Research Article

Preliminary pharmacological evaluation of *Manilkara zapota* stems bark extract for ulcerative colitis in rats

Anuj Kumar, Himanshu Bhushan Sahoo*

RKDF College of Pharmacy, SRK University, Hoshangabad road, Misrod, Bhopal - 462026 (MP) India

Received: 3 July 2020 Revised: 24 June 2020 Accepted: 5 August 2020

Abstract

Objective: The aim of present work was to investigate preliminary pharmacological screening of ethanol extract of *Manilkara zapota* stems barks for Dextran Sulphate Sodium (DSS) induced ulcerative colitis in rats. **Material and methods:** Phytochemical screening of *Manilkara zapota* stems barks (MZSB) extract was performed to detect the presence of different chemical constituents. Selected ethanol extract of MZSB was screened for DSS induced ulcerative colitis in rats. Group I was indicated untreated animals while Group II is control received DSS (3%w/v in drinking water) + 0.9% saline at a dose of 50 ml/kg. Treatment group III received DSS + ethanolic extract suspension 150 mg/kg and group IV received DSS + ethanolic extract suspension 200 mg/kg. Reference group received Sulfasalazine with 500 mg/kg suspension. The effect was observed by the measurement of disease activity Index and weight of colon along with MPO activity and MDA content. Antioxidants parameters were also observed to confirm the action behind protective effect of MZSB. **Results and conclusion**: Phytochemical study revealed that flavonoids and phenolic compounds are present in ethanolic extracts of *Manilkara zapota* ethanolic stem bark. The disease activity index and colon weight for different groups were observed. The disease activity index and weight of colon for observed significantly decreased in dose dependent manner. Results were also comparable to the standard drug treatment group. The ethanolic extract of *Manilkara zapota* stem bark (EEMZ) showed MPO activity significantly elevated whereas inhibited MPO activity with 200mg/kg as similar to standard drug. The value of MDA content was decreased significantly similar to the standard group. Significant increase in GSH level and reduction in MDA level indicated antioxidant activity of EEMA. Hence, the probable mechanism of healing of ulcerative colitis of *Manilkara zapota* stem bark extract may be attributed to antioxidant and free radical scavenging effect may be attributed to presence of flavonoids and phenolic compounds.

Keywords: *Manilkara zapota,* Dextran Sulphate Sodium, Sulfasalazine, antioxidant, ulcerative colitis

Introduction

Inflammatory bowel disease (IBD), which comprises Crohn disease and ulcerative colitis, characterizes a group of chronic diseases illustrated GIT. The chief reason of IBD are not well implicit, but inequities in proinflammatory cytokines like TNF-α, IFN-γ, IL-1, IL-6, and IL-12 and anti-inflammatory cytokines as well as IL-4, IL-10, and IL-11 are consideration to participate an innermost character in mediating and modulating inflammation (Jump and Levine, 2004). Ulcerative colitis (UC) is a rectal and colonic mucosal chronic, idiopathic, inflammatory bowel disease (IBD). It is characterized by colonic inflammation, most likely due to the infiltration of polymorphonuclear cells, lymphocytes, monocytes and plasma cells, accompanied by oxygen-free radicals, which ultimately leads to mucosal alteration and ulceration (Cho *et al*., 2007).

Manilkara zapota L. (Family- Sapotaceae), commonly known as the sapodilla is a long-lived, evergreen tree native to southern Mexico, Central America and the Caribbean (Chopra et al., 1956). The leaves of the sapodilla can also be used as a medicine for inflammatory diseases. The leaves work as an oral anti-inflammatory agent. Take clean sapodilla fruit leaves and then boil them for about ten minutes. This boiled water which contains the extracts of

Address for Corresponding Author:
Dr. Himanshu Bhushan Sahoo
RKDF College of Pharmacy, SRK University, Hoshangabad road, Misrod, Bhopal - 462026 (MP) India
Email: rkdfcp95@gmail.com

DOI: https://doi.org/10.31024/apj.2020.5.4.6

Sapodilla can be used as medicine. It can be used for gargling as well (Wealth of India, 1976). Chikoo or Sapodilla is an excellent source of minerals such as potassium, copper and iron. In addition to these minerals, Chikoo also consists of foliate and niacin acid. These vitamins and minerals help in making the body powerful and energetic. Sapodilla also helps in the treatment of cold and cough. Consumption of this fruit helps to remove nose blockage and also in case of persistent coughs. The simple sugars like fructose and sucrose that are the main ingredients of this fruit rejuvenate the body with natural energy (Chopra et al., 1956). Folic acid contained in this fruit is used in the formation of red blood cells and also help in the development of the fetus during pregnancy. It also helps to prevent the formation of homocysteine which is harmful for health.

Present study consists of phytochemical screening of ethanol extract of *Manilkara zapota* bark for detection of presence of different chemical constituents in the form of spots. Ethanol extract was further investigated for detail anti-inflammatory activity by using carrageenan and histamine induced inflammation in rats.

Material and methods

Identification and Collection of plant Material

The stem bark of *Manilkara zapota* was collected in the month of August from district-Jalgaon, Maharashtra region. Plant was identify and authenticated at the Department of Botany, SRK University, Bhopal (MP). The rhizomes were clean and cut in small pieces sun dried and was powdered moderately.

Extraction and Phytochemical studies

The crude powdered drug (50.0 g) was subjected to successive extraction in Soxhlet apparatus with petroleum ether for defatting and ethanol at 60 – 80^{0}C. After each extraction test was performed to see whether the drug had been completely exhausted or not. All liquid extracts were collected in round bottom flask and subjected to recover in soxhlet apparatus by distillation. Then all the extracts concentrated in water bath until all the solvent had been removed to give an extract in semisolid form.

Acute toxicity study

The acute oral toxicity study of crude extract of *Manilkara zapota* stem bark was carried out as per the guidelines set by Organization for Economic Co–operation and Development (OECD) guideline 423. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 423 was adopted in March 1996 as the second alternative to the conventional acute toxicity test, described in Test Guideline 401.

Pharmacological screening for Dextran Sulphate Sodium (DSS) induced colitis

The administration of DSS contained in water causes haematochezia, body weight loss, shortening of the intestine, mucosal ulcers and neutrophil infiltration. The acute colitis is considered to be induced by innate immunity but not acquired immunity. The Chronic phase on the other hand is said to be caused by lymphocytes that are activated by the cytokines secreted from the activated macrophages (Jurjus et al., 2004).

Ulcerative colitis was induced in rats by adding DSS (Dextran Sulfate Sodium) to water bottles to result in a 3% solution (w/v) (Hirata et al., 2001). The animals were provide to free access to water containing 3% DSS orally for 7 days. All treatment regimens were continued for 7 consecutive days. Drugs were given by oral gavage, once daily and were suspended in Sodium CMC. On the 8th day, clinical activity scores were measured and the animals were anesthetized with ether and blood was collected by retro orbital puncture for biochemical estimation. Body weight, stool consistency and gross bleeding were recorded daily.

Six animals were taken in each group and three groups were made in each wound model. The animals were divided into 5 groups consisting 6 animals in each:

- Group I normal or untreated animals
- Group II is control received Dextran sodium sulfate (3%w/v in drinking water) + 0.9% saline at a dose of 50 ml/kg,
- Group III received Dextran sodium sulfate (3%w/v in drinking water) + ethanolic extract suspension 150 mg/kg
- Group IV received Dextran sodium sulfate (3%w/v in drinking water) + ethanolic extract suspension 200 mg/kg.
- Group V received Dextran sodium sulfate (3%w/v in drinking water) + Sulfasalazine in a dose of 500 mg/kg suspension.

Assessment of colon damage by macroscopic scoring

Disease activity score was quantified with a clinical score assessing weight loss, stool consistency and bleeding of the colon as described by Cooper, which was divided by 3 (Niu et al., 2013). Each score was determined as follows:

- Change in body weight loss (0: none, 1: 1–5%, 2: 5–10%, 3: 10–20%, 4: >20%), Stool blood (0: negative, 1: +, 2: ++, 3: +++, 4: ++++)
- Stool consistency (0: normal, 1 and 2: loose stool, 3 and 4: diarrhea)
- Body weight loss was calculated as the percent difference between the original body weight (day 0) and the body weight on any particular day.

Myeloperoxidase (MPO) assay

MPO activity was determined with the O-dianisidine method (Liu and Wang, 2011; Yang *et al*., 2012) using a MPO detection kit. Blood was collected from eyes and centrifuged. The MPO activity was measured with a spectrophotometer (Shimadzu) by absorbance at 460 nm. MPO activity was defined as the quantity of enzyme degrading 1µmol of peroxide per minute at 37°C and was expressed in units per liter serum.

Determination of malondialdehyde (MDA) content

Lipid peroxidation was assessed as MDA content of the colon according to the method described by Mihara and Uchiyama (1978). In short, the colorimetric determination of MDA is based on the reaction of one molecule of the reactive aldehyde with two molecules of thiobarbituric acid at low pH (2–3) and at a temperature of 95 °C for 45 min. The resultant pink color was extracted by n-butanol, and the absorbance was determined at 532 and 520 nm spectrophotometrically. The difference in optical density between both wavelengths was used as a measure of colonic MDA content. The final value of MDA was represented as nmol/mg protein.

Determination of antioxidants assay

In dead space wound model, one part of granuloma tissue was used for antioxidant assay. Catalase was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in granuloma tissue by the method of Moron et al, (1979).

Statistical analysis

The data were expressed as mean standard deviation (SD). The statistical significance of the difference in each parameter among the groups was evaluated using one-way analysis of variance (ANOVA) followed by the followed by the multiple comparison test of Tukey–Kramer tests. Criterion for statistically significant difference was chosen to be at $P<0.01$.

Results and discussion

Phytochemical studies

The plant material was identified and powdered material was subjected to successive extraction with petroleum ether and ethanol. The percentage yield obtained from non polar (petroleum ether) and polar solvents i.e. ethanol 3.4 and 4.8% w/w respectively. The Preliminary phytochemical investigations revealed the presence of various phytoconstittuents in ethanol extract i.e. glycosides, Tannins and flavonoid compounds. Petroleum ether extract containing steroids, and terpenoids.

Acute toxicity study

Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases no one death was observed within first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and motor activity and behavioral pattern. Attention was also given to observation of tremors and convulsions. We have selected one tenth dose of highest toxic dose level.

Effect of *Manilkara zapota* stem bark (EEMZ) extract on Dextran Sulphate Sodium (DSS) induced colitis

DSS produces severe macroscopic edematous inflammation in the colon. The disease activity index and wet colon weight for different groups were observed. The disease activity index and weight of colon for colitis control group were found to be 8.31±0.43, and 197.65±7.62, respectively. The disease activity index and colon weight for EEMZ were observed significantly decreased in dose dependent manner. EEMZ 200 mg/kg dose decreases disease activity index and weight of colon significantly as 3.28±0.57 and 170.11±5.11, respectively (Table 1). However, the EEMZ 200 mg/kg showed better results in these parameters, indicating its potent activity at the dose tested. These data were also comparable to the standard drug treatment group.

Table 1. Effect of ethanolic extract of *Manilkara zapota* stem bark (EEMZ) on macroscopic observations in rats

Groups	Disease activity Index (% protection)	Weight of colon (mg/cm)
Normal control	0	169.75±5.27
Control (0.9% saline)	8.31±0.43	197.65±7.62
EEMZ 150 mg/kg	5.47±0.61 (34.17)	185.28±5.61
EEMZ 200 mg/kg	3.28±0.57 (60.51)*	170.11±5.11*
Sulfasalazine (500mg/kg)	3.02±0.91 (63.65)*	173.46±4.83*

n = 6 albino rats per group, value represents Mean S.D. *P< 0.01, when compared each treated group with control group.

The effect of *Manilkara zapota* stem bark (EEMZ) ethanolic extract on different biochemical parameters were also observed in dose dependent manner. In the experiment, we found that MPO activity was correlated with the development of colonic inflammation. DSS induced colitis significantly elevated MPO activity, whereas administration of EEMA strongly inhibited MPO activity in rats with 200mg/kg as well as similar to the standard drug (Table 2 and Figure 1). The results of MDA level in tissue also indicated that colonic content of MDA decreased significantly and similar to the standard drug when compared to the DSS model group. Treatment with EEMA exerted, to some extent, effects on reducing the colonic MDA level compared to animals that received DSS alone.

The effect of EEMA on the various antioxidant levels (SOD, CAT and GSH) were observed in table 3. The ethanolic extract of *Manilkara zapota* stem bark (EEMZ) restored up to the normal level of antioxidant parameters, that was confirmed the potent antioxidant effect of ethanolic extract.

Table 2. Effect of ethanolic extract of *Manilkara zapota* stem bark (EEMZ) on MPO and MDA level of colonic tissues of DSS induced colitis in rats

Treatment groups	MPO (OD/g tissue)	MDA (OD/g tissue)
Normal control	27.62±1.42	48.28±2.45
Negative Control (0.9% saline)	58.14±2.68	72.88±3.28
EEMZ 150 mg/kg	38.84±2.63	62.10±3.10
EEMZ 200 mg/kg	29.43±1.24*	50.21±2.92*
Sulfasalazine (500mg/kg)	28.44±1.40*	49.77±2.67*

Values are presented as mean of optical density (OD) ± SD, *P < 0.05, represent significant value compared with control group

Table 3. Effect of *Manilkara zapota* stem bark (EEMZ) ethanolic extract on antioxidants level of colonic tissues in rats

Groups	Antioxidants level		
	SOD(µg/50 mg tissue)	CAT(µmol/50 mg tissue)	GSH(µmol/50 mg tissue)
Normal control	51.50±2.01	73.42±3.01	34.87±1.08
Control (0.9% saline)	21.38±1.53	28.66±1.85	15.62±0.75
EEMZ 150 mg/kg)	34.28±1.60	58.39±2.55*	27.55±1.61
EEMZ (200 mg/kg)	41.92±2.42**	70.11±2.97**	32.62±1.75*
Sulfasalazine (500mg/kg)	48.62±2.64**	71.88±2.64**	33.76±1.54*

n = 6 albino rats per group, value represents Mean S.D. *P< 0.05, **P< 0.01, when compared each treated group with control group

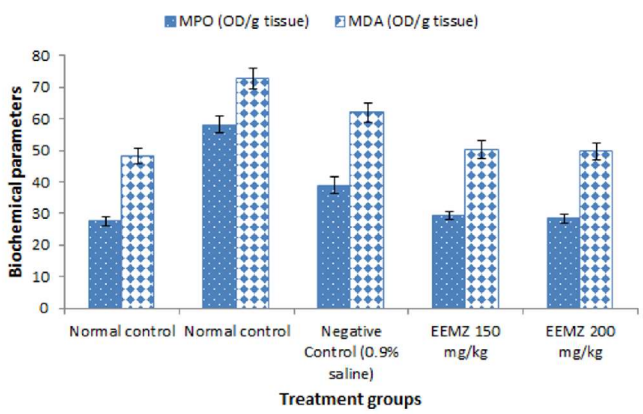

Figure 1. ffect of *Manilkara zapota* stem bark (EEMZ) ethanolic extract on MPO and MDA level of colonic tissues in rats

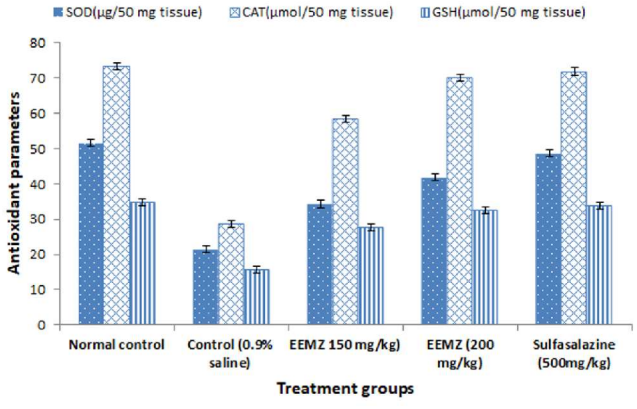

Figure 2. Effect of *Manilkara zapota* stem bark (EEMZ) ethanolic extract on antioxidants level of colonic tissues in rats

The level of antioxidants in colon tissues were observed significant decrease in colitis control group, may be due increasing free radicles generation. This decreasing level of SOD, CAT and GSH was slightly increased in treatment group with 150mg/kg dose of EEMA. But a significant improvement in level of SOD, CAT and GSH was found in treatment group of 200mg/kg dose of EEMA as well as standard drug treated group, when compared to colitis control group (Figure 2).

Conclusion

Phytochemical study revealed that flavonoids and phenolic compounds are present in ethanolic extracts of *Manilkara zapota* ethanolic stem bark. The free radical scavenging property of these flavonoids plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo* antioxidant activity. Hence, the probable mechanism of healing of ulcerative colitis by ethanolic extract of *Manilkara zapota* ethanolic stem bark may be attributed to antioxidant and free radical scavenging property, while free radical scavenging activity may be attributed to flavonoids and phenolic compounds.

Conflict of interest

None

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