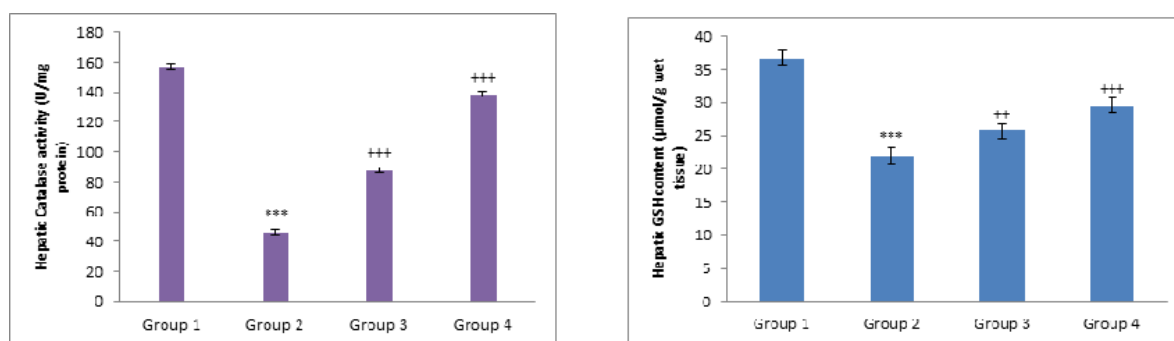
Table 2. Serum lipid profile in control and different treated groups.

Parameters	Group I	Group II	Group III	Group IV
Total cholesterol (mg/dl)	102.00 ± 2.95	221.14*** ± 2.55	152.29*** ± 3.48	103.86*** ± 3.22
Triglycerides (mg/dl)	145.29 ± 3.60	246.00*** ± 2.32	172.57*** ± 3.56	121.57*** ± 2.32
HDL- cholesterol (mg/dl)	37.76 ± 1.34	20.71*** ± 0.92	23.57 ± 0.78	31.00*** ± 0.93
LDL-cholesterol (mg/dl)	35.19 ± 3.48	151.23*** ± 2.96	94.20*** ± 3.43	48.40*** ± 3.35
VLDL-cholesterol (mg/dl)	29.06 ± 0.72	49.20*** ± 0.46	34.51*** ± 0.71	24.31*** ± 0.46

***P<0.001 versus normal control. +++P <0.001 versus AlCl₃ control. Data are expressed as (Mean ± SE).

On the contrary, AlCl₃ intoxicated rabbits showed significantly (P<0.001) decrease in the hepatic content of GSH and catalase activity as compared to normal control group. On the other hand, ginger extract treatment with high

dose (200mg/ml) showed a significant (P<0.01) elevation in hepatic catalase activity and a significant (P<0.01) improvement in hepatic content of GSH when compared with AlCl₃ control one Figure 5.



Results are mean ± SE.

***P<0.001 versus normal control. +++P <0.001; **P <0.01 versus AlCl₃ control.

Figure 5. Hepatic catalase activity and hepatic reduced glutathione content in control and different treated groups

4. Discussion

The exploration of medicinal properties of various plants paying attention, since the last couple of decades due to their forceful pharmacological activities, appropriateness to users, economic feasibility, and low toxicity. Recently, there has been an upgrading of finding natural antioxidants, from plant materials to replace synthetic antioxidants because the previous ones are accepted as green medicine to be safe for health controlling whereas the latter ones are quite unsafe and their toxicity is a matter of concern [23]. Natural antioxidants belonging to the higher plants especially the typical compounds, such as vitamins, carotenoids, and phenolics reveal antioxidant activity and they lessen disease-related chronic health problems. It has been denounced that there is an inverse affiliation between antioxidative status and incidence of human diseases such as malignancy, caducity, neurodegenerative disease, and atherosclerosis [24].

In the current study, Phytochemical screening of ethanolic ginger extract showed the presence of alkaloids, phlobotannins, flavonoids, carbohydrates, tannins, coumarins and terpenoids and absence of anthraquinone Table 1. Similar results were obtained in the study by [25], [26] which showed that the phytochemical screening of ginger shows the presence of carbohydrates, alkaloids, saponins, flavonoids, polyphenols and reducing sugars in both aqueous and petroleum ether extracts. As a rich source, Phytochemical and mineral contents ginger can be considered a potential

source of medicinal herb.

Antioxidant activity of the ginger extract was assessed by determination of total antioxidant capacity and determination of reducing power. Antioxidant compounds and its activity are highly dependent on the concentration of the solvent and type of the solvent [27].

Our results revealed that the antioxidant capacity of the ginger extract increased with increasing concentration of the ginger extract. Regarding, the reducing power assay, there is dose response for the reducing power of the ginger extract where increased absorbance of the reaction mixture indicates increased reducing power. Our results are in agreement with [28], [29]. Moreover, previous studies reported that the reducing power of bioactive compounds is associated with antioxidant activity [30].

Transaminases are intracellular enzymes, released into the circulation after injury of hepatocytes [31]. ALT is the most specific indicator of hepatic injury and hepatocellular necrosis. This liver enzyme catalyzes the transfer of alpha-amino group alanine to the alpha-ketoglutaric acid [32].

Exposure to high concentrations of Al can result in its accumulation in the liver and in turn to alterations in the liver function. The current study provoked significant alterations in the activity of ALT in the blood of AlCl₃-treated rabbits which may be a sign of impaired liver function and disorder in the biosynthesis of these enzymes with modulation in the permeability of the liver membrane. These results are in concordance with findings of [33], [34], [35].

ALP is a membrane-bound enzyme related to the transport of several metabolites so it is a profound biomarker for liver disease. In the present study, AlCl_3 caused a significant elevation in the activity of ALP. This observation is in concordance with the earlier findings of [33], [34]. On the other hand, oral administration of AlCl_3 treated rabbits by ginger extract causes a reduction in the serum ALT and ALP activity. These may be attributed to ginger components which may stabilize hepatocytes plasma membrane and prevent transmission of ALT to the extracellular fluid. This finding is in agreement with results of [36].

The current findings showed that administration of AlCl_3 has induced kidney injury and glomerular dysfunction evidenced by the elevated circulating creatinine and blood urea nitrogen levels. These measurements are often regarded as reliable markers of kidney damage [37] and indicate the loss of a majority of kidney function [38]. These elevated assessments are in agreement with the studies of [32], [33]. Concomitant administration of either dose of ginger extract significantly decreased circulating creatinine and blood urea nitrogen levels. These results are in agreement with the previous studies of [39], [40] whose states that presence of polyphenols and flavonoids in the ginger extract might be responsible for the antioxidant nephroprotective activities and the reduction of serum urea and creatinine levels.

AlCl_3 administration induced a significant increase in serum level of $\text{TNF-}\alpha$ which represents an important mediator of inflammatory tissue damage. Studies presented evidence that nephrotoxics could provoke an inflammatory response leading to organ injury [41]. The significantly elevated $\text{TNF-}\alpha$ reflects the degree of inflammation. In a dose-dependent manner, concurrent administration of ginger extract produced a pronounced decline in serum $\text{TNF-}\alpha$, indicating its anti-inflammatory efficacy. This finding is consistent with that of recent studies on ginger supplementation on anti-inflammatory mediators. Zahra *et al* [42] found that ginger extract had an anti-inflammatory effect on elderly knee osteoarthritis patients. Active ingredients of ginger extract decreased $\text{TNF-}\alpha$ expression by inhibiting I-kappa B alpha phosphorylation, nuclear factor-kappa B (NF-k B) nuclear activation, and protein kinase C-alpha translocation

AlCl_3 administration resulted in dyslipidaemic changes, as illustrated by increasing total cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol and a decrease in serum level of HDL-cholesterol. Concurrent oral supplementation of either dose of ginger extract significantly decreased serum levels of total cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol and increased serum HDL-cholesterol levels. The lipid lowering effect of ginger may come from inhibition of hepatic fatty acid synthesis by lowering key enzymes activities in supplying substrates, thus reducing serum levels of cholesterol and triglyceride. Our finding is in line with a previous report [43], [44]. It was recommended by Nabil *et al.* [45] that presence of phytoconstituents like flavonoid inhibits fat accumulation and ameliorates dyslipidemia and increased antioxidant

defense.

Oxidative stress plays a key contributory role in many diseases including liver damage [46]. The body has anti-oxidative mechanisms to alleviate oxidative molecules, control lipid oxidation and preserve these radicals in balance. When free radicals are produced, the body preserves itself from these radicals by endogenous antioxidants [47, 48]. Catalase enzyme is known to play an important role in scavenging reactive oxygen species. CAT decreases the H_2O_2 into water and oxygen to prevent oxidative stress and in maintaining cell homeostasis. Administration of AlCl_3 reduced the activity of antioxidant enzyme catalase in the liver tissue. The reduction in the activity of catalase enzyme reflects the reduced synthesis of this enzyme due to higher intracellular concentrations of Al and/or accumulation of free radicals and that in agreement with [44].

The administration of ginger extract with AlCl_3 repaired the oxidant/antioxidant balance as reflected by the stimulation of the antioxidants enzyme catalase in the liver. These results are in agreement with [43] who showed that Ginger has an ability to increase the intracellular activities of catalase and have synergistically conflict oxidative stress by scavenging free radicals and boosting endogenous antioxidant activities. This may be related to its active components which motivate free radical scavenging activities [49].

Glutathione is an important biofactor produced in all living cells. It forms an important substrate for GPX, GST, and several other enzymes. In addition, GSH plays an important role in hepatic antioxidation and drug metabolism. High intracellular GSH levels lessen damage and stimulate better persistence under conditions of oxidative stress [50]. Reduced glutathione (GSH) constitutes the first line of defense against free radicals. AlCl_3 treatment resulted in a decrease in the hepatic GSH content. These observations are similar to the data reported by [43]. The administration of ginger extract plus AlCl_3 the GSH content was increased. These results are in agreement with [43], [51].

5. Conclusion

Based on the findings of this study, it was accomplished that ginger extract showed promising antioxidant, anti-inflammatory, hepatoprotective, hypolipidemic and nephroprotective effects against AlCl_3 toxicity. The previous ameliorative properties of ginger make it useful as a therapeutic candidate for the treatment of human diseases.

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