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RENEAL ANTIOXIDANT AND LIPID PEROXIDATIVE ROLE OF
INDIGOFERA TINCTORIA (LINN.) AGAINST PARACETAMOL INDUCED
HEPATOTOXICITY IN RATS

F. ANNIE FELICIA AND M. MUTHULINGAM*
Department of Zoology, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamilnadu, India
*Corresponding author e-mail address: muthuau@rediffmail.com
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ABSTRACT

Antioxidants had been proven to play an important role in the regulation of a vast array of physiological and pathological processes. They principally contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals. Plants play an important role in the maintenance of human health primarily via nutrition and also contribute greatly to the management of various ailments. 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 the administration of paracetamol (3 gm/kg body weight- po) only one day. Paracetamol induced hepatotoxicity and significantly increased the activities of lipid peroxidation (TBARS), decreased the activities of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in kidney as compared with the control group. Treatment with Indigofera tinctoria or silymarin consecutively for twenty eight days could significantly decrease the activity of TBARS where as enhance the activities of SOD, CAT and GSH in the kidney when compared with paracetamol alone treated rats.

Key words: Indigofera tinctoria, paracetamol, antioxidant, lipid peroxidation, kidney.

INTRODUCTION

The liver is the vital organ of metabolism and excretion. About 20 000 deaths were found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 250 000 new cases each year (Meganathan et al., 2011). The use of herbal medicine can be traced back to 2100 BC in ancient China at the time of Xia dynasty and in India during the Vedic period. The first written reports are timed to 600 BC with Charaka Samhita of India, and in China the same became systematic by 400 BC. The basic concept in the medicinal systems is that the disease is a manifestation of a general imbalance of the dichotomous energies that govern life as a whole and human life in particular, and they focus on medicine that can balance these energies and maintain good health. Indian systems of medicine have primarily claimed a curative potential for their medicinal preparations for all kinds of liver diseases. In spite of the significant popularity of these medicinal systems, they are still to be recognized as being universally acceptable treatment modalities for chronic liver disease (Thyagarajan et al., 2002).
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 and glutathione depletion (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, PCM 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 PCM (NAPQI) (Olaleye and Rocha, 2008). The oxidative stress produced by high doses of PCM has also been demonstrated to affect the antioxidant system (Bessens and Vermeulen, 2001). Lipid peroxidation, which is secondary to NAPQI induced GSH depletion and oxidative stress, can cause irreversible membrane injury and cell death (Albano et al., 1985; Kyle et al., 1987). However there are no reports regarding the antioxidant and lipid peroxidative role of Indigofera tinctoria. The present study is aimed to evaluate the renal antioxidant and lipid peroxidative role of an 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 the 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 methonolic 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 was 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 of 24 h after last treatment the animals were killed by cervical decapitation. The kidney tissues were excised immediately and washed with chilled physiological saline.

**Biochemical analysis**

Kidney tissues were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Biochemical parameters such as lipid peroxidation (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities were estimated according to standard methods (Niehaus and Samuelson, 1968; Ellman, 1959; Kakkar *et al.*, 1984; Sinha, 1972) 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 ≤ 0.05 (Duncan, 1957).

**RESULTS**

**Renal lipid peroxidation**

The level of lipid peroxidation in the kidney was estimated in normal and experimental rats. There was a significant elevation in lipid peroxidation in rats treated with paracetamol when compared with the corresponding control rats. Administration of *Indigofera tinctoria* 75, 150, 300 mg/kg and silymarin to paracetamol treated rats caused a significant reduction in lipid peroxidation when compared with paracetamol alone treated rats. No effects were observed on lipid peroxidation activities when extract alone was administered (Table 1).

**Reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in kidney**

The levels of kidney non-antioxidant and antioxidant enzymes such as GSH, SOD and CAT were analyzed in normal and experimental rats. There was a significant decrease in kidney glutathione (GSH), superoxide dismutase and catalase in rats treated with paracetamol when compared with the corresponding control rats. Oral administration of methanolic extract of *Indigofera tinctoria* 75, 150, 300 mg/kg and silymarin to paracetamol induced hepatic damage in rats caused a marked increase in the activities of GSH, SOD and CAT as compared with paracetamol alone treated rats. The extract alone treated rats did not show any significant alterations when compared with control group (Table 1).
Table 1. Activities of SOD, CAT, GSH and TBARS in kidney of control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units(^a))</th>
<th>CAT (Units(^b))</th>
<th>GSH</th>
<th>TBARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.41 ± 0.48(^d)</td>
<td>52.38 ± 3.98(^d)</td>
<td>5.72 ± 0.43(^d)</td>
<td>1.09 ± 0.08(^a)</td>
</tr>
<tr>
<td>Paracetamol (3 g/kg)</td>
<td>3.23 ± 0.24(^a)</td>
<td>29.74 ± 2.26(^a)</td>
<td>3.34 ± 0.25(^a)</td>
<td>3.43 ± 0.26(^a)</td>
</tr>
<tr>
<td>Paracetamol + Indigofera tinctoria (75 mg/kg)</td>
<td>3.63 ± 0.27(^a)</td>
<td>34.60 ± 2.63(^b)</td>
<td>3.92 ± 0.29(^b)</td>
<td>3.06 ± 0.23(^a)</td>
</tr>
<tr>
<td>Paracetamol + Indigofera tinctoria (150 mg/kg)</td>
<td>4.86 ± 0.37(^b)</td>
<td>41.28 ± 3.14(^c)</td>
<td>4.76 ± 0.36(^c)</td>
<td>2.25 ± 0.17(^d)</td>
</tr>
<tr>
<td>Paracetamol + Indigofera tinctoria (300 mg/kg)</td>
<td>6.12 ± 0.46(^cd)</td>
<td>49.86 ± 3.79(^e)</td>
<td>5.60 ± 0.42(^d)</td>
<td>1.31 ± 0.98(^b)</td>
</tr>
<tr>
<td>Paracetamol + Silymarin (25 mg/kg)</td>
<td>5.89 ± 0.44(^c)</td>
<td>45.32 ± 3.45(^d)</td>
<td>5.38 ± 0.40(^d)</td>
<td>1.57 ± 0.12(^e)</td>
</tr>
<tr>
<td>Indigofera tinctoria (300 mg/kg) alone</td>
<td>6.42 ± 0.48(^d)</td>
<td>52.70 ± 4.01(^e)</td>
<td>5.74 ± 0.43(^d)</td>
<td>1.08 ± 0.08(^a)</td>
</tr>
</tbody>
</table>

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).

Units\(^a\) = one unit is as 50% inhibition of NBT/mg protein.

Units\(^b\) = μmoles of H\(_2\)O\(_2\) utilized/min/mg protein.

TBARS= n moles of TBARS/mg protein, GSH= μgm of GSH consumed (min) mg protein.

DISCUSSION

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).

Antioxidants had been proven to play an important role in the regulation of a vast array of physiological and pathological processes. They principally contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals. In addition, they enhance the immune system, modify carcinogen metabolism, alter cell proliferation and stimulate the repair of DNA damage (Barber and Harris, 1994). Antioxidant defense systems have coevolved with aerobic metabolism to counteract oxidative damage by ROS (Reactive Oxygen Species) from various internal and external sources (Puertollano et al., 2011). Presence of other antioxidant enzymes such as SOD and CAT also aids the protection to the tissues. SOD is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide to hydrogen peroxide, while CAT is a peroxisomal heme protein that catalyses the removal of hydrogen peroxide formed by SOD. Thus, SOD and CAT are mutually supportive antioxidative enzymes (Sathyana and Sidduraju, 2013). GSH, a powerful intracellular tripeptide antioxidant found in many mammalian tissues, is a consumer of superoxide, singlet oxygen and hydroxyl radicals by combining with non-protein thiols at their reactive center and abolishes free radical toxicity (Mitchell et al., 1973).

Lipid peroxidation has been identified as one of the basic reactions involved in oxygen free radical induced cellular damages (Halliwell and Gutteridge, 1992). Peroxidation reactions in biological systems are the underlying causes for a variety of pathological conditions (Estuo and Hiroyuki, 1990). Lipid peroxidation is a measurement of function of cellular membranes. The levels of TBARS are an indirect measurement of the lipid peroxidation (Halliwell et al., 1995). The reactive free radicals initiate cell damage through two major mechanisms of covalent binding to...
cellular macromolecules and lipid peroxidation (Slater, 1984; Brattin et al., 1985). The free radicals initiate lipid peroxidation and could produce a range of enzymatically damaging consequences and could result in membrane disorganization by peroxidizing mainly the highly unsaturated and polyunsaturated fatty acids by attacking the methylene bridge hydrogen (Slater, 1972). Lipid peroxidation may produce injury by compromising the integrity of membranes and by covalent binding of reactive intermediates to important biological molecules like GSH; the process leads to necrosis in general. Moreover, the resulting free radicals may provoke inflammatory responses resulting in the release of pro-inflammatory cytokines and chemokines, which may lead to cellular death (Yousef et al., 2010).

In the present study, *Indigofera tinctoria* significantly attenuated the oxidative stress induced by paracetamol. Oral administration of *Indigofera tinctoria* to paracetamol treated rats showed decreased TBARS levels and elevated antioxidant enzyme activity (SOD, CAT and GSH) in the kidney. Similarly oral administration of extracts of *Astracantha longifolia* on carbon tetrachloride treated rats showed minimize the lipid peroxidation levels and enhance the antioxidant activities in kidney (Muthulingam, 2002).

**Conclusion**

It is concluded that treatment with a methanolic extract of *Indigofera tinctoria* decreases the paracetamol induced toxicity in biochemical parameters. These findings suggest that the methanolic extract of *Indigofera tinctoria* was effective in bringing about functional improvement of kidney. The enhancement of the antioxidant effect of this extract was also confirmed by minimizing the lipid peroxidative activities were observed. The study demonstrates that, methanolic extract of *Indigofera tinctoria* has a potential therapeutic approach to renal protective property.

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