Short Communication

Liver histopathological effects of hydroalcoholic, chloroform, butanol and aqueous fractions of *Teucrium polium* in dexamethasone-induced hyperlipidemic rats

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**ABSTRACT**

This study aimed to assess the effect of hydroalcoholic, chloroform, butanol and aqueous fractions of *Teucrium polium* on liver histopathology in dexamethasone-induced hyperlipidemic rats. Animals received 150 mg/kg of different fractions of *T. polium* simultaneously with dexamethasone 10 mg/kg/day for 7 days. Dexamethasone caused fatty degeneration, diffused steatosis and mild cellular swelling. All treatments resulted in a significant reduction in steatosis. However, chloroform and aqueous fractions caused severe inflammation and necrosis in liver tissues. In spite of strong hypolipidemic activity, both chloroform and aqueous fractions of *T. polium* should be considered as the most hepatotoxic portions of this plant.

**Key words:** *Teucrium polium*, hyperlipidemia, dexamethasone, lipid-lowering activity.

**INTRODUCTION**

*Teucrium polium* L (Labiatae) is a sub-shrub and perennial plant characterized by its cylindrical leaves and pink to white small flowers. It grows well in the rocky areas and sandy lands of the Mediterranean regions and is cultivated throughout the west of Asia. *T. polium* possess several bioactive compounds and is used as an herbal tea or a spice (Mirghazanfari et al., 2010). Pharmacological studies have proven various antioxidant, antimicrobial, anorexic, antiabetic, antipyretic, diuretic, antispasmodic, antihypertensive, anti-inflammatory, antinociceptive, cardio- tonic, antiagastic ulcer and cytotoxic properties for *T. polium* (Parsaei and Shafee-Nick, 2006; Bello et al., 1997). Previous studies reported the strong hypoglycemic and hypolipidemic effects for *T. polium* extract, however some hepatotoxic activities have been observed (Rasekh et al., 2001; Vahidi et al., 2010). In order to screen the hepatotoxicity of *T. polium*, this study aimed to estimate the effect of hydroalcoholic, chloroform, butanol and aqueous fractions of *T. polium* aerial parts on liver histopathology in dexamethasone-induced hyperlipidemic rats.

**RESULTS AND DISCUSSION**

Semiquantitative morphological changes of the liver tissues have been presented in Table 1. As shown in Table 1 and Figure 1, no pathological change was seen in the liver architecture from normal control rats (Figure 1A). Morphological inspection of liver sections 1 week after exposure to the high dose of dexamethasone discovered histological alterations as fatty degeneration, diffused steatosis and mild cellular swelling (Figure 1B). Several mechanisms may be involved in the hyperlipidemic activities of glucocorticoids including increase in hepatic lipogenesis and accumulation of triglyceride, differentiation of pre-adipocytes and reduction in the activity of lecithin cholesterol acetyl transferase enzyme (Dourakis et al., 2002, Wang, 2005).

Treatment with atorvastatin (Figure 1C) showed a significant attenuation in the histopathological alterations of liver caused by dexamethasone. Administration of *T. polium* hydroalcoholic extract made significant decrease in fatty degeneration and steatosis compared with dexamethasone-induced hyperlipidemic group (Figure 1D). Treatment with aqueous fraction of *T. polium* also resulted in a notable reduction in fatty degeneration and steatosis but harmful effects including infiltration of lymphocytes and necrosis were observed in some areas of the liver tissue (Figure 1E). The butanol fraction partially reduced the histological changes induced by dexamethasone, however its hypolipidemic effect was less than other
Table 1: Semiquantitative scoring of hepatic injury after treatment with different fractions of T. polium in dexamethasone-induced dyslipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Score of injuries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatty degeneration</td>
</tr>
<tr>
<td>Normal control</td>
<td>0</td>
</tr>
<tr>
<td>Dexamethasone hyperlipidemic control</td>
<td>4</td>
</tr>
<tr>
<td>Dexamethasone + Atorvastatin</td>
<td>1</td>
</tr>
<tr>
<td>Dexamethasone + T. polium Hydroalcoholic fraction</td>
<td>1</td>
</tr>
<tr>
<td>Dexamethasone + T. polium Aqueous fraction</td>
<td>1</td>
</tr>
<tr>
<td>Dexamethasone + T. polium Butanol fraction</td>
<td>2</td>
</tr>
<tr>
<td>Dexamethasone + T. polium Chloroform fraction</td>
<td>0</td>
</tr>
</tbody>
</table>

For generation of hyperlipidemia, subcutaneously injection of dexamethasone (10 mg/kg/day) was done for 7 days. Male Wistar albino rats were randomly used in 7 groups containing 6 rats in each as the following: 1) dexamethasone hyperlipidemic group, 2) normal control group received daily administration of vehicle (normal saline), 3) positive control group received atorvastatin (40 mg/kg) orally simultaneously with dexamethasone, and 4-7) treatment groups received dexamethasone and orally administration of 150 mg/kg hydroalcoholic, chloroform, butanol or aqueous fractions of T. polium for 1 week. At the end of the experiment, the liver were separated from the sacrificed animals and reserved in formalin solution and treated for histopathological inspections. The liver sections were examined for steatosis or fatty degeneration, inflammation, cell swelling and hepatocyte necrosis. The intensity of hepatic injuries was graded as follows: Score 0: No visible cell damage; Score 1: Focal hepatocyte damage on less than 25% of the tissue; Score 2: Focal hepatocyte damage on 25%-50% of the tissue; Score 3: Extensive, but focal, hepatocyte lesions; Score 4: Global hepatocyte necrosis.

Figure 1: Representative hematoxylin and eosin histological sections of the liver tissue. A) Normal control group showing normal histological architecture; B) Dexamethasone-induced hyperlipidemic group presenting mild cellular swelling and diffused steatosis; C) Atorvastatin treated group and D) the group treated with hydroalcoholic extract of T. polium showing small fatty degeneration; E) the group treated with aqueous fraction of T. polium showing small fatty degeneration with inflammation and necrosis; F) Butanol fraction treated group indicating cellular swelling; G) Chloroform fraction treated group presenting less fatty degeneration with several areas of cellular edema, inflammation and necrosis. Black thick arrows, white arrows and black thin arrows show inflammation, necrosis and cellular swelling, respectively (×40 magnification).
fractions and it increased the cellular swelling in hepatocytes (Figure 1F). Although the chloroform fraction of *T. polium* showed the most potent hypolipidemic activity by preventing fatty degeneration and steatosis but it caused the most toxic effects through severe inflammation and necrosis in the liver tissues (Figure 1G). Figure 2 represents the histological sections of the liver tissue after treatment with aqueous (A) and chloroform (B) fractions of *T. polium* with lower magnification. The damaging histological alterations were observed in the perivenular and midzonal areas of some liver lobules (Figure 2A and 2B).

Various phytochemical constituents including terpenoids, flavonoids and polyphenolic compounds may be involved in the hypolipidemic activities of *T. polium* (Bahramikia and Yazdanparast, 2012). Among various fractions of *T. polium*, the most potent effect in diminishing steatosis however undesirably the most hepatotoxic activity was detected by chloroform fraction. Similarly, aqueous fraction showed severe hepatotoxic activity. Butanol fraction resulted in a partially diminution in steatosis and unpleasant rise in hepatocytes swelling. While the hydroalcoholic extract of *T. polium* showed the suitable effect in diminution of steatosis with less hepatotoxicity compared aqueous and chloroform fractions.

Highly increased activities of the liver enzymes have been reported after exposure to aqueous or alcoholic extract of *T. polium* in previous studies (Vahidi et al., 2010; Khazaei et al., 2018). In the investigation of Mehdinia et al. (2013) petroleum ether fraction of *T. polium* caused elevation in liver enzymes in mice.

Zal et al. (2001) detected degenerative alteration in the lobules of liver in diabetic rats treated with aqueous extract of *T. polium* however they reported non-significant infiltration of inflammatory cells. In our study, the occurrence of inflammatory reactions with the existence of mononuclear cells was identified in the liver tissues of animals treated with aqueous and chloroform fractions.
suggesting the possible role of immune response, cytotoxic lymphocytes and release of cytokines in their hepatotoxicity. Polymeros et al. (2002) also reported acute cholestatic hepatitis with temporary presence of anti-mitochondrial antibody induced by *T. polium*. The undesirable histological alterations observed in all of the groups treated with *T. polium* propose possible direct cellular injuries maybe through some phytochemical constituents or their metabolites. Earlier studies have shown the role of neo-clerodane diterpenes, particularly teucrin A and teuchamaedryn A and oxidation of their furan and hydrofuran rings by CYP3A4 and subsequently production of reactive epoxides in hepatotoxicity of *T. polium* (Bedir et al., 1999).

A previous in vitro study by Pacifico et al. (2012) recognized some new methanolic fractions without hepatotoxic properties after more fractionation of methanolic extract. They found that both aqueous and ethyl acetate fractions have cytotoxic activities dose- and time-dependently in hepatoblastoma cell, however, ethyl acetate fraction has more cytotoxicity compared aqueous fraction. After further fractionations, some methanolic fractions containing high amount of poliumoside displayed strong antioxidant capacity without cytotoxicity while chloroform fraction showed higher content of neo-clerodanes than the initial crude extract with the highest cytotoxicity (Pacifico et al., 2012).

**CONCLUSION**

In spite of the helpful effects of hydroalcoholic, chloroform, butanol and aqueous fractions of *T. polium* in eliminating the fatty degeneration and steatosis in dexamethasone-induced hyperlipidemia, all fractions showed hepatotoxic activities particularly aqueous and chloroform fractions. Considering the potential hepatotoxic constituents, bio-guided fractionation for separation of toxic components and preparation of safe efficient medicine from *T. polium* is strongly recommended.

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**REFERENCES**


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