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Role of Berberine as an Adjuvant Response Modifier During Tumour Therapy in Mice

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Abstract

The cytotoxicity and antitumour activity of the isoquinoline alkaloid berberine was studied in-vitro and in-vivo.

Berberine was cytotoxic to L929 cells in culture (IC50 40μg mL⁻¹), and to mice when given as an acute (LD50 50mg kg⁻¹, i.p) or chronic (LD50 15mg kg⁻¹ for 10 days, i.p) dose. At a non-toxic concentration berberine dose-dependently inhibited the tumours induced by Dalton's lymphoma ascites tumour cells in mice. Berberine was more active when given intraperitoneally than orally. The simultaneous administration of berberine potentiated the therapeutic effects of radiation, cyclophosphamide and hyperthermia with a decrease in volume of solid tumours in mice.

The results indicate the beneficial use of berberine as an adjuvant response modifier in cancer therapy.

Materials and Methods

Animals

Swiss albino mice, 20–25 g, were housed in ventilated cages. They were given pelleted mice food (Lipton India Ltd, Bangalore) and had free access to water.

Cells

Dalton's lymphoma ascites cells (DLA) were propagated in Swiss albino mice by injecting 10⁵ cells intraperitoneally. L929 cells were procured from the National Centre for Cell Science, Pune, and maintained by subculturing in Minimum Essential Medium (MEM) (Himedia, Bombay) containing 10% goat serum. (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Spectrochem, Bombay. Berberine hydrochloride was a gift from Dr Rajpal, Kisalaya Herbals Ltd, Indore.

Determination of in-vitro cytotoxicity of berberine hydrochloride

Short-term in-vitro cytotoxicity studies were carried out using DLA tumour cells (Kuttan et al 1985). Berberine hydrochloride was dissolved in hot water and various concentrations (100–1000μg mL⁻¹) were incubated with tumour cells (10⁶) suspended
in phosphate-buffered saline (PBS, pH 7.2) and cytotoxicity was determined after 3 h using the trypan blue exclusion method.

**Determination of cytotoxicity of berberine hydrochloride in tissue culture**

Cytotoxicity of berberine hydrochloride in culture was determined using L929 cells. Cells ($10^4$) were plated in 96-well flat-bottomed titre plates and maintained with MEM containing 10% goat serum. Various concentrations of berberine hydrochloride (10–100 μg mL$^{-1}$) were added to the wells and incubated for 72 h. MTT (5%, 20 μL) was added to the wells 4 h before the end of incubation. Medium and reagents were aspirated, 98% dimethylsulphoxide (DMSO) was added and after shaking for 15 min, the absorbance was measured at 545 nm with a reference wavelength of 630 nm using an Elisa plate reader (Awareness Technology Inc.). The percentage cytotoxicity was calculated and compared with untreated controls.

**Determination of antitumour activity of berberine hydrochloride**

**Ascites tumour.** DLA cells were aspirated from the peritoneal cavity of mice and washed with PBS. Mice were inoculated with a 0.1 mL PBS containing $10^6$ cells. The mice were divided into four groups (6 mice per group). Group 1 acted as an untreated control. Groups 2, 3 and 4 were given berberine hydrochloride (10, 2.5 and 0.5 mg kg$^{-1}$, i.p., respectively) everyday for 10 days.

In another set of experiments, four groups of mice (6 mice per group) were used to determine the oral efficacy of berberine. Group 1 was the control. Groups 2, 3 and 4 received berberine hydrochloride (25, 5 and 1 mg kg$^{-1}$, respectively) dissolved in hot water for 10 days. The pattern of death due to tumour burden was noted and the percentage increase in life span was calculated (Kuttan et al 1988).

**Solid tumour.** DLA cells ($10^6$) were injected intramuscularly into the right hind limb of mice. Mice were divided into seven groups (6 mice per group). Group 1 served as the untreated control. Groups 2, 3 and 4 received intraperitoneal administration of berberine hydrochloride and groups 5, 6 and 7 were treated with berberine hydrochloride (10, 2.5 and 0.5 mg kg$^{-1}$, p.o., respectively) for 10 days. The tumour diameter was measured on every third day and tumour volume was calculated by the formula:

$$V = \frac{4}{3}\pi r_1^2 r_2$$

where $V$ is the volume, $r_1$ and $r_2$ are the radius of the tumour. The results were compared with the untreated control.

**Antitumour activity of berberine hydrochloride in combination with other treatments**

**Berberine and cyclophosphamide.** DLA cells were aspirated from tumour-bearing mice and 0.1 mL containing $10^6$ cells was injected intramuscularly into the right hind limb of Swiss albino mice. Four groups (6 mice per group) were used. Group 1 acted as the control, group 2 received cyclophosphamide (15 mg kg$^{-1}$, i.p.), group 3 received berberine (25 mg kg$^{-1}$, p.o.) and group 4 received both cyclophosphamide and berberine. Drug administration continued for 10 consecutive days. The diameter of the tumour was measured and the volume was determined.

**Berberine and radiation.** Mice were divided into four groups (6 mice per group) and tumour cells ($10^6$) were injected intramuscularly into the right hind limb. Group 1 acted as the control. Group 2 was subjected to irradiation (600 rads) using a CO$^{60}$ gamma source (Theratron 780, Telecobalt unit, Atomic energy Canada Ltd). Group 3 received berberine hydrochloride (25 mg kg$^{-1}$, p.o.) and group 4 received both radiation and berberine. Drug administration continued for 10 consecutive days. The diameter of the tumour was measured on every third day and the volume was determined.

**Berberine and hyperthermia.** Tumour cells ($10^6$) were injected into the right hind limb of 4 groups of mice (20–25 g). Group 1 acted as the untreated control. Group 2 received whole body hyperthermia on day 7 after tumour inoculation by subjecting the mice to whole body heating (43°C for...
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BERBERINE
IN TUMOUR THERAPY

Figure 2. Antitumour activity of oral (A) and intraperitoneal (B) berberine hydrochloride on solid tumour induced by DLA cells. Values are expressed as tumour volumes on different days. Control (●), 1 (■), 5 (▲) and 25 (○) mg kg⁻¹ oral berberine. Control (●); 0.5 (■), 2.5 (▲) and 10 (○) mg kg⁻¹ intraperitoneal berberine.

30 min), group 3 received berberine hydrochloride (25 mg kg⁻¹, p.o.) from day 7–17, and group 4 received both berberine and hyperthermia. The diameter of the tumour was measured and tumour volume was determined every third day.

Results

Berberine hydrochloride was cytotoxic (44%) to DLA tumour cells only at a concentration of 1 mg mL⁻¹ or greater in-vitro. At lower concentrations, it produced a dose-dependent cytotoxic effect to DLA cells. Berberine hydrochloride was cytotoxic to L929 cells in culture at much lower concentration (Table 1). The concentration of berberine hydrochloride needed to produce 50% cytotoxicity to L929 cells in culture was approximately 40 µg mL⁻¹.

Mice treated with berberine hydrochloride had a significantly increased life span (P < 0.001). The life span of mice receiving 10 mg kg⁻¹ berberine hydrochloride intraperitoneally for 10 consecutive days was increased by 32%. Mice treated with 2.5 and 0.5 mg kg⁻¹ berberine hydrochloride intraperitoneally had an increase in life span of 12 and 15%, but this was not significant (Table 2). Oral administration produced a lesser increase in the life span of ascites tumour-bearing mice and even at 25 mg kg⁻¹ it produced only a 5% increase in life span.

Table 1. Cytotoxicity of berberine hydrochloride against L929 cells in culture.

<table>
<thead>
<tr>
<th>Group</th>
<th>Conc (µg mL⁻¹)</th>
<th>Cytotoxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>78.96</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>66.34</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>43.00</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>22.33</td>
</tr>
</tbody>
</table>

Table 2. Antitumour activity of berberine hydrochloride against ascites tumour.

<table>
<thead>
<tr>
<th>Group</th>
<th>Berberine hydrochloride concn (mg kg⁻¹)</th>
<th>Increase in life span (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine hydrochloride concn (mg kg⁻¹)</td>
<td>Intraperitoneal</td>
<td>Oral</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>12.38</td>
</tr>
<tr>
<td>3</td>
<td>0.02</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>0.05</td>
<td>15.20</td>
</tr>
<tr>
<td>5</td>
<td>0.10</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>0.20</td>
<td>32.14*</td>
</tr>
<tr>
<td>7</td>
<td>0.50</td>
<td>–</td>
</tr>
</tbody>
</table>

*P < 0.001

The solid tumour volume of mice treated with berberine hydrochloride intraperitoneally was significantly decreased in mice treated with 10 mg kg⁻¹ for 10 days (P < 0.01). At day 30 the tumour volume of the control mice was 5.5 cm³ while that of mice treated with 10 mg kg⁻¹ berberine hydrochloride was 0.86 cm³. In mice treated with 2.5 and 0.5 mg kg⁻¹ berberine hydrochloride, the tumour volume was reduced to 1.81 and 2.12 cm³, respectively (Figure 2). Oral administration of berberine hydrochloride also reduced the tumour volume significantly (P < 0.001). The tumour volume of untreated control mice was 5.06 cm³ at day 30, while that of mice treated with 25 mg kg⁻¹ berberine hydrochloride was 1.5 cm³. A dose-dependent reduction was found in the remaining groups (Figure 2).

Synergistic effect of berberine hydrochloride in conjunction with other treatments

Cyclophosphamide. The tumour volume of untreated control mice was 5.1 cm³ at day 30 and this was reduced to 1.42 cm³ (72%) by oral administration of berberine hydrochloride. In mice treated...
The antitumour activity of berberine has been reported in mice treated with berberine hydrochloride which induced differentiation and apoptotic death. Oral administration of berberine did not produce any change in the growth of ascites tumour whereas solid tissue growth was significantly inhibited.

Berberine synergistically reduced solid tumour volume when combined with radiation, cyclophosphamide and hyperthermia. Hence berberine could be of use in the treatment of cancer as a response modifier of other treatments.

As yet we do not know the mechanism of action of berberine. Initial studies indicate that berberine inhibits bacterial topoisomerase activity. Similarly, berberine inhibited cdc25 phosphatase activity in vitro (IC50 150 µg mL⁻¹) while cdc2 kinase activity was not inhibited. This indicates that the activity of berberine may be partially due to the inhibition of cell cycle arrest which may produce apoptotic death.

Discussion

The antitumour activity of berberine has been reported in myelocytic leukaemic HL60 cells where it induced differentiation and apoptotic death.

In this study, using DLA cells, the antitumour activity was significant only when the drug was given intraperitoneally. Oral administration of berberine did not produce any change in the growth of ascites tumour whereas solid tissue growth was significantly inhibited.

Radiation. The tumour volume of untreated control mice was 5 cm³ at day 30. In the berberine hydrochloride-treated group, the tumour volume was reduced by 60% to 2 cm³. In the radiation-treated group, the tumour volume was 2.5 cm³ which was 50% lower than the control. There was a synergistic reduction in tumour volume, when berberine hydrochloride was administered simultaneously with radiation. The tumour volume was 1.2 cm³, a percentage reduction of 76% (Figure 3).

Hyperthermia. The tumour volume of untreated control mice was 4.3 cm³ at day 30 which was reduced to 3.43 cm³ after hyperthermia treatment. In mice treated with berberine hydrochloride, the tumour volume was reduced to 1.74 cm³. The effect of berberine hydrochloride was synergistically increased by hyperthermia and tumour volume was significantly reduced to 0.93 cm³ (Figure 3), a percentage reduction of 78%.

References

Inhibition of chemical carcinogenesis by berberine in rats and mice

K. V. Anis, N. V. Rajeshkumar and Ramadasan Kuttan

Abstract

Berberine, an alkaloid isolated from the plant Berberis aristata, has been found to inhibit significantly the carcinogenesis induced by 20-methylcholanthrene (200 µg/0.1 ml/mouse) or N-nitrosodiethylamine (NDEA; 0.02 % NDEA in distilled water, 2.5 ml/animal by gavage, five days a week for 20 weeks) in a dose-dependent manner in small animals. Administration of berberine (0.5, 2.5 or 5.0 mg kg⁻¹) could reduce significantly the incidence of tumour in animals after an injection of 20-methylcholanthrene and increased their life span compared with the control. When berberine (10, 25 or 50 mg kg⁻¹) was administered simultaneously with NDEA, the markers of liver injury (liver weight, γ-glutamyl transpeptidase activity and glutathione S-transferase level) were reduced significantly compared with animals treated with NDEA only, which resulted in all the values being elevated. A similar decrease was noted in the serum levels of lipid peroxide, bilirubin and glutamate pyruvate transaminase. Morphology of liver tissue and levels of marker enzymes indicated that berberine offered protection against chemical carcinogenesis.

Introduction

Hepatocellular carcinoma is widely prevalent in some areas of the Far East and in Africa. The causative agents are reported to be hepatitis B virus and environmental pollutants, of which nitrosoamines have a major role. N-Nitrosodiethylamine (NDEA), a potent hepatocarcinogen, is a byproduct of the nitrosation of primary amines in the acidic condition of the stomach, and is present in many food products e.g., meat, beer etc. NDEA has been shown to be metabolized to its active ethyl radical metabolite (CH₃CH₂⁺) and the reactive product interacts with DNA producing mutation and further oncogenesis. Similar activation is also involved in the carcinogenic action of polyaromatic hydrocarbons.

Berberine, an isoquinoline alkaloid, has been reported to have multiple pharmacological actions (Ckless et al 1995). Antitumour activity of berberine against human and rat malignant brain tumour cells has been reported (Zhang et al 1990). Recently, we reported that berberine could potentiate the antitumour activity of cyclophosphamide and radiation in animals (Anis et al 1999). Chang et al (1990) showed that berberine was able to down-regulate c-ki-ras 2 oncogene expression in human teratocarcinoma cells, indicating that berberine may be able to inhibit the proliferation of cancer cells produced by chemical carcinogens. In this study we have investigated the chemopreventive activity of berberine against 20-methylcholanthrene-induced sarcoma in mice and NDEA-induced hepatocarcinogenesis in rats.
Materials and Methods

Animals
Fifty female Wistar rats (120–150 g) were obtained from the Veterinary College, Mannuthy, India. They were housed in ventilated cages and fed with a pelleted diet (Lipton, India Ltd) with water freely available. The male Swiss albino mice (20–25 g) used were reared in our facility.

Materials
Berberine hydrochloride was a gift from Dr Rajpal, Kisalaya Herbals Ltd. (Indore, India). 20-Methylcholanthrene was purchased from ICN-Pharmaceuticals (New York, NY). N-Nitrosodiethylamine (NDEA) was obtained from Sigma Chemicals (St Louis, MO). 1-Chloro-2,4-dinitrobenzene, glutathione, and 5,5-dithiobis(2-nitrobenzoic acid) were purchased from Sisco Research Laboratory (Bombay, India). Thiobarbituric acid was obtained from E-Merck (Germany). All other chemicals used were of analytical reagent grade.

Drug preparation
Desired concentrations of berberine hydrochloride were dissolved in hot water and administered orally to the animals.

Determination of the effect of berberine hydrochloride on NDEA-induced hepatocarcinogenesis
Liver tumours were induced by the administration of NDEA as described by Narurkar & Narurkar (1989) with slight modifications. Six-week-old female Wistar rats (120–125 g) were divided into five groups (10 animals/group). Group 1 served as untreated normal animals. Group 2 received 0.02% NDEA in distilled water, 2.5 mL/animal by gavage, five days a week for 20 weeks. This dosage was found to induce liver tumours in the rats (Jose Jeena et al 1999) and animals died by 29–31 weeks. Groups 3, 4 and 5 were administered 50, 25 and 10 mg kg⁻¹ berberine hydrochloride, respectively, along with NDEA. This dosage was found to be non-toxic to rats. Berberine hydrochloride was administered 24 h before the first dose of NDEA and was continued for 20 weeks. Animals were kept without any treatment for a further 10 weeks before being killed. At the end of the 30th week animals were administered diethylether anaesthesia, and blood and liver tissues were collected immediately. Change in liver weight and tumour incidence produced by NDEA treatment was noted. Serum was separated and part of the tissue was fixed in formalin and the rest were kept frozen until analysed.

The following serum and liver parameters were measured to assess the effect of berberine hydrochloride on hepatocarcinogenesis. Serum levels of γ-glutamyl transpeptidase activity were measured by Szasz's method using an AVT diagnostics kit (Szasz 1976) and tissue γ-glutamyl transpeptidase was measured using γ-glutamyl p-nitroanilide as substrate (Tate & Meister 1974). Cytosolic glutathione S-transferase activity was determined by its ability to conjugate glutathione with 1-chloro-2,4-dinitrobenzene (Habig et al 1974). Tissue levels of reduced glutathione were determined by its reaction with 5,5-dithiobis(2-nitrobenzoic acid) (Moron et al 1979). Lipid peroxidation in serum was estimated by the thiobarbituric acid method (Ohkawa 1979). Protein was analysed by the method of Lowry et al (1951). Glutamate pyruvate transaminase activity was measured in serum (Bergmeyer & Bernt 1980). Serum total bilirubin level was estimated by the method of Jendrassic & Grof (1938).

Statistical analysis
Results were expressed as mean ± s.d. and evaluated by Student’s t-test.
Inhibition of chemical carcinogenesis by berberine

Results

Berberine hydrochloride could inhibit sarcoma development induced by 20-methylcholanthrene in a dose-dependent manner. Oral administration of the drug (5, 2.5 or 0.5 mg kg⁻¹) was found to inhibit sarcoma development by 60%, 53% and 33%, respectively (Figure 1). Animals treated with berberine and 20-methylcholanthrene were shown to have an increased life span (Table 1). Control animals began dying of tumour burden after 80 days of carcinogen treatment, with all the animals dying by 180 days, whereas in the treated groups only 6, 7 and 10 animals, respectively, had died by 180 days.

The effect of berberine hydrochloride on NDEA-induced liver tumour incidence is shown in Table 2. The NDEA-treated group showed 100% tumour incidence by the end of the 30th week, whereas the berberine-treated groups showed a dose-dependent reduction of tumour incidence. Inhibition of tumour incidence was also reflected in changes in liver weights. Liver weight of normal animals was shown to be 2.3 ± 0.2 g/100 g, which was significantly increased by NDEA treatment to 8.9 ± 2.1 g/100 g (P < 0.001). Liver weight of the berberine-treated groups was found to be reduced significantly in a dose-dependent manner.

γ-Glutamyl transpeptidase enzyme activity, a non-specific marker of liver neoplasm, was significantly elevated from 30.4 ± 5.8 to 152 ± 37 U L⁻¹ in serum and from 0.01 ± 0.002 to 0.15 ± 0.02 nmol min⁻¹ (mg protein)⁻¹ in liver by the administration of NDEA. Administration of berberine (10, 25 or 50 mg kg⁻¹) reduced the serum γ-glutamyl transpeptidase levels to 119, 93 and 82 U L⁻¹, respectively. Liver γ-glutamyl transpeptidase levels were also significantly (P < 0.001) reduced by the administration of berberine hydrochloride (Table 3).

NDEA-treated groups showed significantly elevated liver glutathione S-transferase activity (1769 ± 898) compared with the normal group (377 ± 13; Table 4), which may be due to induced detoxification mechanisms for elimination of NDEA and its metabolites. A dose-dependent decrease in glutathione S-transferase level (837 ± 605 to 549 ± 128 nmol min⁻¹ (mg protein)⁻¹) was observed in the berberine-treated group (10–50 mg

Table 1 Effect of berberine on the survival of animals after induction of sarcoma with 20-methylcholanthrene (200 μg/0.1 mL/mouse).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals that survived (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>15/15</td>
</tr>
<tr>
<td>5 mg kg</td>
<td>15/15</td>
</tr>
<tr>
<td>2.5 mg kg</td>
<td>15/15</td>
</tr>
<tr>
<td>0.5 mg kg</td>
<td>15/15</td>
</tr>
</tbody>
</table>
Table 2  Effect of berberine on NDEA-induced hepatocarcinogenesis in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of animals</th>
<th>% of tumour incidence</th>
<th>Liver weight/100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>10</td>
<td>0</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>2</td>
<td>Control (NDEA alone)</td>
<td>10</td>
<td>100 %</td>
<td>8.9±2.1(^b)</td>
</tr>
<tr>
<td>3</td>
<td>NDEA + berberine (10 mg kg(^{-1}))</td>
<td>10</td>
<td>100 %</td>
<td>5.2±1.2(^a)</td>
</tr>
<tr>
<td>4</td>
<td>NDEA + berberine (25 mg kg(^{-1}))</td>
<td>10</td>
<td>50 %</td>
<td>3.7±1.5(^b)</td>
</tr>
<tr>
<td>5</td>
<td>NDEA + berberine (50 mg kg(^{-1}))</td>
<td>10</td>
<td>50 %</td>
<td>3.5±0.4(^b)</td>
</tr>
</tbody>
</table>

\(^a\)P < 0.01, \(^b\)P < 0.001.

Table 3  Effect of berberine on γ-glutamyl transpeptidase activity in rats treated with N-nitrosodiethylamine (NDEA).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>γ-Glutamyl transpeptidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum (U L(^{-1}))</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>30±5.8</td>
</tr>
<tr>
<td>2</td>
<td>Control (NDEA alone)</td>
<td>152±37(^b)</td>
</tr>
<tr>
<td>3</td>
<td>NDEA + berberine (10 mg kg(^{-1}))</td>
<td>119±34(^a)</td>
</tr>
<tr>
<td>4</td>
<td>NDEA + berberine (25 mg kg(^{-1}))</td>
<td>93±39(^b)</td>
</tr>
<tr>
<td>5</td>
<td>NDEA + berberine (50 mg kg(^{-1}))</td>
<td>87±35(^c)</td>
</tr>
</tbody>
</table>

\(^a\)P < 0.2, \(^b\)P < 0.05, \(^c\)P < 0.02, \(^d\)P < 0.005 and \(^e\)P < 0.001.

Table 4  Effect of berberine on liver glutathione S-transferase and glutathione levels in rats treated with N-nitrosodiethylamine (NDEA).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Glutathione S-transferase (nmol min(^{-1}) (mg protein)(^{-1}))</th>
<th>Glutathione (nmol (mg protein)(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>377±13</td>
<td>3±0.2</td>
</tr>
<tr>
<td>2</td>
<td>NDEA alone</td>
<td>1769±898(^a)</td>
<td>5.3±0.8(^c)</td>
</tr>
<tr>
<td>3</td>
<td>NDEA + berberine (10 mg kg(^{-1}))</td>
<td>837±605(^b)</td>
<td>5±1.8</td>
</tr>
<tr>
<td>4</td>
<td>NDEA + berberine (25 mg kg(^{-1}))</td>
<td>674±370(^c)</td>
<td>4.6±1.6</td>
</tr>
<tr>
<td>5</td>
<td>NDEA + berberine (50 mg kg(^{-1}))</td>
<td>549±128(^c)</td>
<td>4.1±0.8</td>
</tr>
</tbody>
</table>

\(^a\)P < 0.02, \(^b\)P < 0.05, \(^c\)P < 0.005 and \(^d\)P < 0.001.

Reduced glutathione activity was also elevated by the administration of NDEA. However, berberine treatment had only a marginal effect on glutathione levels (Table 4).

Lipid peroxide and glutamate-pyruvate transaminase levels in normal rat serum were found to be 1.3±0.25 nmol mL\(^{-1}\) and 170±52 U mL\(^{-1}\), respectively. These were elevated to 3.0±0.67 nmol mL\(^{-1}\) and 589±168 U mL\(^{-1}\), respectively, after the administration of NDEA. Simultaneous administration of berberine significantly lowered the lipid peroxide and glutamate-pyruvate transaminase level in a dose-dependent manner (Table 5). Similarly, serum bilirubin, which is a biomarker for liver damage, was significantly (P < 0.01)
demonstrated that berberine acted as a calcium channel agonist, of cogenes (Chang et al., 1998). Berberine is an alkaloid widely used in medicinal preparations, for instance as an hepatoprotector (Antarkar et al. 1980). Berberine is an antibacterial agent, active against Gram-positive and Gram-negative bacteria. Zhou et al. (1995) demonstrated that berberine acted as a calcium channel agonist, and Matsumato et al. (1994) reported that it induced platelet aggregation. Kuo et al. (1995) showed berberine to produce aggregation of DNA and induction of apoptosis. Moreover, it was found to produce antitumour activity by down regulation of c-ki-ras2 oncogenes (Chang et al. 1990). Also, berberine was found to inhibit the crypt formation in the rat colon during azoxymethane-induced carcinogenesis (Fukutake et al. 1998).

The results of this study indicated a chemopreventive action of berberine in the two animal models studied. Berberine was found to reduce liver weight, γ-glutamyl transpeptidase, glutamate-pyruvate transaminase and bilirubin, all markers of liver injury, induced by NDEA. Berberine treatment was found to reduce the glutathione S-transferase levels in the liver, but had only a marginal effect on glutathione. Berberine reduced 20-methylnaphthanthrene-induced sarcoma in mice, as seen from the increased life span compared with the control animals and the reduction in the number of tumour-bearing animals. However, at present, the mechanisms of action of berberine are not known and require further investigation.

Table 5: Effect of berberine on lipid peroxide, glutamate-pyruvate transaminase and bilirubin in serum of rats treated with N-nitrosodiethylamine (NDEA).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Glutamate pyruvate transaminase (U mL⁻¹)</th>
<th>Lipid peroxide (nmol mL⁻¹)</th>
<th>Bilirubin (mg/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>170 ± 52</td>
<td>1.3 ± 0.25</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>NDEA alone</td>
<td>589 ± 168*</td>
<td>3.0 ± 0.67*</td>
<td>0.60 ± 0.19*</td>
</tr>
<tr>
<td>3</td>
<td>NDEA + berberine (10 mg kg⁻¹)</td>
<td>542 ± 100*</td>
<td>2.0 ± 0.6*</td>
<td>0.31 ± 0.09*</td>
</tr>
<tr>
<td>4</td>
<td>NDEA + berberine (25 mg kg⁻¹)</td>
<td>315 ± 190*</td>
<td>1.9 ± 0.1b</td>
<td>0.29 ± 0.07c</td>
</tr>
<tr>
<td>5</td>
<td>NDEA + berberine (50 mg kg⁻¹)</td>
<td>295 ± 129*</td>
<td>1.7 ± 0.3d</td>
<td>0.24 ± 0.03j</td>
</tr>
</tbody>
</table>

aP < 0.02, bP < 0.01, cP < 0.005 and dP < 0.001.

Discussion

Berberine is an alkaloid widely distributed in nature. *Berberis aristata* and *Berberis asiatica* are plants rich in this alkaloid. *B. aristata* is used in Chinese medicine and is much used in Indian medicinal preparations, for instance as an hepatoprotector (Antarkar et al. 1980). Berberine is an antibacterial agent, active against Gram-positive and Gram-negative bacteria. Zhou et al. (1995) demonstrated that berberine acted as a calcium channel agonist, and Matsumato et al. (1994) reported that it induced platelet aggregation. Kuo et al. (1995) showed berberine to produce aggregation of DNA and induction of apoptosis. Moreover, it was found to produce antitumour activity by down regulation of c-ki-ras2 oncogenes (Chang et al. 1990). Also, berberine was found to inhibit the crypt formation in the rat colon during azoxymethane-induced carcinogenesis (Fukutake et al. 1998).

The results of this study indicated a chemopreventive action of berberine in the two animal models studied. Berberine was found to reduce liver weight, γ-glutamyl transpeptidase, glutamate-pyruvate transaminase and bilirubin, all markers of liver injury, induced by NDEA. Berberine treatment was found to reduce the glutathione S-transferase levels in the liver, but had only a marginal effect on glutathione. Berberine reduced 20-methylnaphthanthrene-induced sarcoma in mice, as seen from the increased life span compared with the control animals and the reduction in the number of tumour-bearing animals. However, at present, the mechanisms of action of berberine are not known and require further investigation.

References

Matsumato, M., Ishida, S., Ichikawa, T., Sakiya, Y. (1994) Aggregation of DNA enhanced by the protoberberine