The *in vitro* hepatoprotective activity of the flower extract of *Senna alata* against Carbon tetra chloride induced liver damage in rats.

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Abstract : The present investigation has been carried out to evaluate the *in vitro* hepatoprotective activity of different concentrations of *Senna alata* flower extract (100, 250 and 500µg/ml). The *in vitro* hepatoprotective activity was evaluated using estimation of Malondialdehyde , activity of GOT (AST), activity of GPT (ALT) and determination of reduced glutathione (GSH). Ethanolic extract showed the highest hepatoproductive activity when compared to standard due to the presence of phytochemicals present in the flower extract.

InterTerms: Hepatoproductive activity, *Senna alata* , Malondialdehyde, GOT,ALT,

1. INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. And it functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. The bile secreted by the liver has, among other things, plays an important role in digestion. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual (Smuckler, 1975). Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, Carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes are common. Enhanced lipid per oxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis (Agarwal, 2001).

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well documented uses of plantproducts is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent (Ward and Daly, 199). In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell. Many formulations containing herbal extracts are sold in the Indian market for liver disorders (Achuthan *et al*., 2003).

1.1Liver Diseases and Medicinal Plants:

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs

and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailment. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune/disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis (Tortora and Grabowski2003). Among various chemical agents, carbon tetrachloride (CCl_4) has been thoroughly studied for its hepatotoxic properties. Various hepatoprotective agents have been studied to observe the beneficial effects against the chemically induced liver injury produced by carbon tetrachloride. CCl_4 is a well known model compound for producing chemical hepatic injury because it has been administered to humans in vehicles ranging from shampoo to a drug against hook worm. It was even used as an anesthetic and as an analgesic. The inhalation exposure to carbon tetrachloride is the most frequent cause of poisoning by this chemical in industry. It may be readily absorbed through the skin, but while it may also be readily absorbed from the gastrointestinal tract, this is seldom a major problem in industry. The human and experimental toxicology of carbon tetrachloride has been studied for many years and there is an extensive literature on this work. It is known to cause damage to the liver, lungs, kidneys, adrenals and central nervous system in humans and experimental animals. Possible mechanisms of its toxicity have been reviewed (Anders and Jakobson, 1985)

The potentially reactive oxygen species, as ROS, such as superoxide radical($O^{\bullet}_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), are continuously generated inside the human body as a consequence of the exposure to exogenous chemicals in our environment and/or to a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer (Halliwell and Gutteridge, 2007). Under normal circumstances, the ROS generated are detoxified by the antioxidant defenses leading to equilibrium between these two processes. However, owing to ROS overproduction and/or inadequate antioxidant defenses, this equilibrium is hampered, thus favoring as urge of ROS that culminates in oxidative stress. The ROS readily attack and induce oxidative damage to several biomolecules including proteins, lipids, lipoproteins and DNA (Valko *et al*.,2007; Seifried *et al*.,2007), contributing to the development to various diseases such as atherosclerosis, diabetes, cancer, neurodegenerative diseases, hepatic diseases and the ageing process (Halliwell and Gutteridge, 2007; Seifried *et al*., 2007).

To prevent the damage caused by ROS, living organisms have developed an antioxidant defense system that includes the presence of non enzymatic antioxidants (e.g.glutathione, uric acid, bilirrubin, and vitamins C and E) and enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Valko *et al*., 2007). It has been proposed that in addition to these natural antioxidant systems, other synthetic or natural ROS scavengers may reduce the incidence of

free radical-mediated diseases. The use of antioxidants in the prevention and cure of various diseases is expanding, and there is considerable interest in the study of the antioxidant activities of molecules such as plant polyphenolic and carotenoid components (Valko *et al*., 2007; Fang *et al*., 2002).

In the absence of reliable hepatoprotective drugs in modern medicine, a large number of phytochemicals and extracts prepared from folk medicinal plants with proven hepatoprotective properties, could be an alternative in the treatment to liver diseases resulting from high alcohol consumption, exposure to xenobiotics and therapeutic agents and other factors leading to the chronic liver diseases which are very often related to oxidative stress (Gurtsevitch, 2008; Farrell and Larter, 2006; Albano, 2006; Otani *et al*.,2005).

1.2.Cellular damage and lipid peroxidation:

Lipid peroxidation is a highly destructive process and alters the structure and function of cellular membrane (Kale and Sitasawad , 1990). It is involved in a number of diseases and in poisoning of several toxins (Halliwell B and Gutteridge, 1989). Disrupted tissues are known to undergo lipid peroxidation at a faster rate than normal ones. Lipid peroxidation, therefore, can be used as a measure of oxidative damage. Peroxidation brings about change in structure, fluidity and permeability of membranes (Nakazawa and Nagatsuka, 1980; Srivastava *et al*., 1998) inactivates a number of membrane bound enzymes and protein receptors (Yukawa and Naga suka, 1983), induces swelling and alterations of respiratory functions; causes loss of -SH groups from the membrane-bound proteins; mediates DNA damage; and alters RNA transport from nucleus to cytoplasm (Yannarelli and Ward, 1982).

$CC1_4$ is activated by CYP2E1 (Cytochrome P2E1), CYP2B1 or CYP2B2, CYP2A and possibly CYP3A to its intermediate trichloromethylradical $CCl_3^{\bullet}$ and further converted to trichloromethylperoxy radical ($CCl_3O_2^{\bullet}$), a highly reactive species in the presence of oxygen (Weber *et al*., 2003). $CCl_3^{\bullet}$ binds to cellular molecules such as nucleic acid, lipid and protein, impairing very important cellular activities, while $CCl_3O_2^{\bullet}$, a more reactive radical, reacts with polyunsaturated fatty acids (PUFA) to propagate a chain reaction, leading to lipid peroxidation which attacks and destroys poly unsaturated fatty acids, or binds covalently to lipids and proteins, resulting in the destruction of cell membranes and also induction of liver damage. Increased oxidative stress generally describes a condition in which cellular antioxidant defenses are inadequate to completely inactivate the reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated because of excessive production of ROS/RNS (Sevanian and Ursini, 2000).

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlrophyll’s etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment (Kotnis *et al*.,

2004). Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc. (Ashis, 2003). In the present study the chosen medicinal plant *Senna alata* is belonging to the family of Fabaceae. *Senna alata* is widely used as a traditional medicine, particularly valued for its laxative effect and its effective treatment of several skin conditions, including ringworm and scabies. Research has tended to confirm the validity of these traditional treatments.A number of anthraquinone derivatives have been isolated from the leaves, such as aloe-emodin, chrysophanol, isochrysophanol and rhein, as well as the alkaloid tyramine and the common steroid beta-sitosterol.Crude leaf extracts have shown antibacterial activity against a range of bacteria (such as Dermatophilus congolensis, which causes a serious skin condition in cattle), antifungal properties (such as against Pityriasis versicolor in humans), and also antitumour activity].The bark contains tannins.The petals contain anthraquinones, glycosides, steroids, tannins and volatile oil.

In the present investigation deals with in vitro hepatoprotective activity and comprehensive phytochemical studies on root of this plant, including fluorescence analysis, as well as behaviour of root powder with different chemical reagents and histochemical analysis.

2. MATERIALS AND METHODS

2.1Preparation of flower extract

Fresh flower (2Kg) of *Senna alata* collected from Thanjavur district during April were extracted with 85% methanol (4X500ml) under reflux. The alcohol extract was concentrated *in vacuo* from the aqueous concentrate different concentrations of *Senna alata* flower extract (100, 250 and 500µg/ml) was chosen for *in vitro* **hepatoprotective** activity. Acarbose was used as the standard.

2.2..*In vitro* hepatoprotective activity

The livers were excised and weighed in a tared beaker of cold calcium-free Locke's solution. Sufficient solution was .removed to give a ratio of 1 g of liver to 10 ml of final suspension. The liver and solution were then transferred to a homogenizer tube, and the liver broken up by pressing down with a loose-fitting lucite pestle. This was followed by twenty even up and-down strokes by hand. Shreds of connective tissue containing many cells remained after this treatment, but they were readily

removed by straining through bolting silk. Experience has shown that further homogenization to release more whole cells. The isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% newborn calf sample, antibiotics, dexamethasone and bovine insulin. The cell suspension was incubated at 37 ^{o}C for 30 min in a humidified incubator under 5% CO_2. After incubation of 24 hrs, the hepatocytes were exposed to the fresh medium containing CCl_4 (1%) along with different concentration of *Sena alata* flower extract (100, 250 and 500µg/ml). After 60 min of CCl_4 intoxication, the hepatic markers ALT, AST and protein and oxidative markers MDA and GSH were determined.

Experimental Design: Five different groups taken for the study.

Group I : Normal

Group II : Carbon tetrachloride treated alone

Group III : Carbon tetrachloride + 100mg *Senna alata* flower extract treated

Group IV : Carbon tetrachloride + 250mg *Senna alata* flower extract treated

Group V : Carbon tetrachloride + 500mg *Senna alata* flower extract treated

2.3.BIOCHEMICAL ESTIMATIONS

Estimation of Malondialdehyde (MDA/LPO):

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978).

Procedure:

The sample was combined with 2.0ml of TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated for 15minutes in a boiling water bath. The flocculants centrifuged at 1000 ×g for 10 minutes. The absorbance of the sample was read at 535nm against a blank without sample.

Values were expressed as nmol of MDA formed/L

Activity of GOT (AST):-

The GOT was estimated by the method of and Reitman Frankel (1957)

Procedure:-

0.1 ml of sample was mixed with 0.5 ml of substrate reagent and incubated for 60 minutes at 37°C, 0.5 ml of colour reagent was added and further incubation for 20 minutes at 37° C. After the incubation, added 3.0 ml of alkaline reagent stopped the reaction and the colour intensity was read at 505 nm.

The GOT activity was expressed as IU/L

Activity of GPT (ALT):-

The GPT was estimated by the method of Reitman and Frankel *(*1957)

Procedure:-

0.1 ml of sample was mixed with 0.5 ml of substrate reagent and incubated for 60 minutes at 37° C; 0.5 ml of colour reagent was added and further incubation for 20 minutes at 37° C. After the incubation, added 3.0 ml of alkaline reagent stopped the reaction and the colour intensity was read at 505 nm.

The GPT activity was expressed as IU/L

Determination of Reduced glutathione (GSH):

Reduced glutathione was estimated by method of Moron *et al* (1979).

Procedure:

0.5ml of serum sample was precipitated with 1ml of 10% TCA and the precipitate was removed by centrifugation. To 0.5ml of the supernatant 1ml of DTNB was added and the total volume was made up to 3ml with phosphate buffer. The absorbance was read at 412nm.

The level of glutathione was expressed as mg/dl in serum

Statistical Analysis

The results were presented as Mean ± SD. Data was statistically analyzed using student "t" test. P.values set as lower than 0.05 were considered as statistically significant.

3. RESULTS AND DISCUSSION

The present study was carried out to evaluate the *In vitro* antioxidant activity of *Senna alata* flower on CCl_4 induced hepatotoxicity. The observations made on different groups of experimental and control animals were compared as follows.

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($^{\bullet}CCl_3$). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloro methylperoxyl free radical leads to initiate the process of lipid peroxidation., the destruction of Ca^{2+} homeostasis, and finally, results in cell death (De Groot and Noll, 1986; Clawson, 1989; Recknagel *et al*., 1989). These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage (Recknagel and Glende, 1973; Reckengel *et al*., 1991; Wolf *et al*., 1980). MDA is a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions (Ray and Husain, 2002). Hepatotoxic compounds like CCl_4 are known to cause marked elevation in MDA content. In the present study, treatment with *Senna alata* flower attenuated the increased content of MDA in hepatocyte.

Table 1 Effect of *senna alata* on MDA in experimental hepatocyte

Groups	MDA (nmole/dl)
I	5.24±1.03
II	12.01±0.90
III	8.26±1.06
IV	7.31±0.70
V	6.58±0.60

Values were expressed as mean ± SD for triplicate in each group.

Fig 1 Effect of *Senna alata* on MDA in experimental hepatocyte

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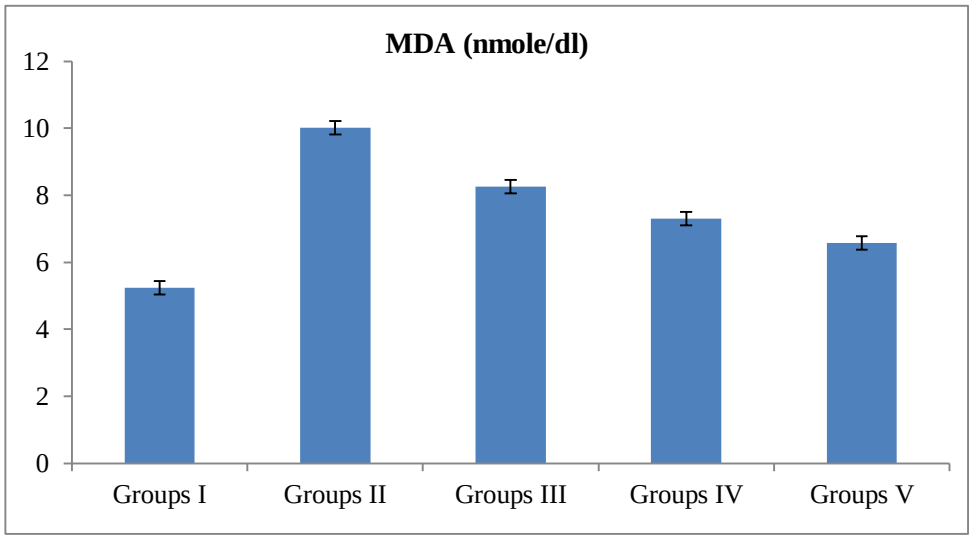


In the assessment of liver damage by carbon tetrachloride, the determination of enzyme activities such as aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) is largely used. Activities of AST and ALT are the most frequently utilized indicators of hepatocellular injury. Necrosis or membrane damage releases the enzymes into circulation; and therefore, they can be measured in hepatocyte.. Elevated levels of hepatocyte enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver (Wolf, 1999). The mechanism by which transaminase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions and the other hypothesis is that the damaged liver fails to excrete transaminase made in the liver (Thapa and Walia, 2007). Total protein leave on other hand, are related to the function of hepatic cells i.e they reveal the functional status of the hepatic cells. Decreased levels of total protein and albumin are indicative of the failure of the biosynthetic function of the hepatocyte (Crawford, 2004).

In the present study, the CCl_4 treated hepatocyte showed a significant elevation (Table- 2 and fig- 2) in the activities of ALT, AST, and significantly decreasing the levels of total GSH (Table – 2 and fig-

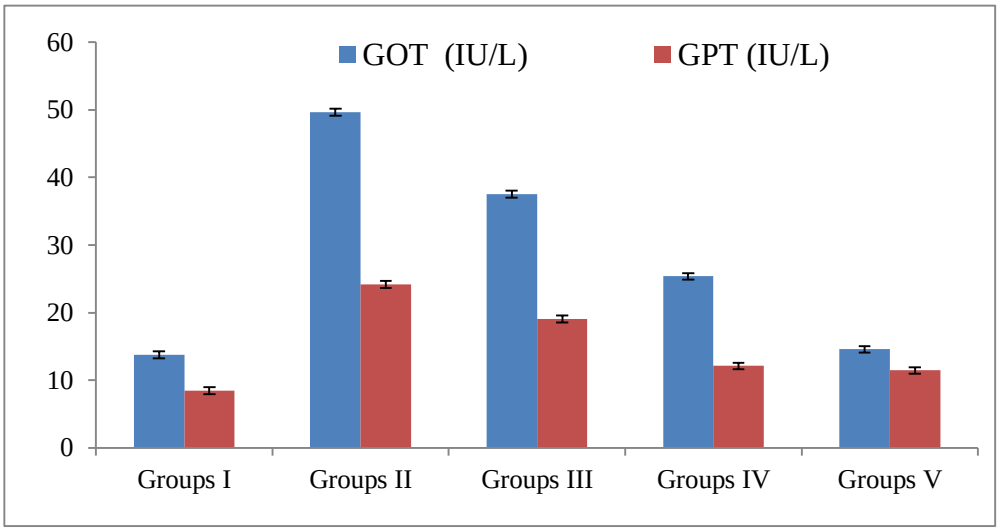
2) as compared to the normal control hepatocyte, thereby indicating oxidative damage. Co-treatment with *Senna alata* flower at doses of 250 and 500µg/kg, significantly prevented the rise in the levels of the marker enzymes, as well as it significantly prevented the decrease in the total protein. The diminished rise of hepatocyte enzymes, together with the diminished fall in the levels of total GSH in the *Senna alata* treated groups, is a clear manifestation of the hepatoprotective effect of the *Senna alata* .

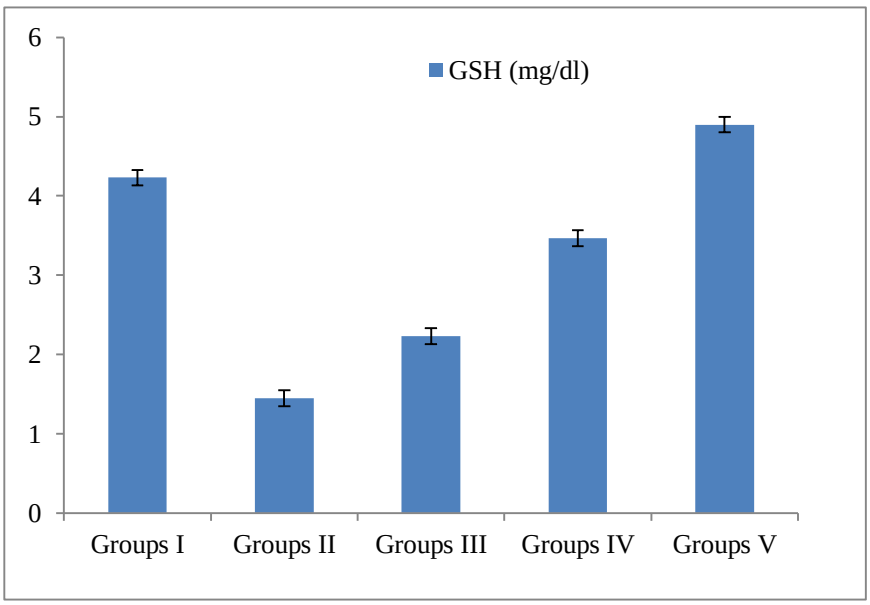
Table 2 Effect of *Senna alata* on GOT and GPT activities in experimental hepatocyte

Groups	GOT (IU/L)	GPT (IU/L)	GSH (mg/dl)
I	13.77±3.94	8.48±2.64	4.23±1.1
II	49.67±3.94	24.17±1.24	1.45±1.10
III	37.54±1.98	19.08±1.27	2.23±2.00
IV	25.37±1.37	12.12±1.27	3.47±4.20
V	14.59±1.46	11.45±1.26	4.90±1.99

Values were expressed as mean ± SD for triplicate in each group

Fig 2 Effect of *Senna alata* on GOT, GPT and GSH activities in experimental hepatocytes





4. SUMMARY AND CONCLUSION

A number of pharmacological and chemical agents act as hepatotoxin and produce variety of liver ailments. Carbon tetrachloride intoxication in rats is an experimental model widely used to study necrotic and steatonic changes in hepatic tissue. Accordingly, the present experiment was designed to use carbon tetrachloride intoxicated rat liver as model. Herbal drugs are playing an important role in health care programs worldwide, and there is a resurgence of interest in herbal medicines for the treatment of various ailments including hepatopathy. Realizing the fact, this research was carried out to evaluate the hepatoprotective activity of methanol extract of *Sena alata* flower against CCl_4 induced toxicity in hepatocytes.

The following conclusion obtained from the study. The biochemical parameters MDA, ALP and AST significantly increased and reduced glutathione and protein were recorded a significant decline on CCl_4 treatment. This indicates that the lipid peroxidation and hepatotoxicity elicited by CCl_4 intoxication had been reversed due to the effect of *Senna alata* flowers.Thus, the *Senna alata* flower co-treatment proved to be hepatoprotective activity. The potential hepatoprotective activity of *Senna alata* flower may be due to the presence of phytochemical such as flavonoids and alkaloid compounds. Further investigation is needed to confirm through *in vivo* experiment.

Over all the *Senna alata* flower as a source of phytochemicals and possess hepatoprotective activity that can be important in oxidative stress mediated diseases like diabetic, cancer and arthritis etc.

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