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RESEARCH ARTICLE

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF CARICA PAPAYA AND FICUS BENGALENSIS LATEX ON THIOACETAMIDE INDUCED HEPATOTOXICITY IN RATS.

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Abstract

Study was designed to evaluate the hepatoprotective activity of Carica Papaya Latex at the dose 400mg/k.g. and Ficus Bengalensis Latex at the dose 300mg/k.g. per oral induced by thioacetamide at the dose 100mg/k.g. S.C. Hepatoprotective activity was observed by using biochemical parameters such as Serum-Glutamate Pyruvate Transaminase and Serum-Glutamate Oxalate Transaminase (SGPT and SGOT), total protein, Bilirubin, Alkaline Phosphate (ALP). Carica Papaya and Ficus Bengalensis Latex produced significant hepatoprotective effect by decreasing the activity of serum enzyme like SGPT, SGOT, Bilirubin, ALP, and elevated total protein level. Both plants latex were found completely prevented the toxic effect of thioacetamide on above serum parameters.

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Introduction:-

Liver is the largest organ in the body weighing 1400-1600 gm in the males and 1200-1400gm in females. Liver performs various multifold functions are metabolism of proteins, lipid, carbohydrate and excretion of bile, detoxification of toxic substance such as alcohol and drugs [1]. It has important role in the maintenance and regulating homeostasis of the body. It is also involve with biochemical pathway to fight against to disease, growth, reproduction and nutrition supply [2].

The morbidity and mortality of different types of liver disease have rises around the world[3]. Alcohol, prescribed drug and over the counter drug which can eventually lead to various liver ailments like alcoholic liver disease, cirrhosis, hepatitis[4],[5]. The untreated liver disease and long term uses of medication sometime cause toxic effect and finally may lead to death[6]. In recent times various plants have been used for prevention of liver disease [7]. Medicinal plants are used in the natural medicines such as Unani medicine and Indian Ayurveda [8].

Ficus Bengalensis plant is commonly known as Banyan tree in Ayurveda[9]. Ficus Bengalensis belong to family Moraceae. Leaves contain crude proteins 9.63%, Cao-2.53% and phosphorus 0.4% [10],[11]. The extracts of Ficus Bengalensis have been reported to decrease insulinase activity from liver and kidney [12].

Various extracts of Ficus Bengalensis were reported anti-allergic and anti-stress potential in asthma by milk induced leucocytosis and milk induced eosinophilia[13]. A milky juice (Latex) of stem bark of Ficus Bengalensis is used for treatment of rheumatism.

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Ficus Bengalensis bark aqueous extracts reported inflammation and swelling in pancreatic tissue and restored the levels of serum electrolytes [13, 14, and 15].

Carica Papaya Linn plant belongs to family Caricaceae is a tropical tree. It is native to the tropics of Americas but now widely cultivated in other tropical regions of the world. Plant is available throughout the year [16]. Carica Papaya is fast growing plant, it measures 7-8m with copious latex and trunk about 20 cm in diameter [17].

Phytochemical studies have been shown Carica Papaya contains different chemical constituents like flavonoids, nicotins, tannins, terpenes, alkaloids and it also contains enzyme like Chymopapain and papain [18][19].

Aqueous extracts of unripe Carica papaya have been reported antisickling and reversal of sickling properties [20]. Latex, juice and fruit of Carica Papaya have digestive properties and it is reported in used habitual constipation and chronic diarrhea [21]. Carica Papaya Latex reported wound healing properties [22].

Material and Methods:-

Plant materials

Carica Papaya and Ficus Bengalensis were collected from Mathura, Uttar Pradesh. The authentication and identification was done by (Prof.) Dr. D.K. Singh, Department of Botany, KR (PG) College, Mathura, Uttar Pradesh.

Carica Papaya Latex Collection

Latex was collected locally in early morning 7:00 to 8:00 am, as the flow of latex is low during the day. Collection was done by making 1-2 mm deep vertical incisions on the skin of unripe fruit, but mature fruit. The latex was then dried at room temperature till it became crumbly and non-sticky. The dried latex was triturated using a mortar and pestle. It was stored at 4-8 °C until use [22].

Ficus Bengalensis Latex Collection

Latex was collected locally in early morning 7:00 to 8:00 am, as the flow of latex is low during the day. Collection was done by making 1-2 mm deep vertical incisions on the skin [22]. Latex was extracted by maceration process (48h) in methyl alcohol after defatted with petroleum ether at (72h) at room temperature. The extracted was dried by rotatory evaporator under reduced pressure [23]. The dried latex was triturated using a mortar and pestle. It was stored at 4-8 °C until use.

Phytochemical studies

Phytochemical analysis was carried out on Carica Papaya Latex and Ficus Bengalensis Latex for the detection of various phytochemicals by following standard methods described in practical pharmacognosy by Trease and Evans.

Phytochemical Screening

Phytochemicals are chemical compounds that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not established as essential nutrients. The presence of tannins, alkaloids, flavonoids, general test for glycosides (reducing sugars), anthraquinones, sterols and saponins were tested by simple qualitative methods (Trease and Evans, 1989) [24].

Animals

Albino rats of either sex (Wistar strain) weighing 150-200g and female albino mice weighing 20-25g were used in this study. Animals were used from animal house of Sanjay College of Pharmacy, Mathura, U.P.

The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27 °C ± 2 °C, relative humidity 65 ± 10% under 12 hours light/ dark cycle. The animals were fed with rodent pellet diet (Gold Mohur Lipton India Ltd.) and water *ad libitum*.

Ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethics Committee (IAEC) and registration number 1334/a/10/CPCSEA of Sanjay College of Pharmacy, Mathura, Uttar Pradesh.

Toxicity study - Determination of acute toxicity LD₅₀

The acute toxicity for Carica Papaya Latex and Ficus Bengalensis latex were determined in female albino mice. The animals were fasted overnight prior to the experiment, fixed dose method of OECD guideline No. 420; (Annexure 2d) of CPCSEA was adopted for this purpose. Group of three mice were taken for each test dose and 1/10th of LD₅₀ cut off value of test latex selected as screening dose for Hepatoprotective activity [25].

Thioacetamide induced hepatotoxicity

Group I:- Serve as control, received Saline (1ml/kg, p.o)

Group II:- Received Thioacetamide (100mg/kg -s.c)

Group III:- Received Thioacetamide (100mg/kg -s.c) and Carica Papaya Latex (400mg/kg/p.o) .

Group IV:- Received Thioacetamide (100mg/kg -s.c and Ficus Bengalensis Latex (300mg/kg/p.o) .

Group V:- Received Thioacetamide (100mg/kg -s.c and Silymarin (100mg/kg, p.o)

Animals were divided into 5 groups of six animals in each.

Group -I serve as a normal control received Saline 1ml/Kg for 10 days. Group -II were administered a single subcutaneous injection of thioacetamide (100mg/Kg. sc) as a 2% w/v solution in distilled water. Group -III, IV and V were treated orally with Carica Papaya Latex (dose 400mg/Kg.), Ficus Bengalensis Latex (dose 300mg/Kg.) and Silymarin (dose 100mg/Kg.) once daily for 10 days followed by single subcutaneous injection of Thioacetamide.

24 hours after the Toxin administration, the animals of each group were anaesthetized, the blood were collected directly from the heart. The blood samples were allowed to clot for 20-30 minutes. Serum was separated by centrifugation at 37°C. Serum was used for estimation of various biochemical parameters such as SGOT, SGPT, Bilirubin, ALP and total protein through the auto analyzer for the study of the toxic effect of thioacetamide.

Biochemical Studies

Estimation of SGPT on blood serum was carried out using AGD Clinipak from AGD Biomedicals Pvt. Ltd. Mumbai.

Estimation of Alkaline Phosphate, Total Protein, SGOT on blood serum was carried out using Diagnostic test kit from Beacon Diagnostic Pvt. Ltd., NAVSARI.

Estimation of Bilirubin on blood serum was carried out using diagnostic kit from SIEMENS Ltd., Vadodara, Gujarat.

Histological studies

The Liver were isolated from the animals and washed with normal Saline. The liver was fixed in Formalin diluted to 10% with normal Saline then processed further for histological studies. The results were analyzed by student t-test.

Statistical analysis

All the values are expressed as mean \pm S.D. result were analyzed statistically by analysis of variance (ANOVA) followed by student T-Test was used for determining significance.

Result And Discussion:-

The result of present study reveals that level of parameters like SGPT, SGOT, Bilirubin, and ALP were found to be elevated and decrease total protein in Thioacetamide treated group (dose 100 mg/kg b.w.) whereas treatment along with Carica Papaya Latex (dose 400mg/kg b.w.) and Thioacetamide (dose 100 mg/kg b.w.) group shows significant reduction in above said parameter SGPT (180.7 \pm 14.80), SGOT (218.40 \pm 12.4), Bilirubin (2.32 \pm 12.40), and ALP (124.8 \pm 7.2) and increased total protein (6.56 \pm 0.34) compare to Thioacetamide treated group.

Thioacetamide (dose 100 mg/kg b.w.) and Ficus Bengalensis Latex treated Group at dose 300mg/kg b.w. were found decrease in Serum Level SGPT (186.3 \pm 10.70), SGOT (227.60 \pm 14.8), Bilirubin (2.68 \pm 0.05), and ALP (132.6 \pm 8.40) and increase total protein (6.12 \pm 0.11) compare to Thioacetamide treated group SGPT (256.4 \pm 12.30), SGOT (338.70 \pm 18.2), Bilirubin (3.88 \pm 0.04), ALP (182.4 \pm 5.20) and total protein (5.43 \pm 0.025).

Table1:-Effect Of Carica Papaya Latex & Ficus Bengalensis Latex In Thioacetamide Induced Hepatotoxicity In Albino Rats

S.No.	Groups	SGPT(1u/L)	SGOT(1u/L)	Bilirubin(mg/dL)	ALP	T. Protein
1.	Control	52.36±2.18	78.32±3.12	0.96±0.04	104.5±6.70	7.78±0.065
2.	Thioacetamide treated(100mg/Kg) S.C.	256.4±12.3	338.7± 18.2	3.88±0.04	182.4±5.20	5.43±0.025
3.	Thioacetamide+ Carica Papaya Latex	180.7±14.80*	218.4±12.4*	2.32±12.40*	124.8±7.2*	6.56±0.34
4.	Thioacetamide+ Ficus Bengalensis Latex	186.3±10.70*	227.6±14.8	2.68±0.05*	132.6±8.40	6.12±0.11
5.	Thioacetamide+ Silymarin(100mg/Kg)	78.16±1.40**	94.86±2.58**	1.24±0.02**	108.8±4.40	7.78±0.43

All values are expressed in mean \pm SD; n=6 Control vs *P<0.05, **P<0.001

Histology:-

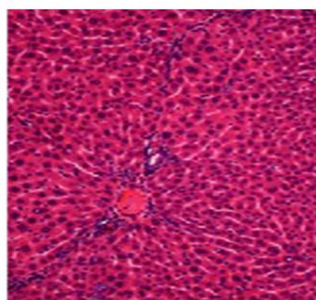


Figure 1:-Control Group

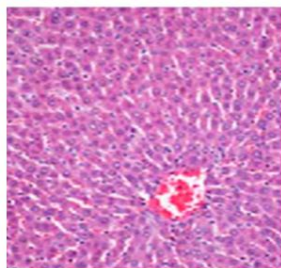


Figure 2:-Thioacetamide Treated Group

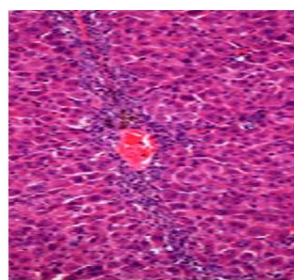


Figure 3:-Thioacetamide and Carica Papaya Latex treated group

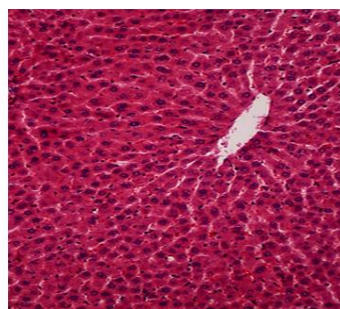


Figure 4:-Thioacetamide and Ficus Bengalensis Latex treated group

The result was shown in Table No.1. represents that Carica Papaya Latex was better reduction in Serum Parameters compare to Ficus Bengalensis Latex.

Phytochemical studies found that both plant carica papaya latex and ficusbengalensis latex contains flavonoid, carbohydrate, tannins, saponins alkaloid, glycoside, protein

We found that Morphological observations showed an increase the size and enlargement of the liver in Thioacetamide treated group. These changes were reversed by treatment with Silymarin(100mg/kg b.w.), Carica Papaya Latex(400mg/kg b.w.) and Ficus Bengalensis Latex (300mg/kg b.w.).

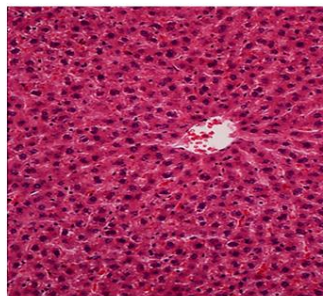


Figure 5:-Silymarin treated group

Histological studies showed that Thioacetamide produced extensive vascular degenerative changes and centrilobular necrosis and Hepatocytes. It was shown in figure no.1 to 5.

Administration of Thioacetamide treated group at the dose of 100 -300 mg/kg by s.c. route results in the Hepatic damage in animals. Thioacetamide is a Hepatotoxic that is metabolized by CYP 450 enzymes present in liver microsomes and is converted to a toxic reactive intermediate called Thioacetamide (s-oxide) due to oxidation process. Thioacetamide s –oxide induced oxidative stress in the Hepatic cells. It interferes with the movement of RNA from the nucleus to the cytoplasm which may cause membrane injury. It reduce the number of viable Hepatocytes as well as rate of oxygen consumption and also decrease the volume of bile and its content. In the present study groups treated with Thioacetamide 100 mg/kg [26].

We found that the treatment with both latex prevented elevation of serum parameter like SGPT, SGOT, Bilirubin, ALT, and decreased total protein due to Thioacetamide challenge models.

Treatment with both latex found that it improved the liver anatomy / hepatic anatomy with normalization of histopathological observation observe after Thioacetamide induced hepatotoxicity.

Conclusion:-

It is thus concluded that both plant *Carica Papaya* Latex and *Ficus Bengalenesis* Latex possesses a hepatoprotective activity against thioacetamide induced hepatotoxicity in rats as evidenced by reduction in serum parameter like SGPT, SGOT, Bilirubin, ALT, and elevation of total protein etc. Whereas *Carica Papaya* latex was found to be more effective compared to *Ficus Bengalenesis* Latex.

A finding from the hepatoprotective study indicates that both latex were showed significantly reduced the elevated serum parameter and enhanced the decreased level of total protein induce by hepatotoxin in rats.

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