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Hypolipidemic Efficacy of Indigofera Tinctoria (Linn.) in Kidney on Paracetamol Induced Fatty Liver in Rats

KEYWORDS: Indigofera tinctoria, paracetamol, lipid profiles, kidney

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ABSTRACT *Plants have been considered as sources of medicinal agents for the treatment of many diseases. The therapeutic potential of Indigofera tinctoria was evaluated by paracetamol induced fatty liver in rats. Male albino wistar rats were orally treated with Indigofera tinctoria (75, 150 and 300 mg/kg body weight) or silymarin (25 mg/kg) daily with administration of paracetamol (3 gm/kg body weight- p o) only one day. Paracetamol induced fatty liver and significantly increased the levels of total cholesterol, free fatty acids, triglycerides and phospholipids in kidney as compared with control group. Treatment with Indigofera tinctoria or silymarin consecutively for twenty eight days could significantly decrease the levels of renal lipid profiles in kidney when compared with paracetamol alone treated rats.*

introduction

Hepatic disorder is one of the major causes of death among the adult population globally (Etuk et al., 2009). Many traditional remedies employ herbal drugs for the treatment of liver ailments (Rao and Mishra, 1998). The plant Indigofera tinctoria belongs to the Fabaceae a family. It is popularly known as Neeli in Tamil and found throughout India, is a common remedy for various ailments. It has been cultivated from worldwide centuries. The Indigo dye is shrub one to two meter height. It may be annual, biennial or perennial. Roots and leaves are used epilepsy and hydrophobia. The phytoconstituents are responsible for the pharmacological screening in the presence of phytochemical constituents. Dry powder is used in the treatment of asthma. (Savithramma ,et. al., 2007), Leaves used for hepatotoxicity and anti-inflammatory respectively (Muthulingam et al., 2010; Tyagi et al ., 2010).

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress (Shah and Deval, 2011). It is a well known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Hurkadale, 2012). At therapeutic doses, paracetamol is considered a safe drug. However, it can cause hepatic necrosis, nephrotoxicity, extra hepatic lesions, and even death in humans and experimental animals when taken in overdoses (Ray et al., 1996). Paracetamol hepatotoxicity is related to excessive oxidative stress mainly caused by the electrophile and highly reactive metabolite of paracetamol (NAPQI) (Olaleye and Rocha, 2008). However there are no reports regarding the lipid profiles in kidney of methanolic extract of Indigofera tinctoria to paracetamol induced fatty liver. The present study was aimed to evaluate hypolipidemic role of Indigofera tinctoria against paracetamol induced fatty liver in rats.

MATERIALS AND METHODS

Procurement and rearing of experimental animals

Adult male albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room temperature (27 ± 2°C). The animals were randomized and separated into normal and experimental groups of body weight ranging from 160-200 g. The animals received a diet of standard pellets (Hindustan Lever Ltd., Bombay). Rats were provided free access to water ad libitum and food through the tenure of acclimatization to the environment for a minimum period of two weeks prior to commencement of experiment. The study was approved by the Institutional Animal Ethical Committee of Rajah Muthiah Medical College (160/1999/CPCSEA, Proposal No. 711), Annamalai University, Annamalainagar, Chidambaram.

Preparation of Methanolic Extract

The collected Indigofera tinctoria leaves were air dried and powdered. The powdered Indigofera tinctoria were kept in airtight containers in a deep freeze until the time of use. A sample containing 1 kg of Indigofera tinctoria was mixed with 4000 mL of methanol and stirred magnetically overnight (12 h) at 37°C. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolved in normal physiological saline and used in the study. The yield of the extract was approximately 42.25 g.

The suitable optimum dosage schedule were identified by administering the aqueous extract of Indigofera tinctoria extracts at different dosages (75, 150, 300 and 600 mg/kg body weight) in a day daily for twenty eight days. The optimum doses were selected as 75, 150 and 300 mg/kg body weight of the animals for twenty eight days respectively.

Experimental design

The animals were divided into 7 groups of 6 rats each.

Group 1 : Control rats given physiological saline solution 10 mL/kg body wt..
Group 2 : Rats given paracetamol (3 gm/kg body wt./po) for one day only.
Group 3 : Rats given paracetamol + Indigofera tinctoria (75 mg/kg body wt.) administered orally using an intragastric tube.
Group 4 : Rats given paracetamol + Indigofera tinctoria (150 mg/kg body wt.) administered orally using an intragastric tube.
Group 5 : Rats given paracetamol + Indigofera tinctoria (300 mg/kg body wt.) administered orally using an intragastric tube.
Group 6 : Rats given paracetamol + silymarin (25 mg/kg body wt.) administered orally using an intragastric tube.
Group 7 : Rats given Indigofera tinctoria (300 mg/kg body wt.) alone administered orally using an intragastric tube.

At the end of the experimental period in 24 h after last treatment the animals were killed by cervical decapitation. The kidney tissues were excised immediately and washed with chilled physiological saline.

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Biochemical analysis
Kidney tissues were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Lipid profiles such as cholesterol, Phospholipids, triglycerides and free fatty acids (Zlatkis et al., 1953; Zilvermit and Davis, 1950; Foster and Dunn, 1973; Falholt et al., 1973) respectively.

Statistical analysis
Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test (DMRT). The level of statistical significance was set at $p \leq 0.05$(Duncan, 1957).

RESULTS
The level of total cholesterol, phospholipids, triglycerides and free fatty acids were estimated in normal and experimental rats. There was a significant elevation of the kidney lipid profiles in rats treated with paracetamol when compared with the corresponding control rats. Administration of methanolic extracts of Indigofera tinctoria 75, 150, 300 mg/kg body weight and silymarin to paracetamol treated rats caused a significant reduction in kidney lipid profiles when compared with paracetamol alone treated rats. No effects were observed on kidney of lipid profiles when extract alone was administered rats (Table 1).

DISCUSSION
Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 2008). Lipid profiles are risk indicators of coronary heart disease (Edem, 2002). Alterations in lipids and lipoprotein levels, especially hypercholesterolemia, result in a variety of chronic complications such as coronary heart diseases and atherosclerosis (McKenney, 2001; Laker, 2006; Gould et al., 2007; Heidarian et al., 2011). The major disorder encountered in hepatitis is the fatty liver. This develops either due to excessive supply of lipids to the liver or interference with lipid transport. Liver is the primary organ concerned with lipoprotein synthesis, phospholipids and cholesterol metabolishm. Formation of fatty liver is mainly due to accumulation of triglycerides which is caused by a decreased synthesis of the apoprotein which is a component of very low density protein. Paracetamol (Acetaminophen) is a widely used analgesic and antipyretic drug (Hinson et al., 1981). Paracetamol when used at normal therapeutic dose level it is considered to be safe and effective analgesic drug. However, high dosage causes acute hepatotoxic and nephrotoxic effects both in experimental animals and in human (Dixon et al., 1975).

Fatty liver is the result of an imbalance between the rate of synthesis and utilization of hepatic triglycerides and also due to deficiency of lipoprotein factor. Murray et al., (2000) have reported that triglycerides synthesized in liver combined with glycoprotein moiety are transported to extra hepatic tissues with the help of VLDL. Cohn and Roth (1996) have reported that phospholipids is present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core.

The present study showed the increasing pattern of cholesterol, triglycerides, phospholipids and free fatty acids in the kidney of paracetamol treated rats. Interestingly, Potter et al (1974) have reported that a reactive metabolite of paracetamol, N-acetyl p-benzoquinoneimine is generated by the hepatic mixed function oxidase system in amounts sufficient to exceed its detoxification. This metabolite binds to critical macromolecules causing disturbed cellular homeostasis and eventual cell death. Excessive formation of N-acetyl p-benzoquinoneimine from paracetamol in hepatic injury resulted in accumulation of lipids. In paracetamol intoxication, a decrease protein synthesis and an inhibition of hepatic triglycerides release may be the basic causes of the syndrome seen in liver cells which progresses to cellular swelling and necrosis (Heimberg et al., 1962). Yoshiji et al., (1998) have reported that triglycerides concentration were significantly increased after Ccl4 injection and further significant increase in free fatty acids, triglycerides in serum, liver and kidney of erythromycin estolate treated rats (Venkateswaran et al., 1997). Oral administration of of methanolic extracts of Indigofera tinctoria 75, 150, 300 mg/kg body weight and silymarin to paracetamol treated rats caused a significant decrease in kidney lipid profiles. Simillarly oral administration of extracts of Astracantha longifolia on carbon tetrachloride treated rats shows minimize the levels of lipid profiles in liver and kidney (Muthulingam, 2002).

CONCLUSION
It is concluded that treatment with methanolic extract of Indigofera tinctoria decreases the paracetamol induced toxicity by minimize the levels of total cholesterol, Phospholipids, Triglycerides and free fatty acids in kidney. These findings suggest that the methanolic extract of Indigofera tinctoria was effectively lowered lipid profiles in kidney. The study demonstrates that, methanolic extract of Indigofera tinctoria have a potential hypolipidemic properties in rats.

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Table 1. Renal lipid profiles in control and experimental groups

Groups	Total cholesterol (mg/g)	Phospholipids (mg/g)	Tryglycerides (mg/g)	Free fatty acids (mg/g)
Control	5.44 ± 0.42^{a}	13.68 ± 1.03^{ab}	3.66 ± 0.28^{a}	6.34 ± 0.48^{a}
Paracetamol (3 g/kg)	8.18 ± 0.62^{d}	22.40 ± 1.71^{e}	6.12 ± 0.46^{e}	11.96 ± 0.91^{e}
Paracetamol + Indigofera tinctoria (75 mg/kg)	7.95 ± 0.60^{cd}	20.74 ± 1.58^{d}	5.98 ± 0.45^{de}	10.58 ± 0.80^{d}
Paracetamol + Indigofera tinctoria (150 mg/kg)	7.42 ± 0.56^{c}	17.16 ± 1.30^{c}	5.55 ± 0.42^{d}	9.33 ± 0.71^{c}
Paracetamol + Indigofera tinctoria (300 mg/kg)	6.12 ± 0.47^{b}	14.55 ± 1.10^{ab}	4.24 ± 0.32^{b}	7.10 ± 0.54^{ab}
Paracetamol + Silymarin (25 mg/kg)	6.38 ± 0.48^{b}	15.28 ± 1.16^{b}	4.86 ± 0.37^{c}	7.62 ± 0.58^{b}
Indigofera tinctoria (300 mg/kg) alone	5.40 ± 0.41^{a}	13.56 ± 1.03^{a}	3.61 ± 0.27^{a}	6.30 ± 0.47^{a}

All the values are mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% level ($P < 0.05$).
Duncan Multiple Range Test (DMRT).

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