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Hepatoprotective Effect of *Azima tetracantha Lam* on Ferrous Sulphate Induced Toxicity in Albino Rats.

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ABSTRACT

Hepatotoxicity induced in albino rats by ferrous sulphate (100mg/b-w) and their hepatoprotective effect were studied by using aqueous extracts of *Azima tetracantha*. Regaining of liver parameters such as glucose, protein, bilirubin, cholesterol, ALP, SOD, CAT, Vitamin E, TBARS, Albumin and globulin to normal level after the oral administration of *Azima tetracantha Lam.*

Keywords: Alkaline phosphatase, superoxide dismutase, tri-barbutaric acid, lipid peroxidation, Reactive oxygen species.

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INTRODUCTION

The liver is a most important organ existing in animals and vertebrates. It lies under the diaphragm exactly in thoracic region of abdomen and it gives an alkaline compound, bile and emulsification of lipids. The liver also achieves and regulates a huge variety of biochemical reactions needs most specialized tissues [1].

The body's primary defense against metabolic poisioning is carried out by the liver. Chemical- driven of liver injury suggested by hepatotoxicity [2]. Liver play a Vitol role in clearing chemicals, transforming and toxicity from respective agents. Organs may be injure due to certain medication when they gives overdoses and most of the time they introduced ranges in therapeutic.

Nowadays, the major public health problems obtain from chemical- induced liver damage caused by drug it leads to cause hepatic disorders [3].

Most of the chemicals are not biologically active but we prompted it as a reactive toxic metabolites which act directly on target cells. However these metabolites affects cell injury and damage the membrane due to direct covalent binding to lipids and protein membrane [4]. The predominant of acute hepatitis are cholestatis and clinical pathological pattern of acute or chronic liver disease [5].

Liver cell and kidney damage mainly occurs due to mitochondria damaged by drugs and respiratory chain disable, lactic acidosis and accumulation of triglycerides [6].

Long term over consumption of iron may cause hemosiderosis, a condition characterized by large deposits of the iron storage protein hemosiderin in the liver and other tissues. Lipid peroxidation and free radical generation is one of the suggested mechanism of hepatotoxicity induced by iron. Catalyzes of hydroxyl radical formation from iron is the best potent- free radical particularly in biological systems and it intimate lipid peroxidation. Therefore greatest risk is found in highest concentrations of iron with tissues [7].

The oxidative breakdown lipids membrane can be originated by a various free radicals. Lipids are characterized by both organic radical intermediates and reactive oxygen species (ROS) [8]. The lipid peroxidation is also described by biochemical oxidative stress [9].

ROS is an accountable for toxic impact through various tissue damage in body. Our biological systems have the defense mechanism against this reactive oxygen species. They are called antioxidants [10] clearly, the diversity of antioxidants matches that of pro-oxidants.

Most current research focuses on one of them, silibinin, which may have specific protective effects on cells in the liver. In multiple human, animal and laboratory studies, it has shown differing degrees of effectiveness for protecting the liver from damage caused by alcohol, chemicals, drugs, diseases and poisonous plants [11].

Traditional medicine are obtained from many medicinal plants around 35,000 - 70,000 species. *Azima tetracantha* is a flowering plant in the family salvandoraceae. A spiny, evergreen shrub with a tendency to scramble. Branchlets blue-green, often densely hairy; with four sharp, axillary spines up to 50 mm long. Leaves simple, opposite, with successive pairs at right angles to each other; elliptic-oblong to almost round; leathery. fruit round, small; yellow to white when ripe. Flowers and fruit are easily overlooked, but this plant cannot be confused with any other species [12].

Mainly occurring in central Namibia, north-west of Windhoek. Two new, isolated records were collected from the south and the north. Uncommon, but locally common to abundant, especially along river banks and on flood-plains **[**13**].** Mainly found on river banks, occasionally on plains. Also found in distributed areas. This plant was found along 'most river banks in the Kunene Region' [14].

MATERIALS AND METHODS

Procurement of Animals

The group of Young healthy albino male rats with weight 130 – 150 gms are kept in stainless steel hoppers separately in conventional laboratory diets and supply unlimited water. Animals should be characterized by source, sex, strain and age.

Hepatotoxic effect induction

Ferrous sulphate was used for induction of hepatotoxicity in rats. The solution was made in administered dose with normal water for 100 mg/kg body weight ,oral for 21 days [15].

Plant materials

Leaves of Azima tetracantha lam were collected from needamangalam . azima tetracantha lam are allowed to dry at 45^{o}C by continuously 48 hours and powdered it by using electric grinder. Powdered should be stored in good air prof container. This fine crude powder was administered with the dosage of 100mg/kg bw/day [16]**.**

Procurement of Standard drug

silibinin was used as standard drug for the treatment of hepatotoxicity and was procured from Micro labs limited, India and was administered with the dosage of 100mg/kg b.w/day [17]**.**

Experimental Design:

The rats were randomly divided into four groups of four animals in each.

Group 1: Four rats were kept as normal control and administrated with 0.9% normal saline once daily for 24 days.

Group 2: Four rats are allowed to injected 100mg ferrous sulphate once in a day for 1-24 days.

Group 3: Four rats are allowed to injected 100mg ferrous sulphate once in a day for 1 – 14 days and administrated 100mg of Azima tetracantha lam powder during 15 – 24 days with interval 24 hours.

Group 4: Four rats are allowed to injected 100mg ferrous sulphate once in a day for 1 – 14 days and administrated silibinin 30 mg during 15 – 24 days with interval 24 hours.

Study protocol

At last rats are sacrificial victim by cervical decapitation and blood is collected along with centrifuged by 2000 rpm × 1 g for 20 minutes apply to separate plasma and anticoagulant.

The following parameters were analysed

- Estimation of Protein
- Estimation of Globulin
- Estimation of Serum Billirubin
- Estimation of Superoxide dismutase
- Determination of A/G Ration
- Estimation of Catalase.
- Estimation of Albumin
- Estimation of Glucose
- Estimation of α-tocopherol
- Estimation of Thiobarbituric Acid Reactive Substance
- Estimation of Cholesterol
- Estimation of Alkaline Phosphatase

RESULTS AND DISCUSSION

Rats liver get damage due to injection of ferrous sulphate which is evident by biochemical parameters such as sugar, bilirubin, alkaline phosphatase and TBARS are significantly increased. Table-1 indicates oral administration of aqueous extract of *Azima tetracantha* decrease the level of sugar, bilirubin ALP and TBARS. When compared to standard drug silibinin.

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Table 1. Effect of aqueous extract of Azima tetracantha on serum biochemical parameters in ferrous sulphate induced hepatic injury in rats.

Parameters	Group 1	Group 2	Group 3	Group 4
Sugar	90.5 ± 4.65	285.5 ± 4.20*	128.5 ± 2.88**	164 ± 7.16**
Cholesterol	115 ± 3.55	348.25 ± 1.70*	172.5 ± 2.08**	212.5 ± 8.73**
Bilirubin	0.4 ± 0.21	1.6 ± 0.34*	0.9 ± 0.16**	1.3 ± 0.25 ns
ALP	135.5 ± 2.88	447 ± 6.48*	163.75 ± 3.59**	242.5 ± 4.12**
TBARS	0.37 ± 0.02	0.80 ± 0.02*	0.46 ± 0.02**	0.53 ± 0.01**

Table 2. Oral administration of aqueous extract of *Azima tetracantha* increase the level of protein and antioxidant.

Parameters	Group 1	Group 2	Group 3	Group 4
Protein	6.97 ± 0.15	2.85 ± 0.20*	6.8 ± 0.29**	6.57 ± 0.34**
Albumin	3.62 ± 0.25	1.85 ± 0.28*	3.57 ± 0.25**	2.82 ± 0.25**
Globulin	2.87 ± 0.09	1 ± 0.40*	3.05 ± 0.23**	3.75 ± 0.41**
A / G	1.44 ± 0.09	2.47 ± 2.02ns	1.17 ± 0.15**	0.75 ± 0.13**
SOD	36 ± 2.16	18.75 ± 5.90*	35.2 ± 3.68**	30.75 ± 5.12 ns
CAT	56.9 ± 0.69	45.37 ± 0.28*	71.7 ± 0.21**	61.27 ± 0.17**
VIT E	152.75 ± 3.59	82.75 ± 10.34*	148.5 ± 6.75**	127.25 ± 6.18**

There was substantial decrease in biochemical parameters such as protein and antioxidants in ferrous sulphate treated animals. The results presented in Table-2 indicates

oral administration of aqueous extract of *Azima tetracantha* increase the level of protein and antioxidant. When compared to standard drug silibinin.

The unsuspected influences between glucose level and iron metabolism has entitled with scientific evidence. Glucose metabolism interrupted on many pathways of iron metabolism. Lesion cytokines and oxidative stress inspired potentiate and amplified the initiated events. The clinical effect mainly depends on exposure period and time frame which is closely connected to signals acts. At present iron stores are increased with increasing glucose levels while depletion was protective against beta cells of Langerhans.

In this present study the Iron hepatotoxicity, resulted in reduction of albumin levels and total protein of serum. This observation leads to morphing in metabolism of free amino acid and protein. Therefore, injured liver cells and protein degradation are raised.

α-linolenic acid (ALA) rich in flaxseed oil resulted the immense secretion of cholesterol into bile leads to hepatic damage and cholesterol turnover increased [18,19].

Iron deficiency enhance the activity of billirubin, it's a main marker for liver damage. During hepatic injury, the direct bilirubin fraction typically is at least 50% of the total serum value, but the total concentration rarely exceeds 500 mmol/L, regardless of severity, because of renal excretion of the direct fraction. The degree of increase in serum bilirubin values has prognostic significance in chronic liver injuries, but not in acute injuries [20]**.**

Serum alkaline phosphatase raised up to some level in several types of liver inflammation occurs. Bile acids account for this increase: They induce alkaline phosphatase synthesis and exert a detergent effect on the canalicular membrane, allowing leakage into serum **.** Mild to moderate increases (less than threefold) are not specific for the type of liver injury [21]**.** The highest concentrations are observed with cholestatic injuries [22]**.**

The iron induced hepatotoxicity mechanism looks like an oxidative stress due to raising of hepatic lipid peroxidation (LPO). Enhanced peroxidation process preceded to tissue injury and consequently antioxidant defense mechanisms failure to stop the excessive free radicals formation. Ferrous salt reacts along with hydrogen peroxide resulted through the achievement of superoxide anion radical and obtain the radical hydroxyl which is highly reactive. All biological molecules are possible to attack by hydroxyl ion to initiate lipid peroxidation.

The oxidative stress was obtain through rise the hepatic lipid peroxidation and decrease the level of antioxidants such as SOD,CAT and Vitamin E is the vital mechanism of induced hepatotoxicity iron. SOD is one of the most attractive metalloprotein present in antioxidant family. Reduced enzyme SOD has recorded in iron intoxicated rats. This is decreased activity of SOD in hepatotoxicity obtain via liver tissues and small level of zinc from plasma [23]. Small level of zinc also avail in alcoholic liver cirrhosis [24].

Catalase is an unusually of hydrogen peroxide to oxygen and reactive molecules. It catalyses the decomposition of hydrogen peroxide to oxygen and water. The antidotal effect of vitamin E in acute iron toxicity may be related to the membrane-stabilizing, antioxidant

and free radical-scavenging properties of vitamin E. Vitamin E is known as a Blocking of chain reaction gives chain-breaking antioxidant which transfer to peroxidation cascade membranes. The iron-catalyzed nonenzymatic auto-oxidation of fatty acids is a reaction that is specifically inhibited by vitamin E [25] although the antidotal effect of vitamin E in acute iron toxicity has been refuted [26].

CONCLUSION

In conclusion, this result showed that orally administerated i Azima tetracantha Lam is an efftective surpressing Ferrous sulphate induced hepatoprotectivity . Azima tetracantha Lam hepatoprptectivity and antioxidant activity mediated byb an important decrease of liver cirosis . liver demage and protect against hepatoxicity and provide better therapeutic agents .

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