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Beneficial Action of Indigofera Tinctoria (LINN.) on Hepatorenal of Glycogen and Protein Levels Against Paracetamol Induced Hepatotoxicity in Rats

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ABSTRACT

The therapeutic potential of Indigofera tinctoria was evaluated by paracetamol induced hepatotoxicity 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 hepatotoxicity and significantly decreased the levels of protein and glycogen in liver and kidney as compared with control group. Treatment with Indigofera tinctoria or silymarin consecutively for twenty eight days could significantly increase the levels of protein and glycogen in liver and kidney when compared with paracetamol alone treated rats.

KEYWORDS : Indigofera tinctoria, paracetamol, glycogen, protein

introduction

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 protein and glycogen levels in oral administration of methanolic extract of Indigofera tinctoria to paracetamol induced liver injury. The present study was aimed to evaluate the liver and kidney of protein and glycogen of extract of Indigofera tinctoria against paracetamol induced hepatotoxicity 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 liver and kidney tissues were excised immediately and washed with chilled physiological saline.

Biochemical analysis

Liver and Kidney tissues were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Biochemical parameters such as glycogen and protein levels were estimated according to standard methods (Kemp and Kits Van Heijininger, 1954; Lowry et al., 1951) 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 protein and glycogen content were estimated in normal and experimental rats. There was a significant minimizing the liver and kidney of protein and glycogen levels 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 elevation in liver and kidney of protein and glycogen when compared with paracetamol alone treated rats. No effects were observed on liver and kidney of glycogen and protein levels when extract alone was administered rats (Table 1).

Table 1. Hepatic and Renal of protein and glycogen levels in control and experimental groups

Groups	Liver Protein (mg/g)	Kidney Protein (mg/g)	Liver Glycogen (mg/g)	Kidney Glycogen acids (mg/g)
Control	164.75 ± 12.54^{d}	135.26 ± 10.29^{d}	33.46 ± 2.55^{e}	20.92 ± 1.59^{d}
Paracetamol (3 g/kg)	118.42 ± 9.02^{a}	106.38 ± 8.09^{a}	14.72 ± 1.12^{a}	11.55 ± 0.87^{a}
Paracetamol + Indigofera tinctoria (75 mg/kg)	126.34 ± 9.62^{a}	111.20 ± 8.47^{ab}	18.34 ± 1.39^{b}	13.10 ± 0.99^{a}
Paracetamol + Indigofera tinctoria (150 mg/kg)	141.74 ± 10.79^{b}	119.66 ± 9.11^{bc}	24.65 ± 1.88^{c}	15.64 ± 1.19^{b}
Paracetamol + Indigofera tinctoria (300 mg/kg)	158.29 ± 12.05cd	131.94 ± 10.04^{d}	30.17 ± 2.29^{e}	19.76 ± 1.50^{d}
Paracetamol + Silymarin (25 mg/kg)	149.67 ± 11.39^{bc}	126.48 ± 9.63^{cd}	27.48 ± 2.09^{d}	18.14 ± 1.38^{c}
Indigofera tinctoria (300 mg/kg) alone	166.37 ± 12.67^{d}	137.14 ± 10.44^{d}	34.52 ± 2.63^{e}	21.34 ± 1.62^{d}

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).

DISCUSSION

Carbohydrates are the main source of energy that is ingested by the human body (Caffall and Mohnen, 2009). Glucose is the major energy source in the body. Glycogen is the storage form of glucose and glycogen is stored in skeletal muscles and liver. If glucose intake exceeds than it is utilized in the body it is converted into fat (Asif et al., 2011). Glycogen, a large and branched polymer of glucose, is the storage form of carbohydrate for virtually every organism from yeast to primates. The major glycogen stores in mammalian vertebrates exist in liver and muscle, smaller amounts of glycogen being present in kidney, intestine and several other tissues. Classically, it is thought that the glycogen stored in liver, kidney and intestine can be made accessible to other organs by virtue of their possession of an enzyme glucose-6-phosphatase (Vornanen et al., 2011). Glycogen levels are found to be highest in liver, as it is the chief organ of carbohydrate metabolism in animals, followed by muscle. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands (Sobha et al., 2007).

The liver is the key organ of metabolism and detoxification. Continuous exposure to a variety of environmental toxic agents enhances hepatic injury (Zimmermann, 1993; Gole and Dasgupta, 2002). A growing interest has emerged around the globe in rediscovering medicinal plants as useful therapeutic agents for the prevention of such injury. Successful liver therapy owes much to the identification of pathogenesis and elaboration of suitable models of hepatic injury, comparable to those encountered in clinical practice (Stavinoha and Soloway, 1990).

The presence of glycogen in vital organs is considered as the major sources of energy for undertaking various metabolic processes and proteins are known as essential components for the formation of tissues and chief sources of enzymes. Most of the physiological actions in the body are motivated through modifications in proteins. Various authors have reported that the administrations of chemicals are known to alter the glycogen levels in the liver and kidney (Chandar and Siddique, 1987; Hussain and Mirza, 1985). Some of the plant extracts and their preparations are also known to change the liver glucogen (Seshadri et al., 1980; Banarjee et al., 1986). Marked reduction of serum and protein levels were observed in paracetamol treated rat. These events could be mediated through the covalent binding of NAPQI to proteins which has been reported as the causative factors for oxidative damage, proteins denaturation and changes in protein structures that contribute to the reduction of serum and liver protein levels (Hazai et al., 2002; Amsath et al., 2011).

In the present investigation the hepatic and renal of glycogen and protein contents were decreased in paracetamol treated rats when compared with control rats. Hawk (1937) has reported that depletion of hepatic glycogen is a common complication of cirrhosis and other liver diseases. Udupa et al., (2000) have reported that hepatic glycogen content was decreased due to paracetamol treatments of the rats. Mandel et al., (1992) have reported that Ccl4 treatment in mice affected the levels of hepatic DNA, RNA and protein content. Atroshi et al., (2000) have reported a decrease in liver enzyme activities and an increase in protein level in the ochratoxin A and T2 toxin intoxicated rats when pretreated with antioxidants such as vitamin E and selenium. In the present study, Indigofera tinctoria significantly attenuated the glycogen and protein levels in liver and kidney induced by paracetamol. Oral administration of Indigofera tinctoria to paracetamol treated rats showed increased protein and glycogen levels in liver and kidney. Simillarly oral administration of extracts of Astracantha longifolia on carbon tetrachloride treated rats shows enhance levels of protein and glycogen in liver and kidney (Muthulingam, 2002).

CONCLUSION

It is concluded that treatment with methanolic extract of Indigofera tinctoria decreases the paracetamol induced toxicity by enhance the levels of protein and glycogen in liver and kidney. These findings suggest that the methanolic extract of Indigofera tinctoria was effective in bringing about functional improvement of liver and kidney. The study demonstrates that, methanolic extract of Indigofera tinctoria have a potential therapeutic approach to hepatorenal protective properties.

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