5. Acute and Chronic toxicity studies of berberine in normal mice

5.1 Introduction

Early toxicity studies may give an opportunity to determine not only maximum tolerated doses but also a means to identify candidate compounds that cause unacceptable effects with reference to the hematological, biochemical and histopathological changes in major target organs. This is important, particularly when the effects are subtle and detectable only microscopically. Such changes may also appear only at high doses but be absent at lower therapeutic doses. Toxicity studies can give an insight into the toxic effects of the compounds under investigation.

This study was carried out according to the guidelines prepared by The Ministry of Health, Government of India.

5.2 Materials and Methods

5.2.1 Experimental animals

Swiss albino mice (25-30g) of both sexes were used for the present study, they were maintained in the laboratory at a temperature 28°C ± 2°C, room humidity 75% ± 5% and having a photoperiod 12 h L:12 h D. Animals were kept on a commercially prepared standardized diet (Gold Mohur, Lipton India Ltd, Bangalore). Animals described as fasted had been deprived of food for at least 18 hrs but allowed to have free access to water.

5.2.2 Drug

The test compound berberine hydrochloride was prepared as described in Chapter 2.
5.2.3 Acute toxicity studies

Swiss albino mice were divided into 9 groups of 10 each. The drug was dissolved in corn oil (depending upon the concentration of berberine) and administered orally to fasted mice in dosage of 200, 400, 1200, 1800 2400, 3000, 4000 and 6000 mg/kg of body weight. All the animals were closely observed for 24 hr and the mortality if any was noted during and after 72 hours, in order fix to lethal dose, \( LLD \), for berberine hydrochloride. All the symptoms including changes in awareness, mood, motor activity, posture, motor co-ordination, muscle tone and reflexes were recorded.

Nine groups were selected for the present investigation (\( n = 10 \))

Group 1 : Normal control

Group 2 -9 : Normal control + Different doses of berberine ranging from 200mg to 6000mg/kg,b.w.p.o.

5.2.4 Chronic toxicity studies

Swiss albino mice were divided into five groups of 6 rats each. The control group was fed on corn oil daily (1 ml/rat) for a period of 45 days. The drug was dissolved in corn oil (1.2 ml) and administered orally to fasted mice in dosage of effective dose (ED)\(_{\text{eff}} \), 2.5 times of ED\(_{\text{eff}} \), 5 times ED\(_{\text{eff}} \) and 10 times ED\(_{\text{eff}} \) levels. Their general behaviour and food intake was recorded. Finally their urine was also collected a day before the sacrifice for the qualitative analysis of urine sugar and albumin. All the animals were sacrificed after overnight fasting and the blood samples were collected. Hematological examinations such as blood hemoglobin, WBC, RBC and platelets and biochemical assays such as blood glucose, urea, serum bilirubin, serum protein, urine albumin serum, cholesterol, phospholipids, triglycerides, lactate dehydrogenase, alanine transaminase, aspartate transaminase, alkaline phosphatase and acid phosphatase and histopathological studies were carried out on major organs viz. liver, kidney, heart, spleen and lungs.

Five groups were selected for the present investigation (\( n = 6 \)).
Single dose of berberine dissolved in corn oil (1.2 ml) was orally administered on a daily basis for 45 days.

| Group 1       | Normal control                                      |
| Group 2       | Normal control + Berberine hydrochloride 50mg/kg.b.w/day p.o. |
| Group 3       | Normal control + Berberine hydrochloride 125mg/kg.b.w/day p.o. |
| Group 4       | Normal control + Berberine hydrochloride 250 mg/kg.b.w/day p.o |
| Group 5       | Normal control + Berberine hydrochloride 500mg/kg.b.w/day p.o. |

5.2.4.1 Collection of plasma and serum

As in section 3.2.5

5.2.4.2 Collection of urine

As in section 3.2.6

5.2.4.3 Glucose

As in section 3.2.7.1.8

5.2.4.4 Urine albumin

Urine albumin was quantitatively assayed by sulphosalicylic acid method. Urine albumin reacts with sulphosalicylic acid and the results were interpreted as follows.

- : No cloudiness
Trace : Cloudiness against a black background
+ : Dense cloudiness (10-50mg)
++ : Cloudiness with granules and definite flocculation (50-200mg)
+++ : Cloudiness with flocculation (200-500mg)
++++ : Cloudiness with precipitation (Above 500mg)
5.2.4.5 Urine sugar

As in section 3.2.7.2.2

5.2.4.6 Hematological parameters

Hemoglobin-as in section 3.2.7.2.9.1, Red blood Cell Count-as in section 3.2.7.2.9.2, White Blood Cell Count-as in section 3.2.7.2.9.3 and platelet Count-as in section 3.2.7.2.9.4

5.2.4.7 Serum proteins

As in section 3.2.7.2.5

5.2.4.8 Liver glycogen

As in section 3.2.7.2.4

5.2.4.9 Estimation of Blood Urea

As in section 3.2.7.4.6

5.2.4.10 Serum bilirubin

Serum bilirubin was estimated by the method Malloy and Evelyn, (1963). This method is based on the formation of a purple compound azobilirubin. When bilirubin reacts with diazo reagent, a coloured complex is formed which can be measured colorimetrically at 540 nm.

Serum bilirubin was expressed as mg/dl.

5.2.4.11 Serum lipids

Cholesterol-as in section 3.2.7.2.10.2, Phospholipids-as in section 3.2.7.2.10.3 and Triglycerides-as in section 3.2.7.2.10.4.
5.2.4.12 Serum enzyme markers

Lactate dehydrogenase-as in section 3.2.7.2.13.2, Alanine transaminase-as in section 3.2.7.2.13.3, Aspartate transaminase-as in section 3.2.7.2.13.4, Alkaline phophatase-as in section 3.2.7.2.13.5 and Acid phophatase-as in section 3.2.7.2.13.6.

5.2.4.13 Histopathological studies

Tissue samples were fixed in formalin, embedded in paraffin wax, cut into 5 μ thickness and stained with haemotoxylin and eosin.

5.2.4.14 Statistical analysis

As in section 3.2.7.4.18

5.3 Results and discussion

There was nil mortality up to a dose of 6000mg/kg during the experimental period. Therefore the LD₅₀ value for berberine may be far above 6000mg/kg. General behaviour pattern in the drug treated groups is normal even after 72 hrs. From the present study it can be concluded that berberine is not lethal in the usual range of oral hypoglycemic drugs i.e. 200 mg to 6000mg/kg body weight in experimental animal models. Berberine hydrochloride is safe when compared to chloropromamide, which has an LD₅₀ value of 760mg/kg (Schneider et al., 1959).

There are no notable changes in the intake of food and body weight gain, urine sugar and albumin when compared to the control rats after 45 days of treatment [Table 35]. Similarly, hematological studies also did not show any pathological changes in the drug treated group [Fig. 40]. Serum proteins and liver glycogen were normal in the entire drug treated group [Table 36]. However there were few biochemical changes in the drug treated group. The serum glucose level was significantly lowered [P<0.05] in all the drug treated groups when compared to the control group [Table 35]. There was a significant increase in the plasma bilirubin levels in 250 and 500 mg treated
Table 35
Effect of berberine on serum glucose, proteins, liver glycogen, urine albumin and sugar in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight [%]</th>
<th>Serum glucose [mg/dl]</th>
<th>Urine albumin</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>100</td>
<td>96.75 ± 2.15 a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Normal + 50 mg</td>
<td>+1</td>
<td>85.10 ± 1.78 b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Normal + 125 mg</td>
<td>+1</td>
<td>80.73 ± 1.78 b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Normal + 250 mg</td>
<td>+3</td>
<td>80.88 ± 1.80 b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Normal + 500 mg</td>
<td>+3</td>
<td>78.38 ± 1.66 c</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- = negative
n = 6.
Values are mean ± S.E.
Group 1, 2, 3, 4 and 5 were statistically compared.
Data followed by different letters are significantly different at \(^*P<0.05\)
Fig. 40 - Effect of berberine on certain hematological parameters in normal rats.

n=6.

Values are mean ± S.E.

Group 1, 2, 3, 4 and 5 were statistically compared.

Data followed by different letters are significantly different at *P<0.05.
groups [Table 36]. Bilirubin is formed from the degradation of hemoglobin by the action of the reticuloendothelial system throughout the body (Grollman, 1957). Increase in the serum bilirubin may be due to inducing the reticuloendothelial system at high dose of berberine. Phospholipids and triglycerides levels were normal in all drug treated groups whereas there was a significant decrease in the serum cholesterol in all the drug treated groups except 25 mg, which showed a marginal reduction [Table 37]. Decrease in plasma cholesterol levels by berberine may be due to two factors (a) increase in cholesterol catabolism (b) stimulation of lipoprotein lipase, which would increase the removal of lipoprotein from plasma, thus decreasing the plasma cholesterol levels.

The activities of lactate dehydrogenase, alanine and asparate transaminase and alkaline and acid phosphatase were normal in the drug treated groups [Fig. 41]. Histopathological results in liver, kidney, heart, pancreas and lungs were found to be normal in the drug treated groups [Plate 2 and 3].

Berberine is generally considered to be nontoxic at doses used in clinical situations. Berberine showed no genotoxicity; it is unable to induce significant cytotoxic, mutagenic or recombinogenic effects during treatments performed under non-growth conditions and therefore it is not a potent mutagenic agent in dividing cells (Pasqual et al., 1993). Most of the berberine containing plants is considered to be uterine stimulant or menogogues, so historically it was recommended to be used with care during pregnancy. A therapeutic dose of 200 mg of berberine two to four times daily is considered to be safe (Shabir and Bhide, 1971).

5.4 Conclusion

The present study reveals that berberine at different doses does not exhibit any toxic action in the above-mentioned system.
Table 36
Effect of berberine on serum proteins, liver glycogen, blood urea and serum bilirubin in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum proteins [g/dl]</th>
<th>Liver glycogen [g/100g wet tissue]</th>
<th>Blood urea [mg/dl]</th>
<th>Serum bilirubin [mg/dl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>7.27 ± 0.19 a</td>
<td>3.06 ± 0.16 a</td>
<td>19.80 ± 0.54 a</td>
<td>1.87 ± 0.07 a</td>
</tr>
<tr>
<td>2</td>
<td>Normal + 50 mg</td>
<td>7.38 ± 0.20 a</td>
<td>3.08 ± 0.16 a</td>
<td>19.92 ± 0.55 a</td>
<td>1.90 ± 0.07 a</td>
</tr>
<tr>
<td>3</td>
<td>Normal + 125 mg</td>
<td>7.36 ± 0.52 a</td>
<td>3.17 ± 0.18 a</td>
<td>19.81 ± 0.56 a</td>
<td>1.99 ± 0.07 a</td>
</tr>
<tr>
<td>4</td>
<td>Normal + 250 mg</td>
<td>7.38 ± 0.52 a</td>
<td>3.18 ± 0.19 a</td>
<td>19.92 ± 0.59 a</td>
<td>2.28 ± 0.09 b</td>
</tr>
<tr>
<td>5</td>
<td>Normal + 500 mg</td>
<td>7.33 ± 0.22 a</td>
<td>3.18 ± 0.20 a</td>
<td>19.95 ± 0.57 a</td>
<td>2.68 ± 0.11 b</td>
</tr>
</tbody>
</table>

n=6.
Values are mean ± S.E.
Group 1, 2, 3, 4 and 5 were statistically compared.
Data followed by different letters are significantly different at *P< 0.05.
Table 37
Effect of berberine on serum cholesterol, phospholipids and triglyceride levels in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum cholesterol [mg/ml]</th>
<th>Serum phospholipids [mg/ml]</th>
<th>Serum triglycerides [mg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>0.67 ± 0.02 *</td>
<td>1.34 ± 0.03 *</td>
<td>0.11 ± 0.004 *</td>
</tr>
<tr>
<td>2</td>
<td>Normal + 50 mg</td>
<td>0.62 ± 0.02 *</td>
<td>1.30 ± 0.03 *</td>
<td>0.11 ± 0.004 *</td>
</tr>
<tr>
<td>3</td>
<td>Normal + 125 mg</td>
<td>0.54 ± 0.02 b</td>
<td>1.28 ± 0.03 *</td>
<td>0.10 ± 0.003 a</td>
</tr>
<tr>
<td>4</td>
<td>Normal + 250 mg</td>
<td>0.50 ± 0.01 b</td>
<td>1.31 ± 0.04 *</td>
<td>0.10 ± 0.003 a</td>
</tr>
<tr>
<td>5</td>
<td>Normal + 500 mg</td>
<td>0.42 ± 0.01 c</td>
<td>1.27 ± 0.03 *</td>
<td>0.11 ± 0.003 a</td>
</tr>
</tbody>
</table>

\[n=6.\]

Values are mean ± S.E.

Group 1, 2, 3, 4 and 5 were statistically compared.

Data followed by different letters are significantly different at \(*P<0.05\).
Type 2 diabetes, *Z. mauritiana*, hypoglycemic, hypolipidemic

Fig. 41 - Effect of berberine on enzyme markers in normal rats.

\( n=6 \).

Values are mean ± S.E.

Groups 1, 2, 3, 4 and 5 were statistically compared.

Data followed by different letters are significantly different at \(*P<0.05\).
Plate 2. LC - Liver Control, LT - Liver Test, KC - Kidney Control, KT - Kidney Test, HC - Heart Control and HT - Heart Test
Plate 3. PC - Pancreas Control, PT - Pancreas Test, LuC - Lung Control and LuT - Lung Test
Summary and Conclusion
Summary and Conclusion

Berberine was identified as the principle compound responsible for the hypoglycemic property of leaves of *Z. mauritiana*. Berberine was isolated by the method of Tsutomu *et al.*, (1972) and its hypoglycemic and hypolipidemic properties were studied in detail in normal, alloxan diabetic and cholesterol fed rats.

**Type 2 diabetes**

1. The fall in serum glucose levels after the administration of berberine was almost 40 percent in normal as well as in alloxan diabetic rats.

2. Berberine stimulated insulin release to significant degree in normal as well as in alloxan diabetic rats.

3. Continuous treatment of diabetic rats with berberine significantly reduced the serum glucose levels by increasing the serum insulin levels in the insulin-deficient alloxan diabetic rats.

4. Decreased levels of liver glycogen, serum proteins and increased levels of blood urea, albumin/globulin ratio and glycosylated hemoglobin were normalized after berberine treatment.

5. Hematological studies in alloxan induced diabetic rats showed significant decrease in hemoglobin. RBC, WBC and platelet count. The above changes were reversed upon berberine and glibenclamide treatment.

6. Enzyme markers such as LDL, ALT, AST, ACP and ALP were significantly increased in the serum and tissues in alloxan diabetic rats. The observed increase in the enzyme levels may be due oxidant stress, which alters membrane structure there by increasing the fragility of the cells. All enzyme levels were significantly reduced to near normal levels upon berberine treatment.
7. Serum and tissue concentration of all the lipid parameters—cholesterol, phospholipids and triglycerides were significantly increased in alloxan diabetic control group. Berberine treatment significantly decreased the lipid profile when compared to the glibenclamide.

8. Cholesterol levels in LDL, VLDL and HDL lipoproteins fraction were significantly elevated in alloxan diabetes. Administration of berberine to diabetic rats significantly increased the HDL levels and at the same time it decreased the elevated levels of LDL and VLDL cholesterol. Berberine effect was better off when compared to the glibenclamide.

9. SOD, catalase, glutathione, glutathione-s-transferase and glutathione peroxidase enzymes were altered in diabetic control rats. The increased oxidant stress observed in the diabetic rats is reduced by berberine treatment, which increases the levels of the antioxidant enzymes, thus reducing the oxidants. In this respect berberine acts as a good free radical scavenger.

10. Tissues and membrane Na⁺, K⁺- ATPase and Ca²⁺-ATPase were significantly decreased in alloxan diabetic group. Berberine administration significantly increased Na⁺, K⁺- ATPase and Ca²⁺-ATPase levels, thus helping the cells to perform their normal functions.

11. Tissue hexokinase levels were significantly increased, at the same time tissue glucose-6-phosphatase and fructose-1,6- diphosphatase were significantly decreased in diabetic rats after continuous berberine treatment.

12. Administration of berberine and glibenclamide significantly increased lipogenic enzyme levels (glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase and malic enzyme) in alloxan diabetic rats.
Hyperlipidemia

1. Treatment with berberine significantly decreased the elevated levels of body, liver weight in cholesterol fed rats. Rats fed with berberine showed significant increase in the hepatic glycogen.

2. The elevated concentration of serum cholesterol, phospholipids and triglycerides in cholesterol fed rats were significantly decreased in berberine treated rats. Similarly the increased concentration of LDL, VLDL and serum free fatty acids and decreased concentration of HDL were reversed after berberine treatment.

3. Elevated levels of lipid peroxides are depressed in the berberine treated cholesterol fed rats. Berberine and gemfibrozil significantly increased the depressed activities of SOD and catalase.

4. Administration of berberine significantly decreased the elevated activities of serum LDH, ALT, AST, ALP and ACP over in cholesterol fed rats.

5. Treatment with berberine significantly decreased the activity of HMG CoA, lipogenic enzymes and increased the total lipolytic activity and LCAT activity is significantly as compared to cholesterol fed rats. The present investigation with cholesterol fed hyperlipidemic animals show that berberine could increase the level of HDL by increasing the activity of LCAT, which play a key role in lipoprotein metabolism.

6. Increased hepatic bile acids levels and further excretion of faecal excretion of bile acids and sterols levels were observed in berberine treated cholesterol fed rats.

Toxicity

Acute toxicity studies have shown that the LD₅₀ value for berberine is beyond 6000mg/kg. Biochemical, hematological and histopathological profiles with reference to chronic toxicity studies were found to be normal.
Therefore, berberine endowed with many therapeutic effects such as cardiovascular, anti-inflammatory and anti-diarrheal, proves to be a good drug with hypoglycemic and hypolipidemic properties. Further comprehensive pharmacological investigations are warranted in this subject to find out the exact mechanism action of berberine with reference to its insulin releasing effect and reduction in the cholesterol levels. Berberine prove superior to many oral hypoglycemic agents because it is a plant product, possesses both hypoglycemic and hypolipidemic properties and with minimal side effects.