ACUTE TOXICITY (LETHAL DOSE 50 CALCULATION) AND HISTOPATHOLOGICAL EFFECTS OF METHANOLIC EXTRACT OF BERBERIS VULGARIS IN MICE

Muhammad Ahmed¹*, Aisha Azmat²

¹Department of Pharmacology, Faculty of Pharmacy, Umm Al-Qura University, P.O. Box 13578, Makkah, Saudi Arabia.
²Department of Physiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.

ABSTRACT
The arithmetic method of Karber for the calculation of LD50 and histopathology were used to study the acute toxicological effects of Methanolic extract of Berberis vulgaris root pulp (BRPM) on albino mice. The study involved different doses (10, 100, 500 and 1000mg/Kg) of the extract by intraperitoneal administration to groups of mice. Signs of toxicity and possible death of animals were monitored for 24 hrs to ascertain the median lethal dose (LD50) of the extract. At the end study, all the animals in all the dose groups were sacrificed and the internal organ-body were compared with values from the control group. The LD50 was found to be 666.66 mg/Kg body weight upon intraperitoneal administration in mice. The histopathological examination indicated that at the dose of 1000mg/Kg, extract induced several histopathological changes in the liver of mice such as necrosis of some hepatocytes, edema, scattered bile canaliculi and damage in septa between lobes of liver. The obtained LD 50 values classify the BRPM (i.p.) as slightly toxic according to Hodge and sterner toxicity scale.

KEY WORDS
Acute toxicity, Berberis vulgaris, Histopathological effects, Lethal dose (LD), Median Lethal dose (LD50).
INTRODUCTION

*Berberis vulgaris* L., or European barberry is a shrub with sour fruits rich in Vitamin C (Dorfler and Roselt, 1989) cultivated in Europe and Asia. Literature reported that *Berberis vulgaris* contain different components like berbamine and berberine (Imanshahidi and Hosseinzadeh, 2008), and derivatives such as berberrubine, bervulcine, columbamine, isotetrandrine, jatrorrhizine, magnoflorine (Pavelka and Sme′kal, 1976), oxycanthine and vulvracine and miscellaneous components, including chelidonic acid, resin, tannin (Hanachi and Golkho, 2009).

Barberries have been used in folkloric medicine for the treatment of different problems as liver dysfunction (Jaundice), gallbadder pain, gall stone diarrhea, indigestion and urinary tract diseases (Foster and Taylor, 1999: Jellin et al., 2000: Chevallier, 1996 and Gruenwald, 1998). Different extracts of Berberis species are used for the treatment of various inflammatory ailments (Yesilada and Kupeli, 2002), antiviral activitie, antimicrobial properties (Musumeci et al., 2003). Aqueous extract from *Berberis vulgaris* fruit was reported for its antihypertensive effects (Fatehi, et al., 2005) while the root bark and root pulp is said to be hypotensive (Azmat and Ahmed, 2014: Azmat et al., 2009). Javadzadeh and Fallah 2012 have reported that use of barberry can cause nausea, regurgitatation, vertigo, convulsion, nosebleed, kidney failure, skin and eye inflammation and decrease blood sugar.

In the view of above reported literatures the current study had planned to the determination of toxicity of Methanolic extract of Berberis root pulp and LD50.

MATERIALS AND METHODS

The study consisted of following steps

Formation of Methanolic extract

Uncrushed root of *Berberis vulgaris* was washed with water and then extracted with methanol for four times at room temperature then evaporated on rotavapour. Yellowish brown colored residue (BRPM) was collected after evaporation (Azmat and Ahmed 2014),used for the determination of LD50.

Animals

30 Adult NMRI mice (20-25g) of either sex were obtained from Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS) and were housed in groups of
6 per cage for seven days prior to experimentation in an ideal laboratory environment (Table 1). Each experimental group consisted of six animals.

**Toxicological/Safety evaluation studies in mice**

Five groups containing six NMR-I mice (25-30 g) in each were used in this study. All animals were treated i.p once as shown in Table 2a.

Animals were weighed before the dose administration. All the animals were kept under continuous observation for 6 hours after the administration of dose, for any change in behavior or physical activities. After 24 hr all survived mice were anaesthetized with pentothal sodium (40mg/kg) and autopsied.

**Calculation of Median Lethal dose (LD50)**

For each mice the observation were made for 24 hr and symptoms of toxicity and rate of mortality in each group were noted. At the end of study period expired animals were counted for the calculation of LD50. The arithmetic method of Karber (1931) was used for the determination of LD 50.

\[
LD50 = \frac{LD100 - \sum (a \times b)}{n}
\]

- \(n\) = total number of animal in group
- \(a\) = the difference between two successive doses of administered extract/substance
- \(b\) = the average number of dead animals in two successive doses.
- LD100 = Lethal dose causing the 100% death of all test animals.

Hodge and Sterner scale (Table 2b) was used for the evaluation of toxicity with the help of LD50 (Hodge and Sturner, 2005). Experimental protocol were approved from the University and Departmental committee for Research and Ethics. Each animal was used only once. For ethical reason all animals were sacrificed at the end of study (AVMA Guideline, 2013).

**Histopathology study**

Immediately after death of animals the organs (kidney, liver, heart and spleen) were fixed in 10% formalin. After dehydration, clearing and infiltration the tissues were embedded in paraffin wax and sectioned (7-μm) by using Leica RM 2145-rotation microtom. These sections were stained with hematoxylin and eosin. After the preparation of slides, photographs were taken through nikon advance trincocular research microscope OPTIPHOT
model X2T-21E equipped with Nikon Microphotography system; model UFX-DX-35 and phase contrast N plan.

**RESULTS**

Although, the *Berberis vulgaris* is used in folkloric medicine for the treatment of various diseases but little research had been done to investigate the safety.

From the experiment the results reveal that the methanolic extract of *Berberis vulgaris* intraperitoneally have been found toxic with LD 100 at 1000 mg/kg body weight of experimental animals as in the first 4 hours of observation 100% morbidity was observed.

The animals received 1000mg/Kg i.p. were suffering from twitching, increase rate of respiration, sedation, abdominal muscle contractions. At the 2nd hour they were drowsy, less responsive and dyspnoeic before death. However, at 4th hour all mice had convulsion and expired (Table 3).

After the administration of dose of 500mg/Kg body weight i.p. mice were suffering from twitching, increase rate of respiration, sedation, abdominal muscle contractions, corner sitting. At the 2nd hour mice had recovered from these symptoms. Only 2 mice were expired at the end of 24 hr (Table 3).

However, methanolic extract at lower limit dose of 100 mg/kg body weight, was not found to cause and mortality and non-significant changes were observed in wellness parameters used for evaluation of toxicity. Behavioral pattern, salivation, sleep of the treated as well as the control animals were found to be normal, diarrhea and coma did not occur in any of the mice (Table 3).

**LD50 Value**

As per observations and calculations (Karber, 1931), the LD50 value of Methanolic Extract of *Berberis Vulgar* after i.p. administration was found to be than 666.66 mg/kg body weight. According to Hodge and Sterner (2005) toxicity scale, the LD50 value of the *Berberis vulgaris* methanolic extract is in the slight toxic category (Table 2). For the future research the proposed retained retained i.p. dose of methanolic extract of *Berberis vulgaris* is 1/20th LD50 that is around 33.5mg/KG body weight.
Histopathological lesions were not found at the dose of 100mg/Kg body weight, hepatocytes are distinct and relatively normal. However, the histopathological examination indicated that the at the dose of 1000mg/Kg tested extract induced several histopathological changes in the liver of expired mice such as necrosis of some hepatocytes, edema, scattered bile canaliculi (Fig 1b). At the dose of 1000mg/Kg doubling of nuclei (Fig 1b) and dilated blood vessels were observed and damage to hepatic cell architecture were more pronounced when compared to control (Fig 1a).

Table 1: Laboratory Conditions were maintained As Per OECD 423, 2001

<table>
<thead>
<tr>
<th>S.No</th>
<th>Condition</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Room Temperature</td>
<td>23°C (±30C)</td>
</tr>
<tr>
<td>2</td>
<td>Humidity</td>
<td>50 to 60%</td>
</tr>
<tr>
<td>3</td>
<td>Light and Dark Period</td>
<td>12/12 Hours</td>
</tr>
<tr>
<td>4</td>
<td>Bedding</td>
<td>Clean Sterilized Husk changed daily</td>
</tr>
<tr>
<td>5</td>
<td>Oral</td>
<td>Feed Conventional Laboratory Diets, Like Standard Pellet Chow.</td>
</tr>
<tr>
<td>6</td>
<td>Distilled Drinking Water</td>
<td>Ad libitum</td>
</tr>
</tbody>
</table>

Table 2a: The Grouping of animal for the Toxicological studies and determination of LD50

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>No of Animals</th>
<th>Dose</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I Control</td>
<td>6</td>
<td>Saline (10ml/kg)</td>
<td>i.p.</td>
</tr>
<tr>
<td>2</td>
<td>II treated</td>
<td>6</td>
<td>BRPM (10mg/Kg)</td>
<td>i.p.</td>
</tr>
<tr>
<td>3</td>
<td>III treated</td>
<td>6</td>
<td>BRPM (100mg/Kg)</td>
<td>i.p.</td>
</tr>
<tr>
<td>4</td>
<td>IV treated</td>
<td>6</td>
<td>BRPM (500mg/Kg)</td>
<td>i.p.</td>
</tr>
<tr>
<td>5</td>
<td>V treated</td>
<td>6</td>
<td>BRPM (1000mg/Kg)</td>
<td>i.p.</td>
</tr>
</tbody>
</table>

Table 2b: Hodge and Sterner Toxicity Scale

<table>
<thead>
<tr>
<th>S.No</th>
<th>Term</th>
<th>LD50 (rat, oral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extremely Toxic</td>
<td>Less than 1mg/Kg</td>
</tr>
<tr>
<td>2</td>
<td>Highly Toxic</td>
<td>1-50 mg/Kg</td>
</tr>
<tr>
<td>3</td>
<td>Moderately Toxic</td>
<td>50-500 mg/Kg</td>
</tr>
<tr>
<td>4</td>
<td>Slightly Toxic</td>
<td>500-5000 mg/Kg</td>
</tr>
<tr>
<td>5</td>
<td>Practically Non Toxic</td>
<td>5000-15000 mg/Kg</td>
</tr>
</tbody>
</table>
Table 3: Toxicological study of different doses of methanolic Extract of *Berberis vulgaris* administered intraperitoneally in mice.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Dose / Day</th>
<th>Mortality (x/N)</th>
<th>Symptoms (2 Hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Saline (10ml/Kg)</td>
<td>0/6</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>BRPM (10 mg/Kg)</td>
<td>0/6</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>BRPM (100 mg/Kg)</td>
<td>0/6</td>
<td>Nil</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>BRPM (500 mg/Kg)</td>
<td>2/6</td>
<td>Twitching, increase rate of respiration, sedation, abdominal muscle contractions, corner sitting.</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>BRPM (1000 mg/Kg)</td>
<td>5/6</td>
<td>Twitching, increase rate of respiration, sedation, abdominal muscle contractions. At the 2(^{nd}) hour drowsy, less responsive and dyspnoeic. At 4(^{th}) hour all mice had convulsion and expired.</td>
</tr>
</tbody>
</table>

x= Number of expired animals  
N= Total number of Animals (Mice)

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Fig. 1  Control Liver (20X)

Fig. 2  Berberis (1000mg/Kg) treated Liver (20X)
DISCUSSION

Herbs and the medicines derived from plants contain different active compounds now researchers took an step to evaluate the toxicity of herbal drugs/ Extract whose adverse effects are mostly unknown (Elvin-Lewis, 2001). Investigation revealed that toxic effect of different herbs such as Kava, germander (Teucrium chamaedrys), chaparral (Larrea tridentate) cause severe liver injury (Stickel et al., 2000), Licorice induced hypokalemic myopathy (Shintani et al., 1992) and Kelp (seaweed) can cause hyperthyroidism (Clark et al., 2003). In the present study acute toxicity, histopathology and determination of LD100 and LD50 of aqueous extract of Berberis vulgaris was conducted.

Acute toxicity and LD50 test determine the range of doses and estimating the therapeutic index of herbal extract (Aniagu et al., 2005). In Present study the LD50 of methanolic extract of Berberis vulgaris was found to be 666mg/Kg body weight. Which was in agreement with another study (Azmat and Ahmed 2014).

This present study is in agreement with another study (Azmat and Ahmed 2014) where it was observed that the Ethanolic extract of berberis vulgaris did not show any signs of toxicity up to 100mg/Kg and the lethal dose was found at 1000mg/kg when given intraperitonially in mice (Azmat and Ahmed 2014). However the oral administration did not cause any mortality up to the dose of 1000mg/Kg when administered daily for 14 days. These variations in the acute toxicity study may be due to difference in route of administration. In the present study the calculated LD50 value of Methanolic extract of Berberis vulgaris is 666mg/Kg body weight. While the LD 100 was observed at 1000mg/Kg.

In the present study toxicological studies were coupled with histological studies at the dose of 1000mg/Kg, because it localize the action of toxin (Sagar and Vidyasagar, 2010). The findings observed at the higher dose of 1000 mg/kg/day in mice, showed changes in liver architecture indicating toxicity and adverse effects on liver. Some reported literature is in agreement with our finding suggest that Berberine present in Berberis vulgaris if used in high quantity 60-100 times as large as a human pharmacological dose, in newborns can cause kernicterus (Chan, 1993). Although, Hermenean et al., 2012 have reported that 50 mg/kg/day oral administration of Berberis vulgaris cause hepatoprotective effects on Carbon Tetrachloride–Induced cute oxicity in mice. Another study showed that Oral administration at the dose of 1000mg/Kg/day did not cause any sign of toxicity and mortality (Azmat and Ahmed 2014), while the i.p. administration at the dose of 300mg/Kg/day reducing the activity
of liver enzymes (Taheri et al., 2012) However in present study i.p administration of 1000mg/Kg/day was found to be LD100 with LD50 at 666 mg/kg body weight. In all above reported literature doses were less than LD50 that why produce significant therapeutic effects.

Although present study confirms that Berberis vulgaris is slightly toxic (Hodge and stern, 2005) In the present study, other organs like kidney, heart and spleen did not show any significant change in cell architecture.

CONCLUSION
In conclusion, the results of present study conclude that Berberis vulgaris (BRPM) is safe or slightly toxic when administered intraperitoneally with median lethal dose (LD 50) at 666.66mg/Kg. For the future research the proposed retained dose of methanolic extract of Berberis vulgaris is 1/20th LD50 that is around 33.5.5mg/KG body weight. Berberis vulgaris at high doses has toxic potential therefore; it should be ingested with caution. This study is preliminary study; in future this research is offering an outset to continue the research in human.

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ETHICAL APPROVAL
Authors hereby declared that the experimental protocol were approved from the University and Departmental committee for Research and Ethics. Each animal was used only once. For ethical reason all animals were sacrificed at the end of study (AVMA Guideline, 2013). Experimental protocol were followed according to Guidelines for Care and Use of Laboratory Animals in Biomedical Research (2010) all rule were followed, as well as specific national laws where applicable.

COMPETING INTERESTS
The authors have no conflict of interest to report.
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