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Hepatoprotective Activity of Aqueous Extract of *Digitalis purpurea* in Carbon Tetra Chloride Induced Hepatotoxicity in Albino Rats

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Abstract

Liver is a vital organ play a major role in metabolism and excretion of xenobiotics from the body. Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Liver cell injury caused by various toxic chemicals (certain anti-biotic, chemotherapeutic agents, carbon tetrachloride (CCL₄), thioacetamide (TAA) etc.), excessive alcohol consumption and microbes is well studied. Herbal medicines have been used in the treatment of liver diseases for a long time. A number of herbal preparations are available in the market. The present review is aimed at compiling data on promising phytochemicals from medicinal plants that have been tested in hepatotoxicity models using modern scientific system. A large number of populations suffer due to various reasons from hepatic diseases of unknown origin. The development of antihepatotoxic drugs being a major thrust area has drawn the attention of workers in the field of natural product research because synthetic drugs may cause serious side effects. The present research was aimed to study the *in vivo* hepatoprotective activity of methanolic extract of *Digitalis Purpurea L.* in CCl₄ induced hepatotoxicity in albino rats. The level of serum aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), alkaline phosphatase (ALP) and total bilirubin (T. BILI.) were determined to assay hepatotoxicity. CCl₄ administration caused severe hepatic damage in rats as evidenced by elevated serum SGOT, SGPT, ALP and T. BILI. Levels. The extract of *Digitalis Purpurea L.* and silymarin administration prevented the toxic effect of CCl₄ on the above serum parameters in both preventive and curative models. The present study concludes that, methanolic extract of *Digitalis Purpurea L.* has significant hepatoprotective activity against CCl₄ induced hepatotoxicity, which support folkloric utilization and further confirmed by the histological investigation. The observed activity may be associated with its high bioactive compounds including Purpurea glycoside -A, Digitoxin (a), Glucoodoroside-H, Gitoxin, Odoroside - H, Verodoxin, Digitalin, β-sitosterol, Cholesterol, Campesterol, Apigenin.

Keywords: Carbon Tetra Chloride (CCl₄), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), serum glutamic-pyruvic transaminase (SGPT), total bilirubin (T. BILI.)

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1. INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role 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 reproduct. The major functions of the liver are carbohydrare, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin¹. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The bile secreted by the liver has, among other things, plays an important role in digestion².

Liver has a pivotal role in the maintenance of normal physiological process through its multiple and diverse functions³.

The liver is a organ of human body which performs detoxification of the exogenous xenobiotic, drug, viral infection and chromic alcoholism. Liver disease have become a worldwide problem and are associated with significant morbidity and mortality⁴. The principal causative factors for the liver disease in developed countries are excessive alcohol consumption and viral induced chronic liver disease while in the developing countries the most frequent causes are environmental toxins, parasite disease, hepatitis B and C Viruses and hepatotoxic drugs⁵.

The most important organs like Liver regulate various physiological processes and play major role in metabolism and have a number of functions in body, including glycogen storage Decomposition of red blood cells, plasma protein synthesis, hormone production⁶.

Hepatotoxicity is the major health problem in world. Hepatotoxicity is the capacity of chemicals, drugs or other exposure to produce injury to the liver. Some of the inorganic or organic compounds produce hepatotoxicity. Inorganic compounds are arsenic, phosphorous, copper, and iron. Moreover, Organic compounds include naturally occurring plant toxins like mycotoxins, bacterial toxins and pyrrazolidine alkaloids. Drug and environmental toxicants enter the hepatic portal vein from the digestive system and liver function can be altering by the injury result from acute and chronic exposure to toxicants. Liver injury or liver dysfunction is a major health problems .It caused by alcohol consumption, toxic substances and certain drugs such as carbon tetra chloride, Thioacetamide high doses of paracetamol, rifampicin, isoniazid, and certain chemotherapeutic agents etc., chronic alcohol consumption and microbes is well studied⁷ . Liver

damage associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in tissue GSH level . Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. During metabolism of ethanol, enhance lipid peroxidation result, development of hepatitis leading to cirrhosis cause death. Drug-induced liver injury may account for as many as 10percent of hepatitis cases in adults overall, 40 percent of hepatitis cases in adults over fifty years old, and 25 percent of Cases of fulminant liver failure⁸.

Thus, to maintain a healthy liver is a crucial factor for overall health and well being⁹. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease¹⁰.

Thus liver diseases are some of the fatal disease in the world today¹¹. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drug available for the treatment of liver disorders¹². Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model¹³.

Treatment options for common liver diseases such as cirrhosis, fatty liver and chronic hepatitis are problematic. The effectiveness of treatment such as interferon colchicines, penicillamine and corticosteroid are inconsistent at best and incidence of side effect is profound through the treatment is worse than the disease. Herbal drugs have gained popularity and much importance in recent years because of their safety, efficacy and cost effectiveness as well as better compatibility. According to world health organization, 80% of the world population relies on the use of traditional medicine, which depends on herbal Plant materials¹⁴.

Although 7,000 different medicinal herbs and 95 species estimated 2, 50,000 flowering plants and their parts are utilized in western medicines. Chinese medicines used 5,000 different plants in their healing system¹⁵.

Several Indian traditional system used in the medicine or herbals formulation for prevent and management liver disorders. 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.

The use of natural remedies for the treatment of liver diseases has a long history and Medicinal plants and their derivatives are still used all over the world in one form or the other for this Purpose.

Hepatoprotective plants contain a deferent variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes . Hepatoprotective herbal drug based on therapeutic effect for liver disorder used long time. Therefore, large number of herbal formulation has been claimed hepatoprotective activity. Development of plants based hepatoprotective drugs given importance in global markets¹⁶.

Digitalis purpurea belonging to the Scophulariaceae family or purple foxglove. The plant's name, *Digitalis* (from the Latin digit, finger) describes the finger-shaped purple flowers it bears. The effects of the plant extract on the heart were first observed in the late eighteenth century by William Withering, who experimented with the extract in fowls and humans.

Digitalis species is distributed in Asia like India, Nepal, Pakistan & Europe & also In Western Asia. Today, around 40 species belonging to different genera of Scophulariaceae family are known. The endemism rate of the species is around 50 %¹⁷.

Foxglove is a bienneial plant with soft, hairy, toothed, ovate and lance-shaped leaves in a basal rosette. The life span of the plant is 2 seasons. The first year growth remains in a basal rosette of leaves. Second year growth produces flowering stems, 3 -6 feet in height. Flower spikes have purple to white spotted thimble-like flowers which hang down and last about six days. The earliest known name for this plant is the Anglo-Saxon "foxes glofa" (the glove of the fox).

It derives its name from the flowers which resemble the fingers of a glove and possibly from a northern legend that bad fairies gave the blossoms to the fox to put on his toes, so that he might soften his tread while he hunted for prey. First year growth has been mistaken for Comfrey (*Symphitum officinale*) with fatal results. Although, ingestion of this plant can be fatal at any time during the life of the plant, it is most toxic just before the seeds ripen.

The upper leaves of the stem are also more toxic than the lower leaves. Foxglove, *Digitalis purpurea* L., is a facultative biennial herb that grows on naturally disturbed soil. The plant produces one or a few

inflorescences, usually 1–2 m in height, and normally with 20–100 flowers.

The self-compatible flowers produce nectar and are mainly visited by bumble-bees. The basal flowers mature first (acropetal flowering)¹⁸. There is little spatial separation of the sexes within flowers, but temporal separation (protandry) is pronounced.

The combination of acropetal flowering and protandry results in inflorescences where the sexes are spatially separated: the lowest flowers are female, usually followed by neuter flowers, male flowers and, at the top open, prefertile flowers.

Nectar reward increases during flower life and consequently induces bumble-bees to visit the lowest flowers first¹⁹. Minerals present in medicinal plants are of great importance to understand their pharmacological actions²⁰.

Mineral elements play an important roles in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living organism which will lead to the formation of active organic constituents²¹.

Their deficiency causes diseases, whereas the excess presence may cause toxicity²². Mineral contents of several medicinal plants have been determined by applying several techniques such as atomic absorption spectroscopy²³, atomic emission spectrometry, electrothermal atomic absorption spectrometry, X-ray emission, X-ray fluorescence, inductively coupled plasma mass spectrometry and neutron activation analysis²⁴.

D.purpurea contains triterpenoids, cardiac glycosides such as digitoxin, gitoxin, and digoxin which are accredited for the insecticidal activity of the plant. *Digitalis* is well known for its cardiac glycosides that has even today retained its place as a powerful cardio active drug. Purpurea glycoside –A, Digitoxin (a), Glucoodoroside–H, Gitoxin, Odoroside – H, Verodoxin, Digitalin, β -sitosterol, Cholesterol, Campesterol, Apigenin, Digitolutein, Chlorogenicacid, Gitaloxin, Verodoxin, Acetyl digitalinum verum, Purpurea glycoside – B²⁵.

Digitalis purpurea (family Scrophulariaceae) are reported to contain various cardiac and steroidal glycosides localized in roots and leaves²⁶. Digitoxin is the main active constituent of these species²⁷. These species are used in the treatment of heart failure²⁸, and extracts are reported to perform cardiovascular²⁹, antitumour³⁰, antimicrobial³¹ and anti-inflammatory activities³².

2. MATERIALS AND METHODS

Animals:

Male Sprague-Dawley rats weighting 200–250 g, were used for the study. The rats were inbred in the central animal house of the Department of Pharmacology, Karavali College of Pharmacy, Mangalore, and kept with a 12 hL : 12 hD light/dark cycle. Rats were housed in cages with food, water freely available & under suitable conditions of housing, temperature, ventilation and nutrition.

3. PLANT MATERIALS

3.1 Collection of Plant Leaves:

The leaves of *Digitalis Purpurea L.* were collected from local market of Uttarakhand in the month of October. It is preserved in the departmental library for future reference.

3.2 Preparation of Extract :

The dry, powdered leaves of *D. purpurea* (1000 g) were extracted first with chloroform and then with methanol in a Soxhlet extractor. The methanol extract, after concentration under vacuum, afforded a syrupy residue (58 g), an overall yield of approximately 4.83% (w:w). The extract gave positive reactions against Fehling, Legal, Keller–Killiani, xanthydrol, antimony trichloride and Kedde reagents. Thin layer chromatography of the extract indicated the presence of the same six digitalis glycosides when the dried plate was sprayed with antimony trichloride. Since digitoxin is present in the majority of Digitalis extracts, it was chosen as the standard glycoside R_f 1.00 in a two-dimensional chromatography system and the relative R of cardiac glycosides were obtained.

The R_{rel} was therefore calculated according to R_{rel} Distance travelled by the substance: Distance travelled by digitoxin. Successive preparative TLC plates of the methanolic extract led to the isolation of the following compounds, named order of decreasing polarity in the two-dimensional system; 1 (ethyl acetate:methanol:water, 16:1:1); and 2 (chloroform:pyridine, 6:1) as solvents: digitalinum verum, (gitoxigenin glucoside digitoxoside) purpurea glycoside A, lanatoside A, strospeside, (gitoxigenin-digitoxoside)

This data was confirmed with that reported in the literature and by direct comparison with authentic samples. The most abundant glycosides detected by thin layer chromatography were digitalinum verum and strospeside. The extract was soluble in methanol, acetone and ethyl acetate moderately soluble in ethanol, insoluble in petroleum ether and benzene.

3.3 Drugs and Chemicals :

Carbon Tetrachloride was collected from Karavali College of Pharmacy. Silymarin was purchased from Microlab. Pvt. Ltd.

3.4 Animal Grouping and Digitalis Purpurea L. Treatment

The animals were divided into 6 groups (I–VI), each group consisting of 6 animals.

Group I received 5% gum acacia suspension and acts as a normal control.

Group II received CCl₄ at a dose of 1 ml/kg orally (p.o.) acts as negative control.

Groups III were treated with selected drugs Silymarin (50 mg/kg p.o.) and plant extract.

Groups IV were treated with Plant Extract (100 mg/kg p.o.).

Groups V were treated with Plant Extract (200 mg/kg p.o.).

Groups VI were treated with Plant Extract (400 mg/kg p.o.).

Groups III–VI were treated with selected drugs for 5 days before the commencement of experiment and on day 6th of the experiment, blood samples were collected (6th day) at 0 hr in all groups and CCl₄ was administered to all groups except group I (normal control) one hour after the administration of drugs. On 7th day blood samples were collected from all groups by retro orbital puncture, serum was separated by centrifugation and used for the estimation of blood serum parameters (SGOT, SGPT, SALP and T. BILI.) according to the standard procedures. The liver sections were also dissected out & subjected to histopathology studies³³.

4. HISTOPATHOLOGICAL STUDIES

All the animals were anesthetized with ethyl ether and livers were dissected out quickly by cutting on the ventral side. The isolated liver specimen was trimmed to small pieces and preserved in neutral buffered formalin (10% formaldehyde in phosphate buffered saline) solution for 24 hrs. The liver specimen was subjected to dehydration with acetone of strength 70, 80, 100 % respectively, each for one hour. The infiltration and impregnation was done by treatment with paraffin wax twice each time for one hour. Specimens were cut into sections of 3–5 μm thickness using microtome and were stained with hematoxylin and eosin and later the microscopic slides of the liver were photographed at 40X magnification^{34,35}.

5. STATISTICAL ANALYSIS

All values are expressed as mean ± S.E.M. Statistical analysis was performed out using a Student's *t* test comparing two groups and by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test for the multiple drug-treated groups versus the control group. *P* < 0.05 was considered to be significant.

6. RESULTS

Sr. No	Treatment Group	Serum Biochemical Parameter SGOT (IU/L)
1	Control (5 % gum acacia 1 ml/kg p.o.)	75.50 ± 1.049
2	Hepatotoxin - CCl ₄ (1 ml/kg p.o.)	509.00 ± 0.894
3	Standard - Silymarin (50 mg/kg p.o.)	132.30 ± 0.816
4	Extract of DP (100 mg/kg p.o.)	321.20 ± 0.752
5	Extract of DP (200 mg/kg p.o.)	224.50 ± 0.547
6	Extract of DP (400 mg/kg p.o.)	148.50 ± 0.836

Table 1: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats.(Serem Biochemical Parameter **SGOT**)

Sr. No	Treatment Group	Serum Biochemical Parameter SGPT (IU/L)
1	Control (5 % gum acacia 1 ml/kg p.o.)	69.17 ± 1.329
2	Hepatotoxin - CCl ₄ (1 ml/kg p.o.)	396.50 ± 1.371
3	Standard - Silymarin (50 mg/kg p.o.)	103.00 ± 0.894
4	Extract of DP (100 mg/kg p.o.)	266.50 ± 1.225
5	Extract of DP (200 mg/kg p.o.)	180.20 ± 0.752
6	Extract of DP (400 mg/kg p.o.)	108.70 ± 1.033

Table 2: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats.(Serem Biochemical Parameter **SGPT**)

Sr. No	Treatment Group	Serum Biochemical Parameter SGPT (IU/L)
1	Control (5 % gum acacia 1 ml/kg p.o.)	135.20 ± 0.753
2	Hepatotoxin - CCl ₄ (1 ml/kg p.o.)	784.80 ± 0.752
3	Standard - Silymarin (50 mg/kg p.o.)	225.50 ± 0.547
4	Extract of DP (100 mg/kg p.o.)	547.30 ± 0.816
5	Extract of DP (200 mg/kg p.o.)	393.20 ± 0.753
6	Extract of DP (400 mg/kg p.o.)	248.50 ± 0.548

Table 3: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats.(Serem Biochemical Parameter **SALP**)

Sr. No	Treatment Group	Serum Biochemical Parameter Total Bili. (mg/dl)
1	Control (5 % gum acacia 1 ml/kg p.o.)	0.478 ± 0.007
2	Hepatotoxin - CCl ₄ (1 ml/kg p.o.)	4.517 ± 0.075
3	Standard - Silymarin (50 mg/kg p.o.)	1.410 ± 0.008
4	Extract of DP (100 mg/kg p.o.)	2.933 ± 0.050
5	Extract of DP (200 mg/kg p.o.)	1.415 ± 0.054
6	Extract of DP (400 mg/kg p.o.)	1.755 ± 0.005

Table 4: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats. (Serem Biochemical Parameter Total Bili.)

7. Graphs

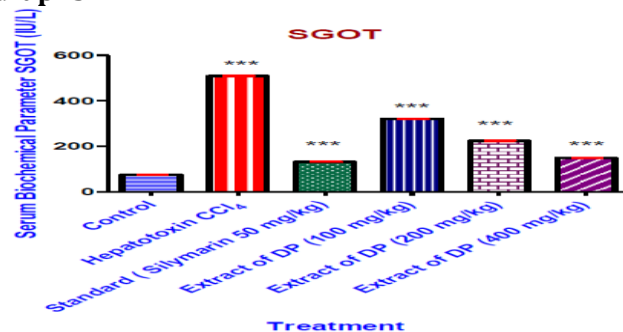


Figure 1: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats.(Serem Biochemical Parameter **SGOT**)

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

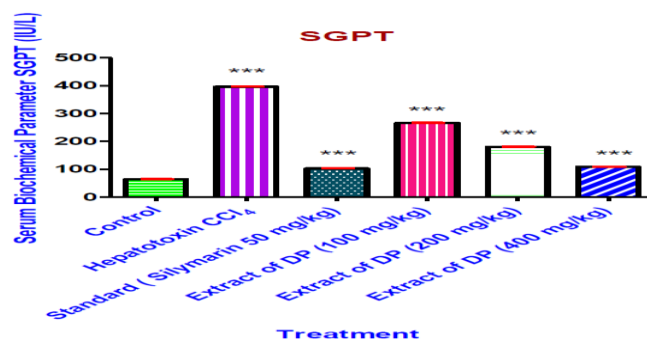


Figure 2: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats.(Serem Biochemical Parameter **SGPT**)

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

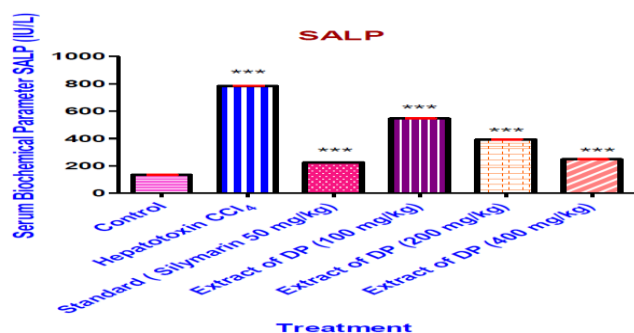


Figure 3: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats.(Serem Biochemical Parameter SALP)

Values were mean \pm S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

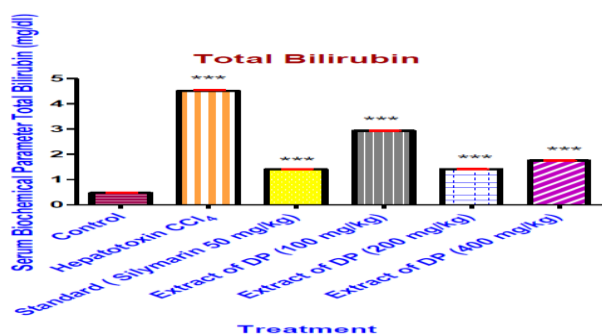


Figure 4: Effect of Methanolic extract of plant of *Digitalis Purpurea L.* against CCl₄ - induced hepatotoxicity in Albino Rats.(Serem Biochemical Parameter Total Bilirubin)

Values were mean \pm S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

8. DISCUSSION

Herbal medicines are free from side effects, adverse effects and they are economical and easily available will be beneficial for the mankind over the centuries. The present studies were performed to assess the hepatoprotective activity in albino rats against CCl₄ as hepatotoxins to prove its claims against liver disorders. CCl₄ induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs.

The selected plant methanolic extract at dose levels of 100 mg/kg b.w, 200 mg/kg b.w and 400 mg/kg b.w, were tested by taking silymarin as a standard. The tested doses exhibited significant hepatoprotective activity against CCl₄-induced liver intoxicated rats by reduction in increased serum levels of SGOT, SGPT, SALP and T. BILL.

Digitalis is well known for its cardiac glycosides that has even today retained its place as a powerful cardio active drug. *Digitalis Purpurea L.* contain Purpurea glycoside -A, Digitoxin (a), Glucoodoroside-H, Gitoxin,

Odoroside - H, Verodoxin, Digitalin, β -sitosterol, Cholesterol, Campesterol, Apigenin, Digitolutein, Chlorogenicacid, Gitaloxin, Verodoxin, Acetyl digitalinum verum, Purpurea glycoside - B.

The tested doses exhibited significant hepatoprotective activity against CCl₄-induced liver intoxicated rats by reduction in increased serum levels of SGOT, SGPT, SALP and T. BILL. However administration of doses at 200 mg/kg b.w and 400 mg/kg b.w produced significant decreasing at serum levels of SGOT, SGPT, SALP and T.BILI³⁶.

Histopathological examination of the liver sections of the control group showed normal architecture of the liver with distinct hepatic cells. The liver section of CCl₄ intoxicated group showed complete Complete disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization, fatty changes, sinusoidal haemorrhages and dilatation .The liver sections of silymarin treated rats showed a normal hepatic architecture with normal hepatocytes. Whereas the rats treated with test methanolic extract of *D. ovatum* (L.) Kraenzl. at doses of 100 mg/kg b.w 200 mg/kg b.w and 400 mg/kg b.w showed recovery from CCl₄ induced liver damage as evident from normal hepatocytes and with higher dose of 400mg/kg b.w showed significant attenuation of inflammatory and necrotic changes and cellular architecture of liver was preserved indicating a marked protective activity similar to that observed in silymarin treated rat liver sections and the effect was found to be dose dependent³⁷.

CCl₄ is a hepatotoxin commonly used for the production of experimental liver toxicity. The carbon tetrachloride mechanism begins with the trichloromethyl radical by the action of the mixed function of cytochrome P-450 oxygenase system. This free radical, which is initially formed as unreactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical. Both these radicals are capable of binding with proteins / lipids or abstracting a hydrogen atom from an unsaturated lipid, thus initiating lipid peroxidation. This process of lipid peroxidation can significantly damage hepatic plasma membranes.

The increased levels of SGOT, SGPT, SALP and T.BILL. are conventional indicators of liver injury. The ability of hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effect.

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscles and kidneys. Elevated levels are

found in myocardial infarction, cardiac surgeries, liver disorders, cirrhosis, acute pancreatitis, acute renal diseases and primary muscle diseases. SGOT catalyses the transamination of L - Aspartate and α -Ketoglutarate to form L Glutamate and Oxaloacetate. In subsequent reaction, malate dehydrogenase (MDH) reduces oxaloacetate to malate with simultaneous oxidation of nicotinamide adenine dinucleotide [reduced] (NADH) to nicotinamide adenine dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm and is directly proportional to SGOT activity in the sample. SGPT is found in a variety of tissues but is mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. SGPT catalyses the transamination of L-Alanine and α -Ketoglutarate to form pyruvate and L-Glutamate. In subsequent reaction, lactate dehydrogenase (LDH) reduces pyruvate to lactate with simultaneous oxidation to nicotinamide adenine dinucleotide (NAD). The rate of oxidation of NADH to NAD is measured as a decrease in absorbance at 340nm which is proportional to the SGPT activity in the sample.

Serum ALP measured is of particular interest in the hepatobiliary disease and in bone diseases. At the pH 10.3, Alkaline phosphatase (ALP) catalyses the hydrolysis of colorless p-Nitrophenyl phosphate (pNPP) to yellow colored p-Nitrophenol and Phosphate. Change in absorbance due to yellow color formation is measured kinetically at 405 nm and is proportional to ALP activity in the sample.

Bilirubin is the main bile pigment which is formed from the breakdown of heme of red blood cells by reticuloendothelial system. Total Bilirubin concentration was increased mildly in chronic haemolytic disease, moderately to several in hepatocellular disease and markedly in cholestasis. Total bilirubin reacts in the presence of caffeine with diazotized sulfanilic acid to form azobilirubin. The color developed is measured at 546 nm and is proportional to the concentration of bilirubin. Phytochemical studies on the selected plant revealed the presence of flavonoids, alkaloids, triterpenoids, glycosides, steroids and carbohydrates. The presence of above constituents in selected plant extract alone or in combination might be responsible for the observed hepatoprotective activity³⁸.

Recent evidence indicates Digitalin, β -sitosterol, Digitoxin, Purpurea glycoside -A helps in CCl₄ induced hepatotoxicity in albino rats. As *Digitalis Purpurea L.* has been successfully used in many health problems

since a long time it provides a wide area of interest for the research purposes in development of newer drug molecules. The therapeutic potential should also be seen in combination with other medicinal agents. It has also been studied extensively from the point of phytochemical and pharmacological aspects which lead to the interest particularly in the area of Neuroprotective, antioxidant, antimicrobial and hypoglycemic actions. In recent years it has been noticed that more emphasis of research is on traditional medicines which have proven its authentication against treating various diseases. Various traditional uses are also known to be possessed by the plant like in rheumatism, bronchial asthma, leprosy, as cardiogenic and many more. Many activities are not studied till date and needs attention to explore further medicinal properties of the plant. Experiments will have to be conducted in future to exploit the full potential activities of this crop and this plant species has to properly identified and conserved to avoid the extinct condition.

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