Protective effect of Oroxylum indicum on acetaminophen induced liver injury in rat

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ABSTRACT
Acetaminophen (APAP) is a common antipyretic drug and leads to liver failure at over dose. In this study, the hepatoprotective effect of Aqueous Methanolic Bark Extract of Oroxylum indicum (L.) Vent. (AMBEOI) has been evaluated in rat model. Rats were treated with 1000 mg kg⁻¹ body weight of APAP alone or with AMBEOI (10, 50 and 100 mg kg⁻¹). Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lipid peroxidation (LPO) in liver tissues were estimated 24 hrs after APAP and AMBEOI treatment. AMBEOI administration effectively reduced serum level of ALT and AST released from liver cells when compared to APAP treated group. AMBEOI also inhibited production of LPO in the liver tissues of APAP treated rats. Histopathological examination of liver samples revealed reduced necrotic areas in AMBEOI treated APAP group compared to APAP alone treated group. Together, this study confirmed the hepatoprotective activities of AMBEOI in APAP induced liver damage in rat.

Key Words: ALT, AST, LPO, hepatotoxicity, AMBEOI, traditional medicine.

INTRODUCTION
Acetaminophen (APAP) is a commonly used analgesic and antipyretic drug and is safe at therapeutic levels, but overdose can lead to potentially fatal hepatic necrosis in humans and experimental model animals (Proudfoot and Wright, 1970; Cobden et al., 1982; Prescott, 1980). APAP overdose is one of the most common/frequent causes of liver failure in western world (Lee, 2004). At overdose APAP is metabolized in the liver by cytochrome P450 (CYP) into reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) (Dahlin et al., 1984; James et al., 2003b). NAPQI is known to deplete cellular glutathione (GSH), a natural antioxidant level and generate oxidative stress that turn into production of free radicals such as reactive oxygen (ROS) and reactive nitrogen species (NOS) (Mitchell et al., 1973). This results in imbalance of cellular antioxidant defense mechanism in liver hepatocyte cells and thus finally leads to hepatotoxicity (Reid et al., 2005). Due to known mode of its hepatotoxicity APAP is widely used as a model liver toxin for experimental validation of hepatoprotective drugs.

The production of various free radicals and successive oxidative stress leads to adverse effect on cellular level of an organ. Herbal antioxidants are widely used for the treatment and prevention of several diseases (Uttara et al., 2009). Current therapeutic research is directed towards finding naturally occurring antioxidants particularly of plant origin. Many plant species are reported to possess potential biomolecules to become a source of hepatoprotective drugs and search is still going on to find the best one. Tangiang et al. (2011) has reported the uses of different plant species as hepatoprotective among the traditional medicine practitioners of Arunachal Pradesh, India (Tangiang et al., 2011).

Oroxylum indicum (L.) Vent. (Bignoniaceae) (Also known Shyonaka, Sonapatha) is a deciduous tree growing in China, Thailand and Southeast Asia including India characterized by sword shaped seed pods. This plant is also found to occur abundantly in the North-Eastern part of India. Oroxylum indicum is widely used in Indian traditional Ayurvedic formulation namely Dasamula, Chyavanprasa, Brahma Rasayana, Dhanamastara, Amla and Narayana Taila (Bhattacharje, 2000). The bark decoction of Oroxylum indicum is a traditionally used by the Adi tribe of Arunachal Pradesh for curing hepatobiliary diseases and cardiac problems (Tangiang et al., 2011). Oroxylum indicum also reported to contain strong antioxidant (Siriwatanam et al., 2010; Tenpe et al., 2009; Kumar et al., 2010), anti-inflammatory (Doshi et al., 2012), antiproliferative and antitumor (Mao, 2002; Lambertini et al., 2004; Brahma et al., 2011) activities. The stem bark of Oroxylum indicum reported to contain flavonoids like baicalein, chrysir and oroxylin A (Sankara and Nair, 1972a; Sankara and Nair, 1972b). Taking this information into account, the present work was carried out to evaluate the hepatoprotective effect of Oroxylum indicum bark extract on APAP induced hepatotoxicity in rat.

MATERIALS AND METHODS

Chemicals
Acetaminophen (APAP) and Thiobarbituric acid (TBA), 1, 1, 3, 4-tetraethoxypropane were purchased from Sigma-Aldrich. Serum ALT and AST enzymes were estimated using commercial kits (Medsource Ozone Biochemicals Pvt. Ltd, India). All other chemicals used during the experiment were analytical grade.

Collection and preparation of Plant extract
The plant material Oroxylum indicum was collected from the Rajiv Gandhi University campus, Arunachal Pradesh and was identified with the help of taxonomist from the Department of Botany, Rajiv Gandhi University. The voucher specimen (LBC/RCU/2013/01) was deposited at Centre with Potential for Excellence in Biodiversity (CPEB), Rajiv Gandhi University for future reference. The

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Table 1: Serum ALT and AST levels in different experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/ml)</th>
<th>AST (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65 ± 6.55</td>
<td>71 ± 14.42</td>
</tr>
<tr>
<td>OI 100</td>
<td>85.66 ± 10.01</td>
<td>92.74 ± 8.96</td>
</tr>
<tr>
<td>APAP 1000</td>
<td>784 ± 107.01a</td>
<td>742.33 ± 56.21a</td>
</tr>
<tr>
<td>APAP 1000 + OI 10</td>
<td>764.66 ± 36.25</td>
<td>717.25 ± 57.49</td>
</tr>
<tr>
<td>APAP 1000 + OI 50</td>
<td>580 ± 54.78 b</td>
<td>604.61 ± 38.68 b</td>
</tr>
<tr>
<td>APAP 1000 + OI 100</td>
<td>367.86 ± 66.80 b,c</td>
<td>374.2 ± 66.52 b,c</td>
</tr>
</tbody>
</table>

Values are mean ± SD of five animals per group. *p<0.001, significantly differ from control,  
bp<0.05, significant differ from APAP group and  
p<0.001, significantly differ from APAP1000 + OI 50 group.

Histopathological analysis
Liver samples were fixed in 10% neutral buffered formalin overnight, washed well in running tap water, dehydrated, cleared in xylene and embedded in paraffin. Liver sections were cut into 5 μm thickness, processed in alcohol grades and stained in haematoxylin and eosin (H&E) (Luna, 1968) for histopathological examinations. Sections were photographed using canon digital image recorder.

Statistical analysis
All data were presented as means ± SEM (n=5). Statistical analysis were performed using one way analysis of variance (ANOVA) followed by Tukey’s post hoc test. A level of p-value < 0.05 was considered for determining statistical significance.

RESULTS
Effects of AMBEOI on APAP induced ALT & AST in serum
Overdose administration of APAP caused significant elevation of serum ALT and AST in treated groups (table 1, p<0.001). In contrast, AMBEOI co-treatment significantly reduced serum ALT and AST elevation in APAP treated mice compared to only APAP 1000 mg/kg treatment group (table 1, P<0.05, 0.001). AMBEOI 100 mg/kg supplementation caused approximately 50% reduction of serum ALT and AST level compared to control group of rat.
Effect of AMBEOI on APAP induced lipid peroxidation (LPO)
APAP treatment caused significant increase in the level of TBRAS, a marker of LPO in the hepatic tissue homogenate compared to control group (figure 1, p<0.001). On the other hand co-treatment of rat with AMBEOI significantly reduces the tissue level of LPO (Figure 1, p<0.05, 0.001).

Effect of AMBEOI on APAP induced liver histology
The liver sections in control rat were looking normal with well-defined hepatocytes surrounding the central vein with clear nuclear architecture and cell membrane (figure 2 A). APAP 1000 mgkg\(^{-1}\) caused severe centrilobular necrosis in rat 24 hours after treatment. The hepatocytes surrounding central vein shows extensive necrosis with nuclear pyknosis and vacuolar cytoplasmic degeneration (figure 2 C.). AMBEOI supplementation reduced the hepatocytes necrosis caused by APAP and restored a near normal morphological feature as was observed in groups receiving 10, 50 and 100 mgkg\(^{-1}\) AMBEOI (figure 2 D).

DISCUSSION
Oroxylum indicum (L.) Vent. is known to have spacious therapeutic applications in traditional medicinal system, and scientific advancement has provided extensive evidence to support most of its medicinal claims. The present in vivo study has demonstrated the hepatoprotective potential of this plant.

Liver is a major target organ for metabolizing the drugs and xenobiotics (Jaeschke et al., 2002). APAP when used at high doses could cause acute liver injury via formation of NAPQI, a toxic metabolite, by cytochrome P450. NAPQI are usually inactivated by hepatic GSH, but when produced excessively, it covalently binds to centrilobular hepatic proteins and elicits hepatic toxicity (Jaeschke et al., 2002; Gardner et al., 1998; Gardner et al., 2002). In this present study hepato cellular damage induced by APAP (1000 mgkg\(^{-1}\)) intoxication in rat was established based on significant elevations in ALT and AST activities as done by previous workers (Walubo et al., 2004; Kim et al., 2009; Jin et al., 2012).
High concentration of ALT is found mainly in the liver hepatocytes and AST which is localized in the mitochondria, are specific serum biomarkers of hepatic injury caused by drugs or toxic metabolites. But when the damage to hepatocytes occur due to exogenous toxic substances or their metabolites, these enzymes are released into bloodstream and cause an elevation in the serum levels of ALT and AST (Rej, 1978; Schmidt, 1978). Administration of APAP overdose in rat caused significant increases in the serum ALT and AST level compared to the control untreated animals as observed in the present study. AMBEOI treatment significantly and dose dependently reduced the release of hepatic ALT and AST as observed from the serum level of these two enzymes in the treated groups. Similar hepatoprotective activities of plant extract such as Acacia nilotica and Moringa oleifera were noted previously and shown to decreases the level of serum ALT and AST in APAP intoxicated rat (Kannan et al., 2013; Fakurazi et al., 2013).

APAP treatment significantly increased the level of TBARS, a marker of lipid peroxidation (LPO) and an indicator of oxidative stress in the treated rat. The oxidative stress caused by APAP overdose was primarily due to excess formation of NAPQI (Jaeschke et al., 2003). In normal circumstances, cellular GSH content of the hepatocytes aids in the process of detoxification of NAPQI (Mitchell et al., 1973). But during overdose GSH content deplete earlier than the rate of its synthesis, which create an imbalance in the cellular antioxidant level. In the absence of sufficient GSH, NAPQI binds to the proteins in the centrlobular hepatocytes and caused necrosis (Rej, 1978). The role of GSH depletion in APAP induced liver injury was further confirmed from observation that rats treated with antioxidant such as N-acetylcysteine was immune to APAP overdose (Lauterburg et al., 1983; Smilkstein et al., 1988). This suggested the role of therapeutic supplementation of antioxidants in attenuating oxidative stress induced liver injury in real life scenario.

The plant selected for the present study is reported to possess strong antioxidant properties (Moirangthem et al., 2013). The decrease in the level of TBARS after administration of AMBEOI in the liver tissue of APAP intoxicated rat suggested the strong antioxidant property present in the AMBEOI. Previously reported studies also confirmed the antioxidant property of the selected plant species under consideration (Moirangthem et al., 2013; Zaveri and Jain, 2007; Shetgiri et al., 2010; Tenpe et al., 2009). The observation that administration of AMBEOI reduces formation of TBARS and similar findings from other researchers confirmed that AMBEOI act as strong antioxidant and protects the hepatocytes during APAP overdose.

CONCLUSION

The findings of the present study indicated that aqueous methanolic bark extract of Oroxylum indicum (L.) Vent. (AMBEOI) protect hepatic tissues against APAP induced liver injury in rat. Together this study further confirmed the potential hepatoprotective action of AMBEOI in drug induced oxidative damage of the liver in experimental models and can be good candidate to bank upon for further research in experimental drug discovery processes.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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