

HEPATOPROTECTIVE ACTIVITY OF *MIKANIA SCANDENS* (L.) WILLD. AGAINST DICLOFENAC SODIUM INDUCED LIVER TOXICITY IN RATS

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ABSTRACT

Comparative hepatoprotective activity of *Mikania scandens* (L.) willd.(MS) against a well-established hepatoprotective drug Silymarin was assessed against Diclofenac sodium (DF) induced liver injury in albino rats. Animals were injected with Diclofenac sodium at the single dose of 150 mg/ kg body weight intraperitoneally once daily for 28 days. Diclofenac was used as the toxicant in hepatoprotective studies, in which various serum biochemical parameters and hepatic oxidative stress parameters were assessed. The hepatoprotective effect of *Mikania scandens* (L.) willd. (500 mg/kg) and its ethyl acetate, n-hexane fractions (500 mg/kg) were comparatively evaluated with Silymarin by measuring levels of serum biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), triglycerides, cholesterol and the hepatic oxidative stress parameters like as levels of, malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), super oxide dismutase (SOD) in rats. Histopathological studies also support the above mentioned parameters. The results indicated that the extract of *Mikania scandens* (L.) willd. possesses significant hepatoprotective activity. The effectiveness of *Mikania scandens* (L.) willd. and its fractions were found to be almost parallel with standard drug Silymarin.

Keywords: hepatoprotective, *Mikania scandens* (L.) Willd, Diclofenac sodium (DF), silymarin, Histopathology.

INTRODUCTION

Liver diseases are considered to be serious health disorders. The liver has one of the highest value of importance for the systemic detoxification and deposition of endogenous and exogenous substances. Liver dysfunction that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Drug-induced liver injury (DILI) possesses a major clinical problem. DILI has become the leading cause of acute liver failure and transplantation in Western countries.^{1,2} Hepatotoxicity associated with most other drugs are idiosyncratic, which implies by definition that DILI develops in only a small proportion of subjects exposed to a drug in therapeutic doses. The risk of acute liver failure associated with idiosyncratic hepatotoxins is usually less than 1 per 10'000 exposed patients. However, more than 1'000 drugs and herbal products have been associated with idiosyncratic hepatotoxicity.³⁻⁷ Diclofenac is used to treat pain, inflammatory disorders, and dysmenorrhea. The name is derived from its chemical name: 2-(2,6-dichloranilino) phenylacetic acid. Inflammatory disorder may include musculoskeletal complaints, especially arthritis, rheumatoid arthritis, polymyositis, dermatomyositis, osteoarthritis, dental pain, spondylarthritis, ankylosing spondylitis, gout attacks, and pain management in cases of kidney stones and gallstones. An additional indication is the treatment of acute migraines. Diclofenac is used commonly to treat mild to moderate post-operative or post-traumatic pain, in particular when inflammation is also present, and is effective against menstrual pain and endometriosis. Liver damage occurs infrequently, and is usually reversible. Hepatitis may occur rarely without any warning symptoms and may be fatal. Patients with osteoarthritis more often develop symptomatic liver disease than patients with rheumatoid arthritis. Liver function should be monitored regularly during long-term treatment. If used for the short-term treatment of pain or fever, diclofenac has not been found to be more hepatotoxic than other NSAIDs.^{8,9} Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Herbal medicines derived from plant extracts are widely utilized to treat a wide variety of clinical diseases including liver disease.^{10,11} The present study was designed to evaluate the hepatoprotective activity of *Mikania scandens* (L.) Willd. against diclofenac sodium induced hepatotoxicity in rats compared with Silymarin a known hepatoprotective drug.

MATERIALS AND METHODS

Plant Material

The whole plant with leaves, stems and roots were collected from rural areas of East Medinipur, West Bengal. The plants were thoroughly washed with water; roots and stems were discarded and the leaves were dried in hot air woven at 35°C for 7 days. The authentication of the plant was done by Central National Herbarium, Botanical Garden, Howrah, Voucher no. CNH/124/2011 /Tech.II/614.

Extraction of the leaves of *Mikania scandens* (L.) Willd

The leaves of *Mikania scandens* (L.) Willd. were dried and powdered. The powdered materials were defatted with petroleum ether (60-80°C). Then subjected to Soxhlet extraction by sufficient volume of ethyl alcohol (95%) to get the ethanolic extract. Then from the ethanolic extract different fractions like ethyl acetate and n-hexane fractions were isolated.¹²

Chemicals

Diclofenac sodium and silymarin were supplied from Institute. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), Total Protein, Total Billurubin, Total cholesterol and triglycerides etc kits were obtained from Span Diagnostic Lab, India. Other chemicals used in this experiment were also of analytical grade.

Phytochemical screening

The freshly prepared crude extract was tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Carbohydrates with Benedict's reagent, Proteins with Biuret test, Alkaloids with Dragendorff's reagent, tannins with ferric chloride and potassium dichromate solutions, saponins with foam test, steroids with Libermann-Burchard reagent and flavonoids with the use of Mg and HCl. These were identified by characteristic color changes using standard procedures.¹³

Experimental animals:

Male Wistar albino rats, weighing about 180 – 200g were obtained from institute animal center and used in the experiments. The protocol was approved by the Institute's Animal Ethical Committee. Animals were kept in animal house at an ambient temperature of

25°C and 45 – 55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and water *ad-libitum*. All the experiment procedures were performed according to the purpose of control and supervision of experiments on animal (CPCSEA), ministry of social justice and empowerment Government of India.

Experimental procedure:¹⁴⁻²²

The rats were divided into the nine groups each containing 6 rats.

Group-I: Control rats, which fed normal diet and water.

Group-II: rats treated with diclofenac (150 mg/ kg, i.p.) for 28 days.

Group-III: rats treated with diclofenac (150 mg /kg, i.p.) + Silymarin (140 mg/kg) orally once daily for 28 days.

Group-IV: rats treated with diclofenac (150 mg/ kg, i.p.) + crude extract of Mikania scandens(500mg/kg) once daily for 28 days.

Group-V: rats treated with diclofenac (150 mg/kg, i.p.) + Ethyl acetate fraction MS(500mg/kg) orally once daily for 28 days.

Group-VI: rats treated with diclofenac (150 mg/ kg, i.p.) + n-hexane fraction of MS(500mg/kg) orally once daily for 28 days.

Group-VII: rats treated with Silymarin (140 mg/kg) orally once daily for 28 days.

Group-VIII: rats treated with MS (500mg/kg) orally once daily for 28 days.

Group-IX: rats treated with diclofenac (150 mg/ kg, i.p.) + MS(500mg/kg) + Silymarin (140 mg/kg) orally once daily for 28 days.

After the completion of the treatment, blood samples were collected from retro-orbital cavity of eye. The serum was used for the estimation of biochemical parameters. Liver was dissected out and washed with saline water. After then the homogenate was prepared in 0.1N Tris HCL buffer. Prepared homogenate was used for the evaluation of Hepatic oxidative stress Parameters.

Biochemical Estimation

The levels of aspartate aminotransferase(AST), alanine aminotransferase(ALT), and alkaline phosphatase(ALP) ,Total Protein(TP), Total Billurubin(TB), cholesterol, and triglycerides were estimated in the serum by using standard kits from Span India ltd, surat, India. Prepared liver homogenate was centrifused for 25 minutes. After then supernatant was used for the estimation of lipid peroxidation (LPO)²³, reduced glutathione (GSH)²⁴, super oxide dismutase(SOD)²⁵, and catalase(CAT)²⁶.

Histopathological studies

The livers were excised quickly and fixed in 10% formalin and paraffin embedded. sections of about 4- 6 μ m were stained with haemotoxylin and eosin(H&E) for Histological evaluation. In brief 4- 6 μ m thick section of paraffin embedded rat liver were dewaxed with distilled water for 2min. then the section were stained with haemotoxylin for 5 min at room temperature. After 15 min, the section were counterstained with eosin for 2min, dehydrated with alcohol, washed with xylene and blocked by eosin. Hemotoxylin and eosin stained studies were observed under microscope²⁷.

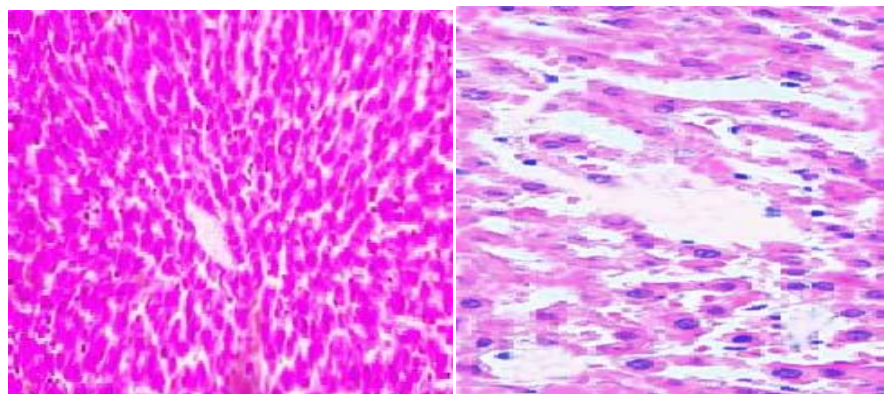
Statistical Analysis

Data for hepatoprotective activity were expressed as Mean \pm SEM from six rats in each group. Hepatoprotective activity were analysed statistically using one way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Test with the help of INTA soft ware. P value of < 0.05 was considered as statistically significant.

RESULTS

Measurement of various biochemical parameters and hepatic oxidative stress parameters are used in the diagnosis and treatment of a variety of diseases involving the liver as well as other metabolic disorders. After 28 days to study the hepatoprotective effect of MS and its fraction, biochemical parameters and hepatic oxidative stress parameters were measured. During experimental study we showed that in diclofenac treated rats(Group-II) AST, ALT, ALP, TB, Cholesterol and Triglycerides levels were sharply increased as compared to normal control group animals. Above results were indicated that severe liver damage occurred by diclofenac. AST, ALT, ALP, TB, Cholesterol and Triglycerides levels were significantly decreased as compared to control group animals when treated with MS and its ethyl acetate, n-hexane fractions (Table1). Results are compared with the standard drug silymarin treated animals (Group-III). Antioxidant enzymes activities of liver are presented in Table 2. SOD, CAT, GSH activities were reduced significantly in the diclofenac treated rats, when compared with the normal rats. In Diclofenac + MS and its fractions treated rats the activities of these enzymes attained a near to normal value.

Animals of silymarin + MS+ DF treated group were shown mostly significant values due to its synergistic effect. But animals of Group-VII (only silymarin without DF) and Group-VIII(only MS without DF) shown values near to normal control group. Histopathological studies showed that the normal control rats had normal hepatocytes and central veins. Necrosis of normal hepatocytes, inflammatory cell and congestions were observed in DF treated animals. The liver sections of the rats treated with silymarin, MS extract and its fractions followed by DF showed a sign of healing which was indicated the absence of necrosis. All results are compared with the silymarin + DF treated animals (Group-III).



Normal control (Group I)

DF treated (Group II)

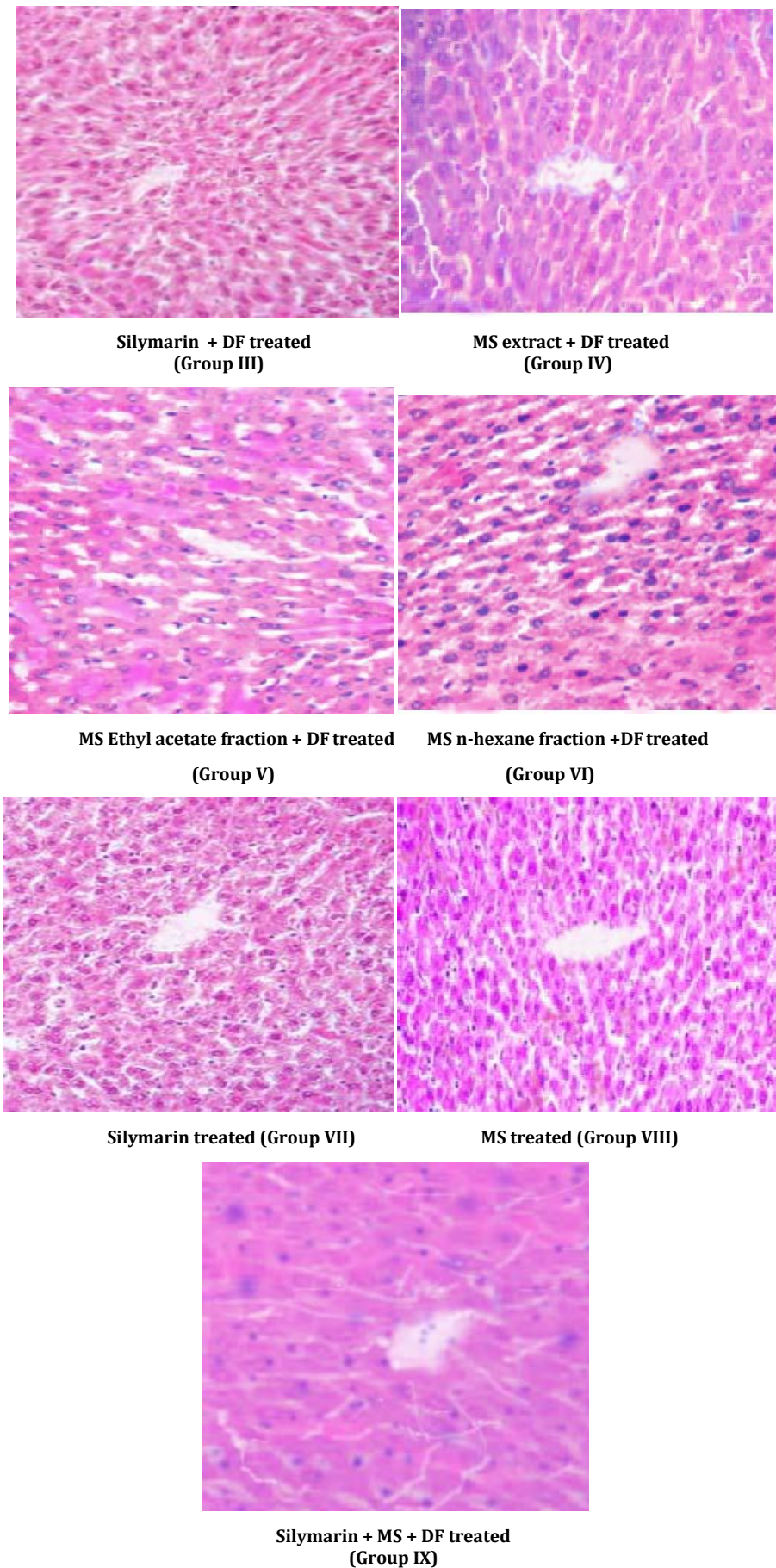


Fig. 1: Histopathological changes in liver of following administration of DF, Silymarin, MS extract and its fractions in rats

Table 1: Effect of *Mikania scandens* (L.) Willd. (MS) and its fractions in Diclofenac(DF) treated rats on serum biochemical parameters

Group	AST U/L	ALT U/L	ALP U/L	TP gm/dl	TB mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
Normal Control	54.28 ± 1.17	51.59 ± 0.91	48.28 ± 1.08	6.68 ± 0.22	0.41 ± 0.20	112.31 ± 0.78	127.46 ± 0.35
DF	134.83 ± 1.046###	127.32 ± 0.22###	139.57 ± 0.68###	2.42 ± 0.15###	1.87 ± 0.02###	198.14 ± 0.69###	163.37 ± 0.54###
Silymarin + DF	67.23 ± 0.82***	62.43 ± 1.87***	59.82 ± 1.46***	5.51 ± 0.94**	0.73 ± 0.01***	134.38 ± 0.46***	141.60 ± 0.38***
MS extract + DF	64.99 ± 1.66***	66.15 ± 1.77***	58.14 ± 0.59***	5.91 ± 0.50**	0.60 ± 0.02***	136.19 ± 0.74***	139.37 ± 0.57***
MS Ethyl acetate fraction + DF	70.43 ± 0.54***	86.48 ± 0.72***	66.18 ± 0.80***	5.65 ± 0.53**	0.70 ± 0.01***	138.34 ± 0.61***	144.80 ± 0.80***
MS n-hexane fraction + DF	69.198 ± 1.28***	86.48 ± 0.72***	59.08 ± 0.56***	5.85 ± 0.25**	1.01 ± 0.01***	135.32 ± 0.55***	144.63 ± 0.83***
Silymarin	54.378 ± 1.087***	52.32 ± 1.29***	47.08 ± 0.22***	6.82 ± 0.74***	0.41 ± 0.01***	112.01 ± 0.36***	128.48 ± 0.62***
MS extract	55.08 ± 1.10***	51.41 ± 0.73***	47.89 ± 2.05***	6.74 ± 0.37***	0.45 ± 0.01***	112.30 ± 0.57***	127.57 ± 0.18***
Silymarin + MS extract + DF	60.39 ± 0.62***	60.64 ± 0.60***	57.56 ± 0.63***	5.83 ± 0.73**	0.59 ± 0.02***	132.30 ± 0.29***	131.37 ± 0.36***

###P<0.001, considered when diclofenac treated group compared to normal control group. ***P<0.001, **P<0.01 are considered statistically significant when other groups are compared to diclofenac treated group.

Table 2: Effect of *Mikania scandens* (L.) Willd. (MS) and its fractions in Diclofenac(DF) treated rats on the hepatic oxidative stress parameters

Group	LPO nm of MDA/mg of protein	GSH µg/mg of protein	CAT U/mg of protein	SOD U/mg of protein
Normal Control	5.64 ± 0.32	15.53 ± 0.28	41.97 ± 0.43	47.21 ± 0.37
DF	26.77 ± 0.31###	5.68 ± 0.35###	29.33 ± 0.23###	22.21 ± 0.36###
Silymarin + DF	7.45 ± 0.36***	11.58 ± 0.35***	38.22 ± 0.77***	42.89 ± 0.30***
MS extract+ DF	7.74 ± 0.08***	11.69 ± 0.40***	39.41 ± 0.58***	44.70 ± 1.30***
MS Ethyl acetate fraction + DF	10.14 ± 0.25***	11.81 ± 0.27***	38.19 ± 0.78***	31.41 ± 0.17***
MS n-hexane fraction + DF	10.19 ± 0.25***	11.51 ± 0.39***	38.24 ± 0.77***	43.24 ± 0.23***
Silymarin	5.70 ± 0.14***	15.51 ± 0.24***	42.57 ± 0.30***	47.35 ± 0.19***
MS extract	5.61 ± 0.14***	15.55 ± 0.26***	42.513 ± 0.25***	47.26 ± 0.18***
Silymarin + MS extract + DF	6.81 ± 0.12***	12.55 ± 0.75***	40.09 ± 0.31***	44.75 ± 0.30***

###P<0.001, considered when diclofenac treated group compared to normal control group. ***P<0.001, considered statistically significant when other groups are compared to diclofenac treated group.

DISCUSSION

The present study evaluates the hepatoprotective role of ethanolic extract *Mikania scandens* (L.) willd. against diclofenac - induced hepatotoxicity in albino rats with the possible involvement of the antioxidants. Hepatotoxicity from NSAIDs can occur at any time after drug administration, most commonly occurs within 6-12 weeks of therapy. There are two clinical patterns of hepatotoxicity due to NSAIDs. The first is an acute hepatitis with jaundice, fever, nausea, eosinophilia etc and other is the chronic active hepatitis.²⁸⁻³¹ The possible mechanism of Diclofenac induced liver injury due to hypersensitivity and metabolic aberration. Diclofenac sodium is a well known anti-inflammatory, antipyretic and analgesic drug which is safe in therapeutic doses but can produce serious liver damage in human and experimental animal with toxic doses.³² The liver damage causes leaking of cellular enzymes into the plasma due to the disturbance of hepatocytes transport functions. A group of enzymes to be found in cytosol is discharge into the blood when liver cell plasma is damaged. So causing increased enzyme levels in the blood serum. Antioxidant marker enzymes are used for estimation of hepatocellular damage³³. After 28 days treatment with Diclofenac, AST, ALT, ALP, TB, Cholesterol, Triglycerides levels were estimated. There was a sharp rise in above biochemical parameters in Diclofenac treated experimental group. The AST, ALT, ALP, TB, Cholesterol, Triglycerides levels were significantly decreased in MS extract and its fractions treated group (Table 1). Measurement of total protein is used in the diagnosis of variety of hepatic diseases. A decrease in total protein was indicated that a massive necrosis of the liver as well as disturbances of liver functions.³⁴⁻³⁵ Total protein level was decreased in diclofenac treated experimental group which indicated that a serious injury of liver. Total protein level was sharply increased at higher concentration when treated with test drugs indicating the liver protective nature of *Mikania scandens* (L)

willd. The rise in TP levels suggests that leading to protein synthesis (Table 1). The above results were well comparable with Diclofenac + silymarin treated groups. Free radicals play an important role in the pathogenesis of liver damage. LPO can be used as the hepatic oxidative stress parameters for measuring the damage that occurs in membranes of tissue due to result of free radical generation³⁶⁻³⁸. In present study, administration of diclofenac, significantly increased the LPO level. Significant elevation of LPO level observed in diclofenac induced hepatotoxic group is possibly due to the generation of free radicals which results superoxide catalysed oxidation process. During treatment with MS extract and its fractions decreased LPO level at a dose of 500 mg/kg body weight as compared to Diclofenac treated rats. Endogenous antioxidant roles in diclofenac sodium- induced rats group was measured here by evaluating the activities of CAT, SOD and GSH. These are important biomarkers for Scavenging free radicals. These reports also suggest that diclofenac produces hepatic injury. The major role of CAT is to scavenge H₂O₂ that has been generated by free radicals or by SOD in its removal of superoxide anions, and convert it to water.³⁹⁻⁴¹ In our experimental results we have showed that significantly decreased levels of GSH, CAT, SOD in diclofenac induced hepatotoxicity rats. Whereas test drug treated rats sharply increased the above hepatic oxidative stress parameters. The above results were well comparable with Diclofenac + silymarin treated groups. The results of the present work indicate that *Mikania scandens*(L.) willd. can protect the liver against diclofenac sodium induced hepatotoxicity.

CONCLUSION

In conclusion, the results of this study demonstrate that *Mikania scandens*(L) willd. has possess a potent hepatoprotective action on diclofenac induced Liver damage in rats. However, further detailed studies are required to isolate the active compound(s) and to establish its clinical application.

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