

IN VIVO INVESTIGATION OF HEPATOPROTECTIVE ACTIVITY OF *ASTERACANTHA LONGIFOLIA* NEES. ON CCL₄ INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Objectives: This study was aimed to investigate the phytochemical constituents, antioxidant activity and hepatoprotective activity of *Asteracantha longifolia* leaf extract.

Methods: Hepatotoxicity was induced in normal albino rats. Carbon tetrachloride (CCl₄) was administered orally at a dosage of 20 mg/kg body weight in physiological saline for 1 day. Group 1 served as Control. Group 2 served as disease control (CCl₄ 20 mg/kg body weight). Group 3 served as animal treated with the aqueous extract *A. longifolia* (150 mg/kg body weight orally administered for 15 days). Group 4 served as animal treated with the aqueous extract *A. longifolia* (300 mg/kg body weight orally administered for 15 days). Group 5 served as animal treated with standard silymarin drug (20 mg/kg body weight orally administered for 15 days). After completed the experiment, the liver markers, lipid profile, and antioxidants were analyzed.

Results: Phytochemical screening of aqueous extract of *A. longifolia* leaves indicates the presence of flavonoids, tannins, glycosides, phenol, steroids, alkaloids, quinone, saponin, and coumarin. Supplementation of *A. longifolia* significantly restored the liver markers, lipid profile, and antioxidant markers on in CCl₄ induced Wistar albino rats.

Conclusions: The results of our study showed that *A. longifolia* possess significant hepatoprotective and antioxidant activity, probably due to its phytochemicals.

Keywords: *Asteracantha longifolia*, Phytochemicals, Carbon tetrachloride, Antioxidant.

INTRODUCTION

Liver is an organ in the upper abdomen that aids in digestion and removes waste products and worn-out cells from the blood. It is a vital organ present in vertebrate and some other animals, which has a wide range of functions including detoxification and protein synthesis. The liver is our greatest chemical factory, it build complex molecules from simple substances absorbed from the digestive tract, it neutralizes toxins, it manufactures bile which aids fat digestion and removes toxins through the bowels [1]. However, the ability of the liver to perform these functions is often compromised by numerous substances we are exposed to on a daily basis; these substances include certain medicinal agents which when taken in over doses and sometimes when introduced within therapeutic ranges injures the organ [2].

Liver disease is a worldwide problem. Conventional, drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety [3]. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver, so the search for effective hepatoprotective drug continues.

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world [4]. Plants have been used in traditional medicine for several thousand years. In India, it is reported that the traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine [5]. During the last few decades, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world [6]. There are considerable economic benefits in the development of indigenous

medicines and in the use of medicinal plants for the treatment of various diseases [7].

The leaves of *Asteracantha longifolia* [Family: Acanthaceae] have been used in the Indian system of a disease, besides hepatoprotective, diabetes. The roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for various ailments such as jaundice, hepatic know obstruction, rheumatism, inflammation, pain, urinary infections, edema, and gout. The leaves of *A. longifolia* have been used in Indian system of a disease, besides hepatoprotective, diabetes. The plant is to possess antitumor [8], hypoglycemic [9], antibacterial [10], free radical scavenging and lipid peroxidation (LPO) activity [11]. This plant contains a diversity of biologically active compounds such as alkaloids [12], Waxy substances, and gum [13]. Minerals as Ca, Mg, K, Fe, Cu, Zn, Mn, Co, and Cr [14], and phytosterols [15], essential oil, a straight chain ketone [16]. Flavonoids, terpenoids, manganese salts, potassium chloride and sulfate, and fixed oils [17]. The aim of the present work is to investigate the phytochemical constituents, antioxidant activity, and hepatoprotective activity of *A. longifolia* leaf extract on carbon tetrachloride (CCl₄) induced hepatotoxicity.

METHODS

Collection of plant

The plant *A. longifolia* N. leaves were collected from Ayyampet, Thanjavur District.

Drying

The collected plant leaves were allowed for shade drying for 1 week completely. The dried leaves ground into powder which was used for further extraction.

Preparation of aqueous extract

150 g of plant powder *A. longifolia* was mixed with 1200 ml of water and boiled. Boiling was continued until the content was reduced to

one-third and filtered. The filtrate was evaporated to dryness until it reaches a thick paste consistency. Paste from of the extract obtained was used for the experiments. Qualitative analysis of phytochemical constituents [18].

Experimental animals

Healthy adult Wistar strain of albino rats, weighing 100-120 g was used as experimental models. Animals were kept in well-ventilated cages and fed with standard rat chow pellet obtained from Sai Durga Food and Feeds, Bangalore, India and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (approval No: SAC/IAEC/BC/2015/006).

Induction of hepatotoxicity in rats

Hepatotoxicity was induced in normal albino rats. CCl₄ was administered orally at a dosage of 20 mg/kg body weight in physiological saline for 1 day. The induced rats were chosen and grouped for further studies.

Experimental design

Animals were divided into 5 groups for 4 rats. The experimental design given below has been followed for the present study.

Group 1: Control

Group 2: Disease control (CCl₄ 20 mg/kg body weight)

Group 3: Animal treated with the aqueous extract *A. longifolia* (150 mg/kg body weight orally administered for 15 days).

Group 4: Animal treated with the aqueous extract *A. longifolia* (300 mg/kg body weight orally administered for 15 days).

Group 5: Animal treated with standard silymarin drug (20 mg/kg body weight orally administered for 15 days).

At the end of experiment period, the animals were sacrificed by cervical decapitation. The blood was collected and serum was separated. The liver was washed in ice cold saline, homogenized, and it was used for various experiments.

Statistical analysis

The data of results obtained were subjected to statistical analysis and expressed as mean \pm standard deviation. The data were statically analyzed by one-way Analysis of Variance, and $p < 0.05$ was considered to be significant.

Biochemical estimation

The serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) level were assayed using the method of King [19]. The serum protein level was assayed using the method of Lowry *et al.* [20]. Serum bilirubin content was estimated by the method of Malloy *et al.* [21]. Cholesterol was estimated in serum and tissue by the method of Zlatkis *et al.* [22]. Triglycerides were estimated in serum and tissue by the method of Foster and Dunn [23]. The lipid peroxides content was estimated by the method of Ohkawa *et al.* [24]. Superoxide dismutase content was estimated by the method of Misra and Fridovich [25]. The assay of glutathione peroxidase (GPx) was carried out using the method of Rotruck *et al.* [26]. Glutathione (GSH) reductase activity was measured by the method of Carlberg and Mannerivik [27]. Reduced GSH was estimated by the method of Ellman [28].

RESULTS AND DISCUSSION

Medicinal plants are an important source for the therapeutic remedies of various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. Since time immemorial, different parts of medicinal plants have been used to cure specific ailments in India. Now-a-days, there is widespread interest in evaluating drugs derived from plant sources. This interest primarily stems from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects. Natural antimicrobials have been often derived from plants, animal tissues, or microorganisms. The

adverse effect of the drugs available today, necessitates the discovery of new harmless pharmacotherapeutic agents from medicinal plants [29].

The traditional system of continues to be widely practiced in India. It is estimated that 50-75% of the population used traditional drugs, some because of the lack of easy access to drugs of the modern system, but many by deliberate choice on account of their faith in them. Furthermore, there is a strong belief that the systems possess drugs for certain conditions, particularly chronic condition for which the modern system offers inadequate or no remedies. However, the safety of any medicinal plants cannot be taken for granted merely because they have this sanction of centuries of use. Hence, it is necessary to confirm their safety by toxicity studies and activity by pharmacological and biochemical studies. This fact has led to detailed chemical and pharmacological investigation and general biochemical screening programs of these plants not only in India but all over the world [30].

Table 1 represents the qualitative analysis of methanol and aqueous extract of leaves of *A. longifolia*. Phytochemical screening revealed the presence of flavonoids, tannins, glycosides, phenol, steroids, alkaloids, quinone, saponin, and coumarin in both the leaves extract.

Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom. Flavonoids are hepatoprotectives. Some isoflavones widely used in insecticides. They might also play a role in disease resistance. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity [31]. As antioxidant flavonoids from the plant provide anti-inflammatory activity.

Alkaloids are very important in medicine and constitute most of the valuable drugs. They have marked physiological effect in animals [32]. Alkaloids such as soladine have been indicated as a starting material in the manufacture of steroidal drugs [33]. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antibacterial effects.

Glycosides are certain molecule in which a sugar part is bound to some other part. Glycosides play numerous important roles in living organisms. Many plants store important chemicals in the form of inactive glycosides. If these chemicals are needed, the glycosides are brought in contact with water and an enzyme and the sugar part is broken down, making the chemical available for use. Many such plant glycosides are used as medications. In animals (including humans), poisons are often bound to sugar molecules to remove them from the body [34].

Tannins are well-recognized due to their hepatoprotective action [35]. Tannins were reported to exhibit antidiabetic [36], anti-inflammatory, antibacterial, and antitumor activities. It has also been reported that certain tannins were able to inhibit HIV replication selectively besides use as diuretics. Coumarin is used in certain perfumes and fabric conditions. Coumarin has been used as an aroma enhancer in pipe tobaccos and certain alcoholic drinks, although in general it is banned

Table 1: Phytochemical screening of plant extract of *A. longifolia* leaves

S. No	Components	Methanol extract	Aqueous extract
1.	Terpenoid	-	-
2.	Flavonoid	+	+
3.	Steroid	+	-
4.	Glycoside	+	+
5.	Alkaloid	+	-
6.	Quinone	+	+
7.	Phenol	+	+
8.	Tannin	+	+
9.	Saponin	-	+
10.	Coumarin	+	+

-: Absence, +: Presence, *A. longifolia*: *Asteracantha longifolia*

as a flavoring food additive, due to concerns regarding its hepatotoxicity in animal models.

It has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl₄ or by inhibition of LPO induced by CCl₄. Phytoconstituents, such as flavonoids and triterpenoids, are known to possess hepatoprotective activity [37].

Table 2 represents the effect of *A. longifolia* leaves on the liver marker enzymes AST (113.50±1.44) were markedly elevated in CCl₄ treated animals, indicating liver damage. Administration of *A. longifolia* leaves extract decrease the activities in Group III (150 mg/kg) 74.50±1.32 and Group IV (300 mg/kg) 49.50±1.04 to near normal, and it is closer with silymarin treated group 52.25±1.25. Analysis of AST showed a significant increase in the CCl₄ treated rats. Treatment with *A. longifolia* leaves extract (150 mg/kg) and (300 mg/kg) significantly (p>0.05) prevented the increase in AST level which was brought to near normal.

ALT (97.25±1.38) was markedly elevated in CCl₄ treated animals. Indicating liver damage administration of *A. longifolia* leaves extract decrease the activities of Group III ALT (150 mg/kg) 62.50±1.19 and Group IV (300 mg/kg) ALT 40.25±1.49 to near normal, and it is closer with silymarin treated groups of ALT 40.50±0.65. Analysis of ALT showed a significant increase in the CCl₄ treated rats. Treatment with *A. longifolia* leaves (150 mg/kg) and (300 mg/kg) significantly (p>0.05) prevented the increase in ALT level which was brought to near normal.

The changes in the marker enzyme levels will reflect in hepatic structural integrity. The rise in the AST is usually accompanied by an elevation in the levels of ALT which play a vital role in the conversion of amino acids to keto acids. This plant might have potent hepatoprotective activity. The elevated serum enzyme levels, such as AST and ALT, are indicative of cellular leakage and functional integrity of cell membrane in liver [38]. Treatment with *A. longifolia* decreases the serum levels of AST and ALT toward their respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl₄.

ALP (309.50±2.50) was markedly elevated in CCl₄ treated animals. Indicating liver damage administration of *A. longifolia* leaves extract decreases the activities of Group III (150 mg/kg) ALP 212.50±2.10 and Group IV (300 mg/kg) ALP 121.50±2.60 to near normal, and it is closer with silymarin treated group of ALP 118.75±1.11. Analysis of ALP showed a significant increase in the CCl₄ treated rats. Treatment with *A. longifolia* (150 mg/kg) and (300 mg/kg) significantly (p>0.05) prevented the increase in ALP level which was brought to near normal.

The ALP is widely distributed throughout the body, and clinically important for diagnostic reason in bone, liver, placenta, and intestine. Increase in the serum level of ALP is due to increase synthesis in presence of increasing biliary pressure [39].

Table 3 indicates that the oral administration of CCl₄ causes a significant decrease in the protein (3.93±0.03) in serum administration of *A. longifolia* to CCl₄ intoxicated animals increase the serum protein Group III (150 mg/kg) 5.94±0.02 and Group IV (300 mg/kg) 6.85±0.05 to near normal, and it is closer with silymarin treated group 6.48±0.09.

The liver is the largest organ in the vertebrate body and the site for intense metabolism. In living system, liver is considered to be highly sensitive to toxic agent. The liver is an important site for protein synthesis. CCl₄ is commonly used in animal models for the screening of hepatoprotective drug because it damages liver tissue. Rate of protein synthesis decrease in liver damage induced by CCl₄. The rise in serum protein has been attributed to the damage in structural integrity of liver [40].

Table 4 reveals that the levels of bilirubin in the serum of the control and experimental groups of albino rats. The CCl₄ intoxicated group shows the increased level of bilirubin when compared to normal. The levels are significantly decreased due to the administration of *A. longifolia* leaves extract when compared to silymarin treated groups. Bilirubin is an endogenous organic anion, which binds reversible to albumin and is transported to the liver. When it is conjugated to glucuronic acid and excreted in bile [41]. Bilirubin assay is a sensitive test to substantiate the functional integrity of the liver and severity of necrosis. Bilirubin also measures the binding, conjugating, and excretory capacity of hepatocytes and is proportional to the erythrocyte degradation rate [42].

Table 5 reveals that the levels of triglycerides in the serum of the control and experimental groups of albino rats. The CCl₄ intoxicated group shows the decreased level of serum triglycerides when compared to normal. The levels of significantly increased due to administration of *A. longifolia* N. extract when compared to silymarin treated groups.

Table 6 reveals that the levels of cholesterol in the serum of the control and experimental groups of albino rats. The CCl₄ intoxicated group shows the decreased level of cholesterol when compared to normal. The levels of significantly increased due to administration of *A. longifolia* N. extract when compared to silymarin treated groups.

Table 2: Effect of aqueous extract of *A. longifolia* on hepatic marker enzymes in CCl₄ induced hepatotoxic rats

Particulars	AST (U/L)	ALT (U/L)	ALP (U/L)
Group 1	40.75±1.25*	34.50±1.32*	96.25±1.93*
Group 2	113.50±1.44 [#]	97.25±1.38 [#]	309.50±2.50 [#]
Group 3	74.50±1.32*	62.50±1.19*	212.50±2.10*
Group 4	49.50±1.04*	40.25±1.49*	121.50±2.60*
Group 5	52.25±1.25*	40.50±0.65*	118.75±1.11*

Data are expressed as mean±SEM (n=6), *p<0.05 when compared with disease control, [#]p<0.05 statistically significant when compared with normal and treated group, CCl₄: Carbon tetrachloride, SEM: Standard error mean, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, *A. longifolia*: *Asteracantha longifolia*

Table 3: Effect of aqueous extract of *A. longifolia* on the level of protein in CCl₄ induced hepatotoxic rats

Particulars	Protein (g/dl)
Group 1	7.24±0.02*
Group 2	3.93±0.03 [#]
Group 3	5.94±0.02*
Group 4	6.85±0.05*
Group 5	6.48±0.09*

Data are expressed as mean±SEM (n=6), *p<0.05 when compared with disease control, [#]p<0.05 statistically significant when compared with normal and treated group, CCl₄: Carbon tetrachloride, SEM: Standard error mean, *A. longifolia*: *Asteracantha longifolia*

Table 4: Effect of aqueous extract of *A. longifolia* on level of bilirubin in CCl₄ induced hepatotoxic rats

Particulars	Bilirubin (mg/dl)
Group 1	0.77±0.01*
Group 2	3.84±0.05 [#]
Group 3	1.08±0.02*
Group 4	0.97±0.02*
Group 5	1.06±0.01*

Data are expressed as mean±SEM (n=6), *p<0.05 when compared with disease control, [#]p<0.05 statistically significant when compared with normal and treated group, CCl₄: Carbon tetrachloride, SEM: Standard error mean, *A. longifolia*: *Asteracantha longifolia*

Damage of liver cell is reflected by an increase in the levels of hepatospecific enzymes; these are cytoplasmic and are released into circulation after cellular damage [43]. In this study, significant increase in the total bilirubin content and in the serum glutamic oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and ALP activities in the CCl₄ treated group could be taken as an index of liver damage.

Tables 7 and 8 show the activity of the antioxidant enzymes and non-enzyme. A significant decrease in the LPO, GPx, and GSH reductase

Table 5: Effect of aqueous extract of *A. longifolia* on the level of triglycerides in CCl₄ induced hepatotoxic rats

Particulars	Triglycerides (mg/dl)
Group 1	183.75±1.93*
Group 2	76.00±1.58 [#]
Group 3	126.73±1.70*
Group 4	181.25±1.11*
Group 5	157.75±1.11*

Data are expressed as mean±SEM (n=6), *p<0.05 when compared with disease control, [#]p<0.05 statistically significant when compared with normal and treated group, CCl₄: Carbon tetrachloride, SEM: Standard error mean, *A. longifolia*: *Asteracantha longifolia*

Table 6: Effect of aqueous extract of *A. longifolia* on level of cholesterol in CCl₄ induced hepatotoxic rats

Particulars	Cholesterol (mg/dl)
Group 1	163.75±1.75*
Group 2	67.75±1.31 [#]
Group 3	153.00±1.96*
Group 4	161.25±1.49*
Group 5	140.50±0.65*

Data are expressed as mean±SEM (n=6), *p<0.05 when compared with disease control, [#]p<0.05 statistically significant when compared with normal and treated group, CCl₄: Carbon tetrachloride, SEM: Standard error mean, *A. longifolia*: *Asteracantha longifolia*

Table 7: Effect of aqueous extract of *A. longifolia* on the LPO and glutathione reductase in CCl₄ induced hepatotoxic rats

Particulars	LPO (nM of MDA formed/g of tissue)	Reduced glutathione (µg/g tissue)
Group 1	21.83±1.83*	26.33±0.15*
Group 2	169.17±0.57 [#]	10.41±0.18 [#]
Group 3	123.17±1.29*	14.24±0.33*
Group 4	52.17±0.57*	19.65±0.02*
Group 5	87.17±1.26*	22.04±0.16*

Data are expressed as mean±SEM (n=6), *p<0.05 when compared with disease control, [#]p<0.05 statistically significant when compared with normal and treated group, CCl₄: Carbon tetrachloride, SEM: Standard error mean, *A. longifolia*: *Asteracantha longifolia*, LPO: Lipid peroxidation

Table 8: Effect of aqueous extract of *A. longifolia* on antioxidant enzyme activity in CCl₄ induced hepatotoxic rats

Particulars	GPx (µg of GSH hydrolyzed/g tissue/minutes)	SOD (mg of epinephrine oxidized/g tissue)	Glutathione reductase (µg of GSH produced/g tissue/minute)
Group 1	0.84±0.02*	9.64±0.58*	3.82±0.02*
Group 2	0.24±0.018 [#]	213.83±2.92 [#]	1.04±0.02 [#]
Group 3	0.65±0.01*	86.52±2.33*	0.05±0.01*
Group 4	0.80±0.01*	56.67±0.92*	2.14±0.01*
Group 5	0.81±0.01*	65.20±0.34*	2.76±0.03*

Data are expressed as mean±SEM (n=6), *p<0.05 when compared with disease control, [#]p<0.05 statistically significant when compared with normal and treated group, GPx: Glutathione peroxidase, GSH: Glutathione, SOD: Superoxide dismutase, CCl₄: Carbon tetrachloride, SEM: Standard error mean, *A. longifolia*: *Asteracantha longifolia*

were observed in CCl₄ treated groups, which was prevented with the treatment of the plant extracts Group III (150 mg/kg) LPO 123.17±1.29, GPx 0.65±0.01, and GSH reductase 1.05±0.01. Group IV (300 mg/kg) LPO 52.17±0.57, GPx 0.80±0.01, and GSH reductase 2.14±0.01, to near normal, and it is closer with silymarin treated groups. Superoxide dismutase (SOD), reduced GSH significant increase in the antioxidant enzyme non-enzyme were observed in CCl₄ treated groups, which was prevented with the treatment of the plant extracts Group III (150 mg/kg) SOD 86.52±2.33 and reduced GSH 14.24±0.33. Group IV (300 mg/kg) SOD 56.67±0.92 and reduced GSH 19.65±0.02 to near normal, and it is closer with silymarin treated groups.

The increase in LPO level in induced by CCl₄ suggests enhanced LPO leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with *A. longifolia* significantly reverses these changes. Hence, it is likely that the mechanism of hepatoprotective of *A. longifolia* is due to its antioxidant effect.

GSH is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals, and maintains membrane protein thiols. Furthermore, it is substrate for GPx [44]. Decreased level of GSH is associated with an enhanced LPO in CCl₄ treated rats.

GSH is the most important intracellular thiol antioxidant and a major determinant of the intracellular redox status. Intracellular GSH levels and GSH redox status play a central role in regulating a wide variety of cell responses, including signal transduction, immune regulation, maintenance of protein structure cell proliferation, and apoptosis [45]. The GSH antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species [46].

GPx is a selenium dependent enzyme has high potency in scavenging reactive free radicals. The levels of GPx activity in liver were elevated during alcohol intoxication to compensate the free radical scavenging effect utilized by the GSH. The levels of antioxidant enzymes return to normal by treated with *A. longifolia* extract. This result indicates that the herbal drug promoted the hepatoprotection by elevating free radical scavenging activity. It was also observed in silymarin treated rats [47].

Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live injury [48]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. In *A. longifolia* causes, a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

SOD plays an important role in eliminating reactive oxygen species formed during bioactivation of xenobiotics in hepatic tissues. The SOD is easily inactivated by lipid peroxide or reactive oxygen species [49]. SOD major scavenging enzymes that remove the toxic-free radical *in vivo*. Reduced activities of SOD in liver, kidney, and pancreas have been observed during diabetes and a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Similarly, oral administration of *Hygrophila auriculata* to streptozotocin treated rat showed SOD level were increased [50].

This study confirmed that the leaves of *A. longifolia* possess various phytoconstituents which may contribute its antioxidants and hepatoprotective activity. This study paved a way for the future investigations to analyze and to isolate a novel pharmacologically active compound from the leaves of *A. longifolia* which is used for treating infectious disease and metabolic disorder.

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