

Antitubercular effect of 8-[(4-Chloro phenyl) sulfonyl]-7-Hydroxy-4-Methyl-2H-chromen-2-One in guinea pigs

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ABSTRACT

Objective: To evaluate the antitubercular efficacy and safety of New Chemical Entity (NCE): 8-[(4-Chloro phenyl) sulfonyl]-7-Hydroxy-4-Methyl-2H-chromen-2-One (CSHMC) in guinea pigs. **Materials and Methods:** This pilot study was carried out in guinea pigs. They were infected with *M. tuberculosis* H₃₇Rv (1.5×10^4 cfu/guinea pig) via intramuscular route. After 30 days, infections were confirmed in 6 guinea pigs by histopathology of spleen, lung, and liver. After that CSHMC (5 and 20 mg/kg) was administered for 1 month and its effect was compared with vehicle, rifampicin (60 mg/kg) and isoniazid (30 mg/kg). Efficacy of CSHMC was evaluated on the basis of histopathologic scoring of lesion in lung, spleen, liver, and safety on the basis of measuring hemogram, liver and renal function parameters. **Results:** Isoniazid, rifampicin, and CSHMC (20 mg/kg) significantly reduce the median lesion score in lung, spleen, and liver as compared to disease control group. Reduction in median lesion score for lung and spleen were not statistically significant for CSHMC 5 mg/kg. CSHMC (20 and 5 mg/kg) did not produce any changes in hemogram, liver and renal function parameters with respect to normal values. **Conclusions:** CSHMC had shown significant antitubercular efficacy comparable to isoniazid and rifampicin and did not show hematological, hepato- and nephrotoxicity.

Key words: Isoniazid, rifampicin, tuberculosis

INTRODUCTION

Mycobacterium tuberculosis is the world's largest cause of death from a single microorganism after human immunodeficiency virus (HIV). Globally, there are almost nine million new cases of tuberculosis (TB) each year, two million of which results in death.^[1] It is estimated that about 1/3 of the current global

population is infected asymptotically with *M. tuberculosis* of whom 5%–10% will develop clinical disease during their lifetime and approximately two million deaths are attributable annually.^[2,3] TB continues to be a leading cause of mortality in spite of the availability of an effective chemotherapeutic regimen. Chemotherapy of TB consists of three or four drug regimen, administered for 6–9 months. The long duration of therapy results in poor compliance, produces unwanted side effects and therapeutic failure leading to emergence of multidrug resistant strains of *M. tuberculosis*.^[4-6] Association with noncompliance, HIV, and increasing multidrug resistant tuberculosis (MDR-TB) appears to be a serious issue, especially for the developing nations.^[7] Extensive drug resistant tuberculosis (XDR-TB) spreading all over the world is defined as a resistance to all first-line drugs and to fluoroquinolones

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Quick Response Code:	Website: www.jpharmacol.com
	DOI: 10.4103/0976-500X.85951

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and all of the injectables.^[8] The XDR-TB strain is mixing with the acquired immuno deficiency syndrome (AIDS), causing nearly 100% mortality.^[9,10]

Chemotherapy is being the mainstay of TB control. Development and evaluation of new chemical entity (NCE) against TB is a challenging task. 8-[(4-Chloro phenyl) sulfonyl]-7-Hydroxy-4-Methyl-2H-chromen-2-One (CSHMC) used in the present study is derivative of coumarin [Figure 1a] and heterocyclic analogue of dapsone (diaminodiphenyl sulfone [DADS]). Coumarin has a wide range of pharmacological properties, such as anticoagulant, antibacterial, diuretic, vasodilator and antiallergic.^[11] Several coumarin substitutions have shown antitubercular activity.^[12-14] Dapsone [Figure 1b] was found to be effective in suppressing experimental infections with tubercle bacilli.^[15-18] Minimum inhibitory concentration (MIC) of dapsone against *M. tuberculosis*, *M. avium* complex (MAC), and *M. leprae* are 50–250 mg/L, 2–100 mg/L, and 1.5–4 µg/L, respectively.^[19-21] So, a higher dose of dapsone is required in the treatment of tuberculosis. Metabolism of dapsone by hepatic oxidation to hydroxylamine and its subsequent reduction to amine within red cells leads to persistence of drugs in red cells and which is suspected for its hematological toxicity.^[16] An attempt was made to reduce its toxicity by replacing free amino (NH₂) group with chloro group. Due to antibacterial activity against a variety of microorganisms by sulfones, 7-hydroxy-4-methyl coumarin was selected for the synthesis of CSHMC. It has been shown to have an antitubercular activity *in vitro* on culture of sensitive strain of *M. tuberculosis* in Lowenstein–Jensen Media. The MIC was found to be 0.99 mg/L.^[22] The present study was planned to evaluate antitubercular efficacy and safety of CSHMC in isoniazid and rifampicin-sensitive strain in guinea pigs. If infected with *M. tuberculosis*, they show striking similarities to natural infections in humans, thus making it a good model for testing the effects of drug therapy.^[23-25]

MATERIALS AND METHODS

The study (experiment protocol no. 05/2007) was approved by the Institutional Animal Ethics Committee, Government Medical College, Bhavnagar, Gujarat, India.

Drugs and chemicals

Isoniazid (Alfa Aesar, A Johnson Matthey Company, Lancaster), rifampicin (Tokyo Chemical Industry Co. Ltd., Japan), purified protein derivative (PPD RT 23 Tween 80; Radiant Parenterals Ltd., Vaghodia, India) were purchased and used in the study. CSHMC was a kind free gift sample from the Department of Chemistry, Bhavnagar University, Bhavnagar, India. Its chemical structure is shown in Figure 1c. Chemical formula and molecular weight of CSHMC are C₁₆H₁₁O₅SCl and 350.5,

respectively. Spectral data (IR) confirmed the structure of the synthesized compound.

Bacterial strain and culture media

M. tuberculosis H₃₇Rv in Middle Brook 7H9 broth media was obtained from State Tuberculosis Demonstration Centre (STDC), Ahmedabad, Gujarat, India, as a free gift sample.

Animals

Dunkin–Hartley guinea pigs (250–300 g) and Swiss albino mice (20–30 g) of both genders were obtained from central animal house of the institute. Male and female guinea pigs were housed in separate stainless steel cages and mice in polypropylene cages under standard condition; temperature-controlled room (24°C ± 2°C) with 12 h light:dark cycles and were given standard laboratory diet and water *ad libitum*.

Acute oral toxicity determination

The nonpregnant, nulliparous female albino mice of 8–12 weeks old and 20–25 g body weight were selected to find out the acute oral toxicity of the CSHMC. The mice were fasted for 3 h prior to the experiment and were administered with CSHMC in the range of doses 300, 1000, and 2000 mg/kg and observed for mortality up to 14 days according to OECD guidelines.^[26] The LD₅₀ of CSHMC was found to be 300 mg/kg. Equivalent dose for guinea pig (200 mg/kg) was calculated as per Ghosh.^[27] 1/10th (20 mg/kg) and 1/40th (5 mg/kg) dose of it were selected for further study.

Mantoux test

All the guinea pigs were tested with Mantoux test using 0.1 mL of purified protein derivatives (PPD RT 23 Tween 80) injected intradermally into the lower left side of abdomen. All the guinea pigs examined (up to 72 h) were Mantoux negative and hence used for experiment.^[28]

Chemotherapeutic studies

Antitubercular study was carried out on guinea pigs and they were divided into 6 groups of either gender with 6 in each group.

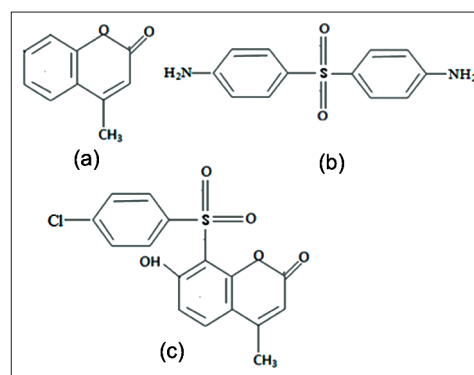


Figure 1: Chemical structure of (a) coumarin; (b) dapsone (diaminodiphenyl sulfone [DADS]); (c) 8-[(4-Chloro phenyl) sulfonyl]-7-hydroxy-4-methyl-2H-chromen-2-One

Cultures of *M. tuberculosis* H₃₇Rv were harvested from a 2-week culture in Middle Brooke 7H9 broth media. All the guinea pigs were infected with 0.1 mL (1.5×10^4 cfu/guinea pig) suspension via intramuscular route in the left thigh muscle. Qurrat-ul-Ain *et al.* and Challu *et al.* have produced a tuberculosis infection in guinea pigs via intramuscular route.^[29-31] This suspension was matched with McFarland standard. After 30 days, infections were confirmed in 6 guinea pigs by histopathology of spleen, lung, and liver. Other 30 guinea pigs were randomly divided into 5 groups (Groups II–VI) by random allocation software version 1.0, May 2004, by simple randomization. Drugs and CSHMC were administered by mouth once a day for 30 days in Groups III–VI. Human equivalent dose of isoniazid (30 mg/kg) and rifampicin (60 mg/kg) was calculated by extrapolation as mentioned by Ghosh.^[27] Shang *et al.* took a human-equipotent dose of isoniazid and rifampicin in guinea pigs as 30 and 50 mg/kg, respectively.^[32] Group II received vehicle (10% ethanol) by mouth once a day for 30 days. Food was withdrawn 12 h before the experiments.

Group I: Normal control group: Did not receive the injection of *M. tuberculosis* H₃₇Rv and received vehicle (10% ethanol) by mouth once a day for 30 days.

Group II: Disease control group: Received vehicle by mouth once a day for 30 days.

Group III: Standard control group: Received isoniazid (30 mg/kg) by mouth once a day for 30 days.

Group IV: Standard control group: Received rifampicin (60 mg/kg) by mouth once a day for 30 days.

Group V: Test compound group: Received CSHMC (20 mg/kg) by mouth once a day for 30 days.

Group VI: Test compound group: Received CSHMC (5 mg/kg) by mouth once a day 30 for days.

Safety parameters

Blood samples were collected in ethylene diamino tetra acetic acid (EDTA) and plain bulb from the carotid artery under pentobarbitone anesthesia intraperitoneally (30 mg/kg) 24 h after the last dose of drug. EDTA sample was used for estimation of hemoglobin (Hb), total white blood cell (WBC) count, total red blood cell (RBC) count, total platelet count, differential count (polymorphs, lymphocytes, eosinophils, monocytes), blood indices (packed cell volume, mean corpuscle volume, mean corpuscle hemoglobin, mean corpuscle hemoglobin concentration, red cell distribution width, and erythrocyte sedimentation rate). Complete blood count was analyzed by using celltac alpha cell counter (Nihontohdem, Japan). Serum was separated for estimation of biochemical parameters: Serum glutamic oxaloacetic transaminase (SGOT),^[33] serum glutamic pyruvic transaminase (SGPT),^[33] alkaline phosphatase (ALP)^[34] by enzyme kinetic method, bilirubin^[35] by Jendrassik–Grof method (total, direct, and indirect) and serum creatinine^[36] by Jaffé creatinine method in a fully automated analyzer.

Histopathologic lesion analysis

The animals were sacrificed soon after blood collection. Thorax and abdomen were cut open to remove lung, spleen, and liver. Their gross appearance was noted and they were placed in 10% formalin for 24 h. Then 5 mm thick pieces of the lung, spleen, and liver were embedded in paraffin, cut into 5 μ m thick sections, stained using hematoxylin–eosin dye (H and E), and finally mounted in dibutyl diesterate parathylate xylene.^[37] Each slide was coded and observer masked to evaluate histopathological changes in the lung, spleen, and liver. The scoring was done based on a previously described method and photomicrographs were taken.^[38] The guinea pig lungs were scored based on the following three criteria: (1) Primary lesion (epithelioid cells and other inflammatory cells): 0, no primary lesions present; 1, single primary lesion; 2, two or more primary lesions; multifocal; 3, two or more primary lesions; multifocal to coalescing; 4, multiple primary lesions, coalescing, and extensive. (2) Secondary lesion (granuloma formation, giant cells, and caseous necrosis): 0, no secondary lesion present; 1, up to 25% of lung involved; 2, up to 50% of lung involved; 3, up to 75% of lung involved; 4, above 75% of lung involved. (3) Pneumonitis (alveolar wall thickness, interstitial, and peribronchial inflammation): 0, alterations in less than 10% of the fields; 1, alterations in 10%–30% of the fields; 2, alterations in 30%–50% of the fields; 3, alterations in 50%–70% of the fields; 4, alterations in more than 70% of the fields are scored based on severity as follows: 0, none; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe. All individual scores were added for the final total score for each organ.

The spleen was scored based on two criteria (same scales as lung); (1) Primary lesion and (2) Secondary lesion. The liver was scored based on one criterion; ballooning degeneration: 0, no ballooning degeneration; 1, minimal enlargement in few hepatocytes; 2, mild enlargement in many hepatocytes; 3, moderate enlargement in most hepatocytes; 4, severe enlargement in most hepatocytes.

Statistical analysis

The histopathologic score of organs are presented as Median (Interquartile Range). The nonparametric Kruskal–Wallis test followed by Dunnett’s test was used to assess statistical significance between total lesion score of lung, spleen, and liver. The hematological, liver function, and renal function parameters are expressed as mean $\pm$ standard error of mean (SEM). Statistical differences between means were determined by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison tests using GraphPad Prism software demo version. $P < 0.05$ was considered as significant.

RESULTS

Organ histopathology

In normal control group (Group I); gross examination and

histology of lung [Figure 2a], spleen [Figure 3a], and liver [Figure 4a] of guinea pig showed the normal morphology.

Isoniazid, rifampicin, and CSHMC treated groups showed less white caseous granulomatous foci as compared with disease control group in lung and spleen by gross examination. Liver did not show any gross changes in Groups II–VI.

As shown in Figure 2b, lesion of lung consisted of epithelioid cells, lymphocytic infiltration, multinucleated giant cells, caseation necrosis, granuloma, and pneumonitis. Isoniazid [Figure 2c], rifampicin [Figure 2d], and CSHMC (20 mg/kg) [Figure 2e] treated group had shown a minimal epithelioid cells and alveolar wall infiltration with no caseation necrosis and giant cell. The lesion of spleen consisted of epithelioid cells, lymphocytic infiltration, multinucleated giant cells, caseation necrosis, and granuloma [Figure 3b]. Isoniazid [Figure 3c], rifampicin [Figure 3d], and CSHMC (20 mg/kg) [Figure 3e] treated group had minimal to mild epithelioid cells infiltration with no caseation necrosis and giant cell. As shown in Table 1, isoniazid, rifampicin, and CSHMC (20 mg/kg) significantly reduces the median lesion score in lung and spleen. Reduction in median lesion score of lung and spleen were not statistically significant for CSHMC (5 mg/kg)

[Figures 2f and 3f]. The lesion of liver consisted of ballooning degeneration [Figure 4]. CSHMC (20 and 5 mg/kg) treated group had shown a minimal or no ballooning degeneration. Isoniazid [Figure 4c], rifampicin [Figure 4d], and CSHMC [Figure 4e and 4f] significantly reduce the median lesion scores of liver [Table 1].

Safety analysis

The hemogram [Table 2], liver [Table 3], and renal [Table 3] function parameters were monitored after 30 days of isoniazid, rifampicin, and CSHMC treatment. The result indicates that CSHMC did not produce any changes in hemogram, liver functions, and renal function parameters with respect to normal values.

DISCUSSION

The main drawback of antitubercular chemotherapy is the lack of patient compliance, therapeutic failure, long duration of therapy, emergence of multidrug and extensive drug resistance tuberculosis, increasing incidence of HIV and toxic side effects, such as hepatotoxicity.^[39] The XDR-TB is virtually untreatable with high mortality and now treatment of it will depend on sensitivity of drugs. The second-line drugs

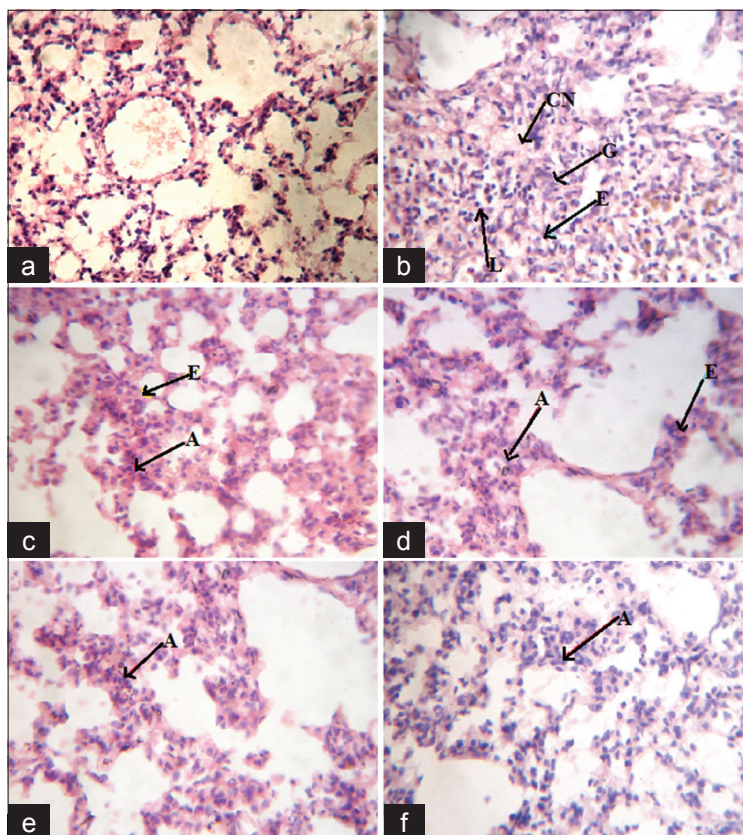


Figure 2: Histopathology of lung (H and E stain, $\times 40$). (a) Normal control group—normal alveolar morphology; (b) disease control group—severe (score 6) epithelioid cells (E), lymphocyte (L), alveolar wall infiltration (A), caseation necrosis (CN), and giant cells (G); (c–e) isoniazid, rifampicin, and CSHMC (20 mg/kg) treated group—minimal (score 0.5–1) epithelioid cells and alveolar wall infiltration with no caseation necrosis and giant cell; (f) CSHMC (5 mg/kg) treated group—mild (score 1.5) epithelioid cells and alveolar wall infiltration with no caseation necrosis and giant cell

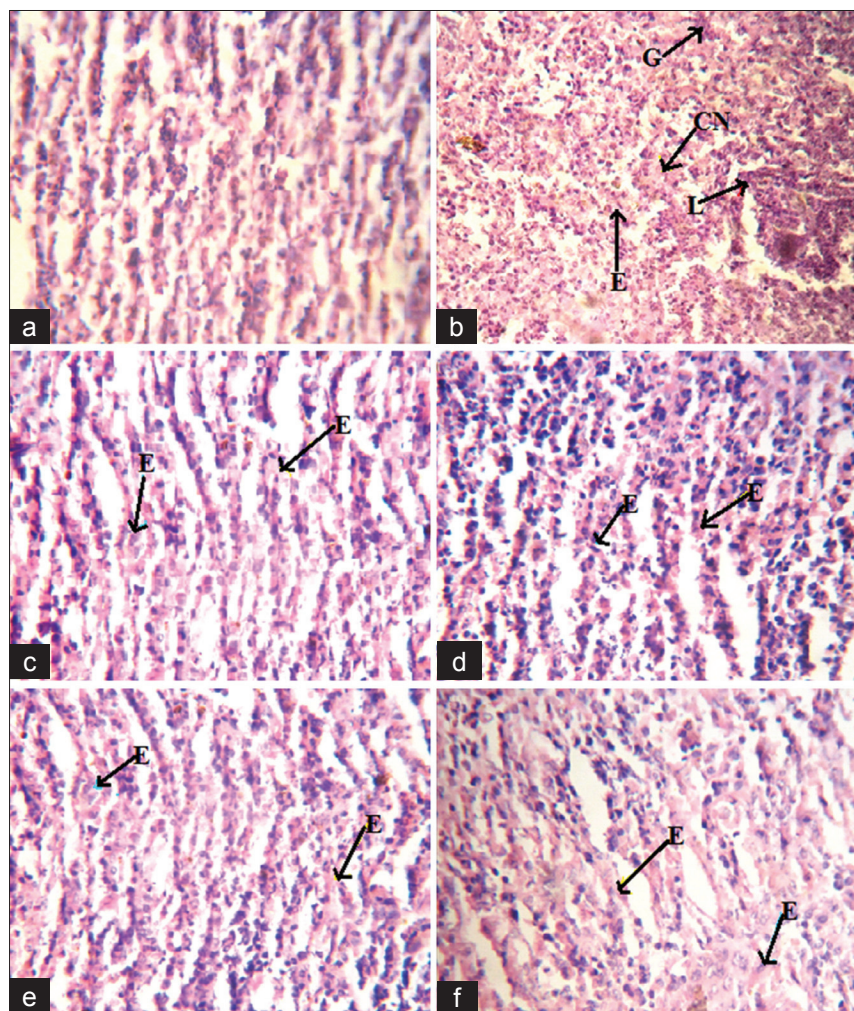


Figure 3: Histopathology of spleen (H and E stain, ×40). (a) Normal control group—normal morphology; (b) disease control group—severe (score 6) epithelioid cells (E), lymphocyte (L) infiltrate with caseation necrosis (CN), and giant cells (G); (c–e) isoniazid, rifampicin, and CSHMC (20 mg/kg) treated group—minimal to mild (score 1–1.5) epithelioid cells infiltration with no caseation necrosis and giant cell; (f) CSHMC (5 mg/kg) treated group—mild to moderate (score 2.5) epithelioid cells infiltration with no caseation necrosis and giant cell

are less efficacious, less convenient, more toxic, and more expensive. These drugs have to be given for 18–24 months.^[40] In view of this, we investigated the antitubercular efficacy and safety of coumarin derivatives: CSHMC in guinea pig model. The antitubercular efficacy was evaluated in terms of histopathological score of the organs of infected guinea pigs after daily administration of CSHMC for 30 days. Higher reduction of the histopathological score of lesion from the lung, spleen, and liver with CSHMC in the dose of 20 mg/kg than with 5 mg/kg suggests clearance of tubercle bacilli from the lesion in a dose-dependent manner. CSHMC-20 mg/kg had shown similar reduction in median score lesion of lung, spleen, and liver as compared to isoniazid and rifampicin. Antitubercular efficacy can be better measured by colony counting in the culture of the organs (lung, spleen, and liver) of the guinea pigs infected with *M. tuberculosis*. This is the limitation of our study.

The safety of CSHMC was evaluated in terms of alteration

on hematological, liver and renal function parameters of the infected guinea pigs. Antitubercular drugs, such as isoniazid, rifampicin, and ethambutol have been shown to induce hematological toxicity.^[41] In the present study, no alteration in hematological parameters with NCE in the dose of 20 and 5 mg/kg compared to normal control group. Most of the antitubercular drugs, such as isoniazid, rifampicin, and pyrazinamide have been shown to induce hepatotoxicity particularly when used in combinations.^[42] Levels of SGPT, SGOT, ALP, and total bilirubin were monitored as an index of hepatotoxicity. In the present study, no alteration in the levels of liver function parameters with CSHMC in the dose of 20 and 5 mg/kg were observed compared to normal control group. Antitubercular drugs, such as streptomycin and ethambutol cause nephrotoxicity.^[43,44] In this study, serum creatinine was used to detect nephrotoxic potential of CSHMC. No alteration in the level of renal function parameters with CSHMC in the dose of 20 and 5 mg/kg were observed compared to normal control group. This study indicates the CSHMC in the dose

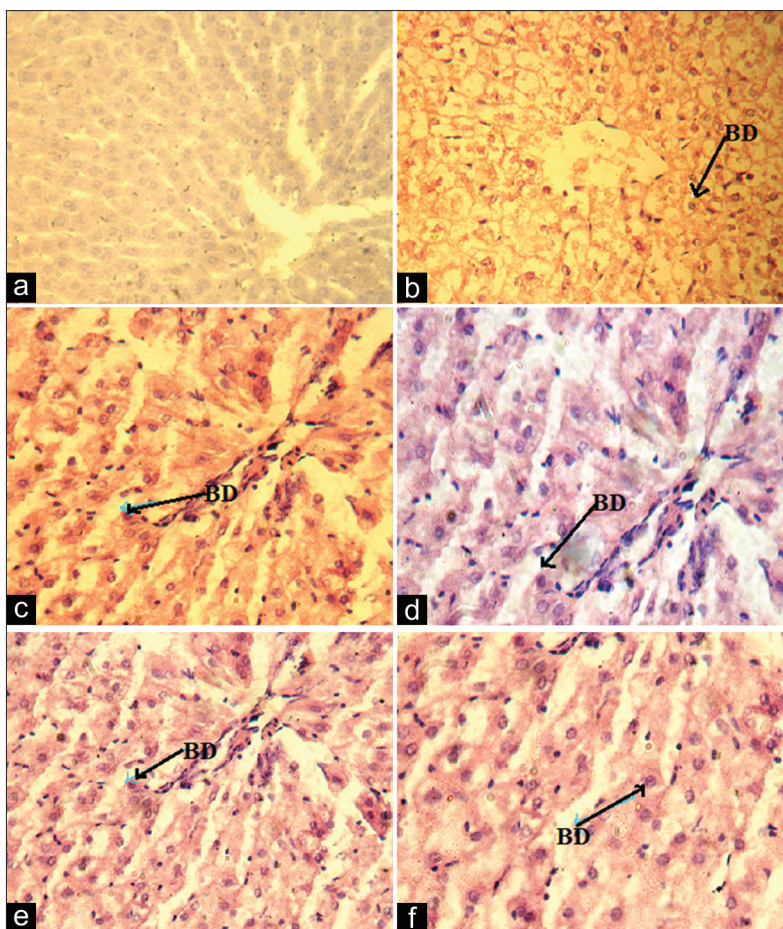


Figure 4: Histopathology of liver (H and E stain, x40). (a) Normal control group—liver shows normal morphology; (b) disease control group—a mild (score 2) ballooning degeneration (BD); (c–f) isoniazid, rifampicin, and CSHMC (20 and 5 mg/kg) treated group—a minimal or no (score 0.5) ballooning degeneration (BD)

Table 1: The lesion scores of lungs, spleen, and liver were calculated in all the 5 groups after 30 days of the treatment

Groups	Scoring of lesion		
	Lung	Spleen	Liver
Disease control	6 (4,8)	6 (5,9)	2 (1,3)
Standard control—isoniazid (30 mg/kg)	1 (0,3)**	1.5 (1,2)**	0 (0,1)**
Standard control—rifampicin (60 mg/kg)	1 (0,2)**	1.5 (1,3)**	0 (0,1)**
Test control—CSHMC (20 mg/kg)	0.5 (0,1)***	1 (1,2)**	0 (0,1)**
Test control—CSHMC (5 mg/kg)	1.5 (1,3)	2.5 (1,4)	0 (0,1)*

Values are presented as median (interquartile range) for 6 guinea pigs in each group. Statistical analysis Kruskal–Wallis followed by Dunnett's test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ treatment group compared to disease control group

of 20 and 5 mg/kg did not show the hematological toxicity, hepatotoxicity, and nephrotoxicity.

NCE is a coumarin derivative and heterocyclic analogue of dapsone (DADS) was attached to it. In DADS free amino group was replaced by chloro group, which may reduce the toxicity. Antimycobacterial effect of this compound may be due to its 8-[4-Chloro phenyl] sulfonyl] group and/or due to

coumarin ring. This study supports the role of chlorophenyl moiety at C4 for the antimycobacterial activity.^[45] Quantitative structure–activity relationship (QSAR) study remains open for the further studies.

CONCLUSIONS

CSHMC has shown an antitubercular effect. CSHMC in the dose of 20 mg/kg had shown more efficacies against the *M. tuberculosis* infection in guinea pigs compared with a dose of 5 mg/kg, which is comparable to isoniazid and rifampicin by histopathological data. In both doses, it did not produce hematological, hepatic and renal function toxicity.

ACKNOWLEDGMENT

Authors are sincerely thankful to Dr. K. R. Joshi, Senior officer, Research and Development Department, Excel Crop Care Limited, Bhavnagar, and Dr. N. K. Undavia, Ex-Professor and Head, Department of Chemistry, Bhavnagar University, Bhavnagar for providing NCE for research work; Dr. Nabendu Joshi, M. D., State

Table 2: Effect of isoniazid, rifampicin, and CSHMC on hemogram parameters in blood of guinea pigs after 30 days of treatment

Parameters	Groups						P value
	Normal control	Disease control	Standard control Isoniazid (30 mg/kg)	Standard control Rifampicin (60 mg/kg)	Test control CSHMC (20 mg/kg)	Test control CSHMC (5 mg/kg)	
Hemoglobin (g/dL)	14.5 ± 0.44	15.1 ± 0.38	14.1 ± 0.27	14.6 ± 0.25	14.3 ± 0.25	14.7 ± 0.32	0.39
Total RBC (million/m ³)	5.3 ± 0.17	5.2 ± 0.2	5.1 ± 0.19	5.2 ± 0.11	5.0 ± 0.13	5.2 ± 0.11	0.90
Total WBC (/m ³)	6916.7 ± 1180.2	6166.7 ± 734.2	4983.3 ± 384.2	5833.3 ± 705.5	4866.7 ± 440.9	6416.7 ± 1037.8	0.42
Total platelet (million/m ³)	0.31 ± 0.04	0.26 ± 0.01	0.28 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0.32 ± 0.03	0.64
ESR (mm/h)	5.2 ± 2.02	3.2 ± 0.6	3.3 ± 0.49	2.3 ± 0.42	2.8 ± 0.6	3.5 ± 0.4	0.49
Polymorphs (%)	45.2 ± 3.6	44.2 ± 4.5	46.0 ± 3.7	44.2 ± 4.2	43.5 ± 2.6	43.7 ± 4.2	0.10
Lymphocytes (%)	47.2 ± 2.07	47.2 ± 4.09	52.3 ± 1.8	53.0 ± 3.8	51.2 ± 2.6	49.7 ± 3.13	0.63
Eosinophils (%)	6.3 ± 1.6	5.8 ± 1.3	3.8 ± 0.6	3.0 ± 0.5	4.0 ± 0.6	5.2 ± 1.01	0.19
Monocytes (%)	3.2 ± 0.5	2.3 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	2.5 ± 0.2	2.3 ± 0.2	0.20
PCV (%)	42.1 ± 1.2	43.1 ± 1.3	38.1 ± 1.1	39.8 ± 1.4	40.9 ± 1.5	40.7 ± 1.95	0.23
MCV (Femtoliter)	80.02 ± 0.5	81.9 ± 0.7	78.3 ± 1.1	79.7 ± 1.3	80.7 ± 1.07	80.4 ± 1.04	0.24
MCH (Picogram)	27.6 ± 0.2	28.4 ± 0.2	27.5 ± 0.5	27.5 ± 0.3	27.7 ± 0.4	27.7 ± 0.3	0.37
MCHC (%)	34.4 ± 0.2	34.4 ± 0.2	34.8 ± 0.3	34.7 ± 0.3	34.5 ± 0.3	34.4 ± 0.3	0.80
RDW (%)	13.9 ± 0.4	13.97 ± 0.4	14.02 ± 0.3	14.95 ± 0.3	14.4 ± 0.3	14.7 ± 0.3	0.17

One-way ANOVA

Values expressed as Mean ± SEM for six guinea pigs in each group. RBC, Red blood cell; WBC, White blood cell; ESR, Erythrocyte sedimentation rate; PCV, Packed cell volume; MCV, Mean corpuscle volume; MCH, Mean corpuscle haemoglobin; MCHC, Mean corpuscle haemoglobin concentration; RDW, Red cell distribution width; ANOVA, Analysis of variance

Table 3: Effect of isoniazid, rifampicin, and CSHMC on liver and renal function parameters in blood of guinea pigs after 30 days of treatment

Parameters	Groups						P value
	Normal control	Disease control	Standard control Isoniazid (30 mg/kg)	Standard control Rifampicin (60 mg/kg)	Test control CSHMC (20 mg/kg)	Test control CSHMC (5 mg/kg)	
SGOT (u/L)	115.8 ± 15.9	128.8 ± 9.9	95.8 ± 8.8	110.3 ± 9.1	106.3 ± 4.9	101.5 ± 6.2	0.25
SGPT (u/L)	60.3 ± 5.1	52.8 ± 9.3	55.7 ± 11.2	61.7 ± 15.4	52.2 ± 3.5	67.2 ± 9.3	0.60
ALP (u/L)	262.2 ± 37.9	368.2 ± 50.6	352.5 ± 62.7	517.8 ± 85.5	421.5 ± 100.8	498.5 ± 83.5	0.16
Total serum bilirubin (mg/dL)	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.03	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.03	0.20
Direct serum bilirubin (mg/dL)	0.2 ± 0.03	0.3 ± 0.07	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.06	0.22
Indirect serum bilirubin (mg/dL)	0.3 ± 0.07	0.3 ± 0.09	0.2 ± 0.03	0.3 ± 0.03	0.3 ± 0.08	0.3 ± 0.04	0.72
Serum creatinine	1.0 ± 0.07	1.0 ± 0.07	0.9 ± 0.09	1.0 ± 0.08	1.0 ± 0.07	1.0 ± 0.14	0.72

One-way ANOVA

SGOT, Serum glutamic oxaloacetic transaminase; SGPT, Serum glutamic pyruvic transaminase; ALP, Alkaline phosphatase; ANOVA, Analysis of variance. Values expressed as mean ± SEM for six guinea pigs in each group

Tuberculosis Demonstration Centre, Ahmedabad, for providing *M. tuberculosis* bacilli H₃₇Rv strain.

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How to cite this article: Patel PB, Patel TK, Baxi SN, Acharya HR, Tripathi C. Antitubercular effect of 8-[(4-Chloro phenyl) sulfonyl]-7-Hydroxy-4-Methyl-2H-chromen-2-One in guinea pigs. *J Pharmacol Pharmacother* 2011;2:253-60.

Source of Support: Nil, **Conflict of Interest:** None declared.