Ind. J. Pharm. Edu. Res., 2023; 57(3):773-781.
https://www.ijper.org

Original Article

Lupeol in Functional Gastrointestinal Disorders: An Evidence-based Preclinical Study

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ABSTRACT

Objectives: The current study assessed the possible use of lupeol in the treatment of intestinal inflammation for the initial instance and for the anxiolytic activity for the management of Functional Gastrointestinal Disorder (FGID) by two different models of colitis (IBD) TNBS and DSS and by two anxiolytic models EPM and OFT. **Materials and Methods:** The intestinal anti-inflammatory models of colitis were performed by using TNBS and DSS to obtain the possible use of lupeol at a dose of 25µg/mL and 50µg/mL in Irritable Bowel Disease (Ulcerative Colitis). In both experiments, Albino Wistar rats were divided into 5 groups and each group contain six animals. During the experiment change in body weight, stool consistency and colon weight/length as well as a histological examination of the colon were performed. For the anxiolytic activity EPM and OFT models were used. In both, the model's Swiss albino mice were used and they were divided into four groups and each group containing six animals. In EPM model, each mouse was positioned in the centre of the maze, facing an enclosed arm. For 5 min, the number of entries and duration spent in open arms was monitored. Increased open-arms entry and time spent in open arms were viewed as indicators of possible anxiolytic activity. In OFT model animals were brought separately to the centre of the arena just after intervention and then exposed to a 5-min open field test. Throughout the experiment, the mice's overall social behaviours were recorded, including the total number of crossings, time spent in the arena, as well as central arena. In both models of anxiolytic, all the interventions were given intraperitoneal (i.p.) 30 min before the experiment began. **Results:** In this research work both the screening procedures of colitis were linked to up-regulation of pro-inflammatory enzymes in inflamed mucosal tissues in the current investigation, indicating that it has considerable anti-inflammatory impacts in the intestine. The findings of this study enhance the rationale for additional research into the medicinal benefits of lupeol in the management of human IBD. Anxiety, sleeplessness, and psychosis are all common in today's fast-paced world, which is filled with a variety of high-stress situations. According to the findings, the bioactive phytoconstituent triterpenoid lupeol had a substantial antianxiety impact in mice. The current study found that colonic inflammation was linked to increased free radicals in the CNS and ENS, as predicted by the results of the IBD and anti-anxiety screening models. Furthermore, the findings demonstrated that lupeol was able to alleviate experimental colitis and anxiety symptoms, both of which are important FGID phenomena.

Keywords: Functional Gastrointestinal Disorder, Irritable Bowel Disease, Trinitrobenzene sulfonic acid, Dextran Sodium Sulfate, Lupeol.

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Received: 20-10-2022;
Revised: 01-03-2023;
Accepted: 01-05-2023.

INTRODUCTION

A subset of gastrointestinal illnesses known as Functional Gastrointestinal Disorders (FGIDs) is defined by persistent or recurrent gastrointestinal symptoms lacking quantitative as well as anatomical defects. It is also described as a disorder of gut-brain interactions and is a group of multifactorial illnesses affecting visceral hypersensitivity and motility problems that affect various regions of the intestinal tract.[1] FGID aren't caused by a single pathophysiological mechanism, but rather by a complicated interaction of genetic, psychological, as well as social aspects. Deformities in gastrointestinal motion, epithelial or immunological responses, gut microbiota, but also Central Nervous System (CNS) processing and even some tensile strengths are genetic mutations that can exist in diverse combinations across various users. Psychological symptoms like stress, sadness, young childhood traumas, or social life lessons could work alone and sometimes in concert with physical responses to cause FGIDs to emerge, impacting the patient's health, physical, as well as social consequences.[2] Functional Dyspepsia (FD) and

DOI: 10.5530/ijper.57.3.94

Irritable Bowel Syndrome (IBS) are well FGIDs, but many other disorders like functional constipation, abdominal bloating or functional abdominal pain syndrome are now becoming more universally acknowledged. FGIDs are exceptionally common with IBS announced in about 10% to 15% of North American inhabitants and some reports finding upto 20% recurrence.[3] IBS is a functional bowel condition for that abdominal pain and discomfort are related with evacuation or perhaps an alteration in stool pattern as well as the symptoms of disordered evacuation, as per the Rome III definition.[4] Ulcerative colitis and Crohn's disease are the two most prevalent inflammatory bowel illnesses and each of these frequently includes chronic diarrhoea, discomfort, exhaustion or loss of weight. IBD is exhausting but frequently it might result in life-threatening problems.

Nowadays, the wide use of synthetic drugs like-corticosteroid, immunosuppressant and aminosalicylates is routinely used to cure IBD. They show therapeutic potential in most cases but are also associated with various side effects which ultimately affect the patient's Quality of Life (QOL) as well as the patient's compliance. So, in this regard, the present work is focused towards the bioactive constituents from the herbal source. Lupeol is a triterpene also called Fagarsterol found in numerous fruits such as olive, fig, mango, strawberry, and grape as well as in bioactive compounds along with American ginseng, Shea butter plant, *Tamarindus indica, Celastrus paniculatus, Crataeva nurvala, Zanthoxylum riedelianum, Bombax ceiba,* and *Sebastiania adenophora*.[5-7] Lupeol is pharmacologically effective in healing a variety of illnesses in preclinical settings because of its vast diversity of biological activities. Much more *in-vitro* as well as preclinical animal research indicates that somehow lupeol might also have anti-inflammatory, anti-microbial, anti-protozoal, anti-proliferative, anti-invasive, anti-angiogenic or cholesterol-lowering properties. Over the last 15 years, investigators all over the world have worked tirelessly to produce remarkable chemicals for therapeutic application in the treatment of a wide range of illnesses. These investigations likewise give an understanding of the system of activity of lupeol and propose that it is a multi-target specialist with tremendous anti-inflammatory potential by focusing on key sub-atomic pathways which include Nuclear Factor kappa-B (NFκB), Cflip, Fas, Kras, Phosphatidylinositol-3-kinase (PI3K), and Wnt/ß-catenin in an assortment of cells.[8] With this goal in perspective, the current study assessed the possible use of lupeol in the treatment of intestinal inflammation for the initial instance and the anxiolytic activity.

In this research study, two different experimental models of colitis (IBD) TNBS and DSS-induced colitis were used. For the anxiolytic activity EPM and OFT models were used. In both activities, anti-inflammatory and anxiolytic lupeol at doses of 25µg/mL and 50µg/mL were used.

MATERIALS AND METHODS

Animals

In this research study, Wistar albino rats of 8-12 weeks old with 180-200g of weight (either sex) were used for anti-inflammatory activity and Swiss albino mice of 6-9 weeks old with 25-35g of weight were used for Anxiolytic activity. They were obtained from Central Animal House of Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida. The animals were housed in the standard condition such that temperature was maintained at 25±2°C with a relative humidity of 55-65%. They were treated to 12-hr light and 12-hr dark cycles. All of the animals in this research study had free access to standard laboratory supplies, including food and water. All experiments adhered to the ethical standards set out by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Procurement of Lupeol

This synthetic active pharmaceutical ingredient Lupeol is a biologically effective triterpene found in a variety of species was collected and purchased from Indiamart.com.

Drugs and Chemicals

Trinitrobenzene Sulfonic acid (TNBS) and Dextran Sodium Sulfate (DSS) was supplied by Sigma Chemical Co. (St. Louis, MO, USA). The drug Sulphasalazine, Carboxymethylcellulose and Buffered Formaldehyde is provided from Central Drug House, New Delhi and Ethanol was purchased from Shivi Enterprises, Ghaziabad (U.P.). The drug Diazepam was purchased from Indiamart.com.

Experimental Work

Anti-inflammatory activity

Induction of Colitis by Trinitrobenzene Sulfonic acid (TNBS) model of rat colitis.

Trinitrobenzene Sulfonic acid (TNBS) is used as a hapten. Whenever TNBS is coupled to material containing high molecular tissue proteins, it becomes an antigen.[9] Colonic inflammation is generated in the sensitive strain of rats in this model by administering the TNBS in combination with ethanol intrarectally.

In this experiment, the rats were allowed to acclimate for at least 7 days at the animal house and before the induction of colitis; all of the animals fasted 24 hr. Then using ethanol (50% v/v) as an anaesthetic to begin the procedure. The experimental colitis was induced with a single dose of TNBS (10mg/kg) mixed in ethanol (50% v/v) per rectum. After that, an appropriate medical polyurethane catheter is used to administer TNBS to the anal

verge, which is roughly 4-8 cm away. The rats were then held vertically for 30 sec to prevent fluid ejection and assure uniform dispersion of the drug. Throughout the experiment, the rat's body weight, stool consistency, as well as rectal bleeding were then observed daily for up to 7 days.[10]

Experimental Procedure

In this experiment, Wistar albino rats were separated into five groups and each group containing six animals.

Group I: Served as Negative Control which received 2 mg/kg Carboxymethylcellulose (CMC) via oral gavage.

Group II: Served as Positive control which received 10 mg/kg Trinitrobenzene Sulfonic acid (TNBS).

Group III: Taken as test compound I which received lupeol 25 µg/mL along with 10 mg/kg TNBS.

Group IV: Taken as test compound II which received lupeol 50 µg/mL along with 10 mg/kg TNBS.

Group V: Served as Standard Group which received Sulphasalazine 30 mg/kg along with 10 mg/kg TNBS.

On the seventh day, all the animals were sacrificed with an overdose of ethanol (50% v/v). During the experiment, the weights of the animals, the prevalence of diarrhoea, and the amount of water and food ingested were all recorded regularly. After that, the animal's colons were dissected aseptically and placed on an ice-cold plate, then longitudinally opened and washed with cold saline to eliminate any luminal secretions. The colon was viewed under a microscope very away, and any obvious damage was graded on a scale of 0 to 5, according to Morris *et al*, 1989.[10] For histological evaluation, small slices of the colon were removed from two different locations of each colon and put in 10% formalin.

Induction of Colitis by Dextran Sodium Sulfate (DSS) model of rat colitis

Dextran Sodium Sulfate is a sulfated polysaccharide which is water-soluble and negatively charged with a molecular weight that varies from 5 to 1400 KDa. This sulfated polysaccharide does not cause intestinal inflammation straightforwardly in the DSS model, but instead works as a specific chemical irritant to the colonic epithelium causing mucosal cell damage. The postulated and most commonly recognized method through which DSS causes intestinal inflammation is the disruption of the epithelial monolayer lining which permits the luminal bacteria and related antigens to enter the mucosa along with the spread of proinflammatory gut elements within the surrounding tissues.[11]

In this experiment mark the rats and measure their body weight. After that DSS powder was weighed and stirred unless a transparent mixture is formed. The cage water bottles were filled with DSS water and the negative control rat's water bottles does not contain DSS. Throughout the experiment rat's body weight and the occurrence of blood in their stool were examined daily. The residual water and water consumption in each rat group were monitored daily.

Experimental Procedure

In this experiment, Wistar albino rats were separated into five groups and each group containing six animals.

Group I: Served as Negative Control and this received 2 mg/kg Carboxymethylcellulose (CMC) via oral gavage.

Group II: Served as Positive control and this received 30 mg/mL Dextran Sodium Sulfate (DSS).

Group III: Taken as test compound I which received lupeol 25 µg/mL along with 30mg/mL DSS.

Group IV: Taken as test compound II which received lupeol 50 µg/mL along with 30 mg/mL DSS.

Group V: Served as Standard Group which received Sulphasalazine 30 mg/kg along with 30 mg/mL DSS.

On the seventh day, all of the animals were sacrificed with an overdose of ethanol (50% v/v). During the experiment, all the rats received DSS in their drinking water which induced severe colitis with weight loss, bloody diarrhoea, ulcer formation, epithelial cell loss and neutrophil infiltration comparable to symptoms seen in human ulcerative colitis. The weight of the animals, as well as their food and water consumption, were recorded regularly. After that animals were sacrificed and colons were dissected aseptically and placed in an ice-cold plate, then longitudinally opened and washed with cold saline to eliminate any luminal secretions. After that, the colon was viewed under a microscope very away and any obvious damage was on a scale of 0 to 5 according to Morris *et al*, 1989.[10] For histological evaluation, small slices of the colon were removed from two different locations of each colon and put in 10% formalin.

Anxiolytic Activity

Gross Behaviour Study

The gross behavioural modifications in mice due to the administration of lupeol (25 µg/mL and 50 µg/mL) were conducted for observing information on their effect on CNS of mice. The effect on the reflexes of mice like – righting, pinna, and corneal reflexes were observed carefully. At the same time, the effect of lupeol on muscle tone, stereotyped behaviour, cataleptogenic activity, motor activity and awareness were evaluated to get maximum information on behavioural effects of lupeol in mice.

Elevated Plus Maze

In this model, two closed arms (50 cm × 10 cm × 40 cm) and two open arms (50 cm × 10 cm) are attached to a central platform (10 cm × 10 cm). Both open as well as closed arms were opposite each other and were enclosed with a detachable lid. The maze was raised to a height of 50 cm above the ground.[12]

Experimental Procedure

In this experiment, animals were divided into four groups and each group containing six animals.

Group I: Served as Positive control group and were given 0.9% (w/v) saline.

Group II: Taken as test group I and received low dose of lupeol (25µg/mL).

Group III: Taken as test group II and received high dose of lupeol (50 µg/mL).

Group IV: Served as the Standard group which received Diazepam (1 mg/kg i.p.).

In this experiment, all the interventions were given intraperitoneal (i.p.) 30 min before the experiment began. During the experiment, each mouse was positioned in the centre of the maze, facing an enclosed arm. For 5 min, the number of entries and duration spent in open arms was monitored. When the mice's paws reached an open or closed arm, it was considered as one entry. Increased open-arms entry and time spent in open arms were viewed as indicators of possible anxiolytic activity.

Open Field Test

Open-field exploration was first used to study emotional behaviour in rats and was subsequently shown to be just as effective in studying emotional behaviour in mice. The open field equipment consisted of an opaque Plexiglass cage (72×72 cm) having sidewalls 35 cm high and a white-lined surface split into 16 equal squares (18×18 cm).[13] To record the mice's activity, a digital recording device was put just above the cage.

Experimental Procedure

In this experiment, animals were divided into four groups and each group containing six animals.

Group I: Served as Positive control group and they were given 0.9% (w/v) saline.

Group II: Taken as test group I and received low dose of lupeol (25 µg/mL).

Group III: Taken as test group II and received high dose of lupeol (50µg/mL).

Group IV: Served as the Standard group which received Diazepam (1mg/kg i.p.).

In this experiment, all the interventions were given intraperitoneal (i.p.) 30 min before the experiment began, after that the animals were brought separately to the centre of the arena just after intervention and then exposed to a 5-min open field test. Throughout the experiment the mice's overall social behaviours were recorded, including the total number of crossings, time spent in the arena, as well as central arena.The study of statisticsFor this study, the arithmetic mean and Standard Error of the Mean (SEM) are used. Prism® Version 2.01 Software was used to conduct an investigation. ANOVA with Dunnett's "*t*" test and *p* values of 0.05 were used to examine the statistical significance of any differences in the variables between the groups.

RESULTS

Anti-inflammatory activity

Induction of Colitis by Trinitrobenzene Sulfonic acid (TNBS) model of rat colitis

The result of this experiment showed changes in the weight of test group rats at both the doses of lupeol 25 µg/mL (19.12± 1.18) and 50µg/mL (17.94±1.24), (Table 1) in comparison to positive control group rats (22.04±1.97) which was comparable to the standard group (20.4±1.04) (Table 1). For stool consistency in the Positive Control group, bloody diarrhoea was observed (0.79±0.19) in the test groups the frequency of diarrhoea reduced at both the doses of lupeol 25 and 50 µg/mL (0.68±0.10) and (0.52±0.9) respectively, which was comparable to the standard group (0.35.0±0.1). The colon weight and length increased in the test group which was (0.26±1.2) (0.18±0.12) and in the standard group (0.22±0.14) in comparison to the positive control group (0.20±0.1).

Histological studies of Dextran Sodium Sulfate (DSS) Rat Colitis

Histopathological observations of colon tissue after the treatment with Lupeol (25 µg/mL and 50 µg/mL) in TNBS rat colitis: (A) Negative Control (CMC), the mucosa is normal. (B) Positive Control (TNBS 10 mg/kg), inflammatory cell infiltration, apoptosis of epithelium at luminal interface, gross ulceration and proliferous granulomas. (C) TNBS (10 mg/kg) + Lupeol (25 µg/mL), epithelial ulceration, inflammatory effusion, proliferous granulomas, and cell infiltration are all symptoms of mucosal ulcers. (D) TNBS (10 mg/kg) + Lupeol (50 µg/mL), Crypto abscess development and minor mucosal ulceration. (E) TNBS (10 mg/kg) + Standard (Sulphasalazine 30 mg/kg), the architecture of mucosal lining is intact, no epithelial damage and ulcers were seen (Figure 1).

Induction of Colitis by Dextran Sodium Sulfate (DSS) model of rat colitis

The result of this experiment showed changes in the weight of test group rats at both the doses of lupeol 25 µg/mL (13.2±1.9) and 50 µg/mL (21.0±1.3) in comparison to positive control group rats (20.5±1.4) which was comparable to the standard group (21.0±1.3). For stool consistency in the Positive Control group, bloody diarrhea was observed (0.59±0.12) in the test groups the frequency of diarrhea reduced at both the doses of lupeol 25 and 50 µg/mL (0.49±0.1) and (0.42±0.11) respectively, which was comparable to the standard group (0.31±0.1). The colon weight and length increased in the test group which was (0.20±0.5) and (0.19±1.3) and in the standard group (0.22±0.2) in comparison to the positive control group (0.18±0.12) (Table 2).

Histopathological observations of colon issue after the treatment with Lupeol (25µg/mL and 50 µg/mL) in DSS rat colitis: (A) Negative Control (CMC), displaying the histology of a normal rat bowel. (B) Positive Control (DSS 30mg/mL), revealing a substantial inflammatory cell infiltration in the basal lamina and submucosa, as well as moderate colonic ulcers. (C) DSS (30 mg/mL) + Lupeol (25 µg/mL), with no inflammatory cell, infiltrate in the basal lamina and submucosa, the rat has the fewest colon ulcers. (D) DSS (30 mg/mL) + Lupeol (50 µg/mL), the inflamed response in the rat has recovered without ulceration of the gut wall; only modest localised infiltration can be seen in the basal lamina. (E) DSS (30 mg/mL) + Standard (Sulphasalazine 30mg/kg), rat showing no intestinal ulcerations and intact mucosal lining with very good histology of lamina propria and submucosa (Figure 2).

Anxiolytic activity

Gross Behaviour Studies

There is no change in any parameter (like-righting, pinna and corneal reflexes) followed to check the gross behavioural changes in mice. On administration of Lupeol at a dose of 50 µg/mL showed less change in righting and corneal reflexes. Lupeol at a dose of 50 µg/mL as compared to lupeol at a dose of 25 µg/mL was observed to be a down response for skeletal muscle relaxant activity as well as the cataleptogenic activity when compared with that of the control group. The test drug lupeol at both the selected doses was found to significantly change the stereotyped behaviour i.e. by increasing the rearing, sniffing and grooming activities of treated mice. From the above findings, lupeol was indicated as an anti-anxiety agent when compared to the standard drug diazepam (1 mg/kg b.w.). There is no significant change in any of the parameters seen during the gross behavioural studies.

Elevated Plus Maze Test

The behavioural alteration patterns showed by lupeol at a dose of 50 µg/mL and 25 µg/mL after performing the gross behavioural activities the experimental animals were selected for EPM model to reflect the psychopharmacological changes by anxiolytic effect as given in Table 4. For this investigation, the behavioural changes generated by lupeol at larger concentrations were equivalent to those produced by the reference medication (Diazepam). Mostly in EPM model, the behavioural changes generated by lupeol (50 µg/mL and 25 µg/mL) substantially increased total arm inputs in open arms (12.5 ± 1.6) as well as reduced the hrs spent as well as arm entrants in closed arms (123.1±9) in a comparable manner. Diazepam enhanced the average time spent in the open arms (85.1± 10.1) and the number of arm entrances (13.1 ± 0.9) (Table 3; Figure 3). As compared to the control group mice, Lupeol exhibited a considerable rise in open and closed-arm entry as well

Table 1: Effect of Lupeol in TNBS-induced rat colitis.

Groups	Number of Animals (n)	Body weight changes (g)	Stool Consistency (rating 0-1)	Colon weight/length (g/cm)
Negative Control (CMC)	6	18.14±1.07**	0	0.35±0.18
Positive Control (TNBS)	6	22.04±1.97	0.79±0.19***	0.20±0.1
TNBS+ Lupeol 25µg/mL	6	19.12± 1.18*	0.68±0.10*	0.26±1.2***
TNBS+ Lupeol 50µg/mL	6	17.94±1.24***	0.52±0.9**	0.18±0.12*
TNBS+ Sulphasalazine 30mg/kg (Standard)	6	20.4±1.04***	0.35.0±0.1***	0.22±0.14**

Colonic variables were quantified in the control group ($n = 6$), which received CMC application. TNBS group received the TNBS intra-rectally in a vehicle of 50% (v/v) ethanol. Data are expressed as mean ± SEM(*) $p< 0.05$, (**) $p< 0.01$, and (***) $p< 0.001$ vs. control vs. TNBS group (One-way ANOVA followed by "Tukey's multiple comparison *post hoc* tests).

Table 2: Effect of Lupeol in DSS-induced colitis rat model.

Groups	Number of Animals (n)	Body weight changes (g)	Stool Consistency (rating 0-1)	Colon weight/ length (g/cm)
Negative Control (CMC)	6	11.1±0.1	0	0.29±0.9
Positive Control (DSS)	6	20.5±1.4	0.59±0.12	0.18±0.12
DSS+ Lupeol (25µg/mL)	6	13.2±1.9*	0.49±0.1	0.20±0.5
DSS+ Lupeol (50µg/mL)	6	18.1±1.87**	0.42±0.11*	0.19±1.3*
DSS+Sulphasalazine 30mg/kg (Standard)	6	21.0±1.3***	0.31±0.1**	0.24±0.2**

Colonic variables were quantified in the control group (n = 6), which received CMC application. DSS group received the DSS orally in feeding water bottle. Data are expressed as mean ± SEM (*) p< 0.05, (**) p< 0.01, and (***) p< 0.001 vs. control vs. DSS group (One-way ANOVA followed by "Tukey's multiple comparison *post hoc* tests).

Table 3: Anxiolytic Effect of lupeol in EPM Model.

Groups	Number of Animals (n)	No. of entries (n)		Time spent (n)	
		Open arms	Closed arms	Open Arms	Closed Arms
Positive Control (Saline)	6	5.3 ± 0.1	11.6 ± 1.1	39.1±7.3	181.2±16.6
Test drug I (Lupeol 25µg/mL)	6	9.8 ± 1.3	8.3 ± 0.1	56.4±9.0*	151.2±11.2
Testdrug II(Lupeol 50µg/mL)	6	12.5± 1.6**	6.1 ± 0.3**	78.2±12.6**	123.1±9**

Time spent and open arm enteries were quantified in the control group, which received CMC application. Test drug groups (n = 6) received the Lupeol at a dose of 50µg/mL and 25µg/mL via i.p. Data were expressed as mean ± SEM (*) p< 0.05, (**) p< 0.01, and (***) p< 0.001 vs. control vs. standard group (One-way ANOVA followed by "Tukey's multiple comparison *post hoc* tests.

Table 4: Anxiolytic effect of lupeol in OFT Model.

Groups	Number of Animals (n)	No.of squares crossed in central area	No. of rearing in the central area
Positive Control (Saline)	6	110.02 ± 9.1	24.1 ± 3.3
Test drug I (Lupeol 25µg/mL)	6	123.1 ± 12.2	39.1 ± 6.1
Test drug II (Lupeol 50µg/mL)	6	145± 10.4**	51.2± 4.1**
Standard drug (Diazepam 1mg/kg)	6	167± 9.3***	45.2 ± 3.2***

No. of squares crossed in central area and no. of rearing in the central area were quantified in the control group, which received saline application. Test drug groups (n = 6) received the lupeol at a dose of 50 µg/mL and 25µg/mL via i.p. Data were expressed as mean ± SEM (**) p< 0.01, and (***) p< 0.001 vs. control vs. standard group (One-way ANOVA followed by "Tukey's multiple comparison *post hoc* tests).

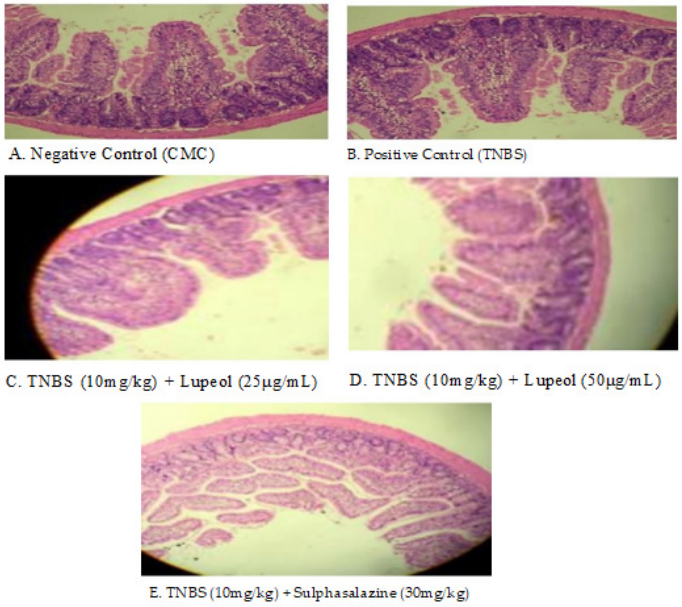

Figure 1: Photomicrographs showing Hematoxylin and Eosin-stained segments of rat colons in TNBS-induced rat colitis model.

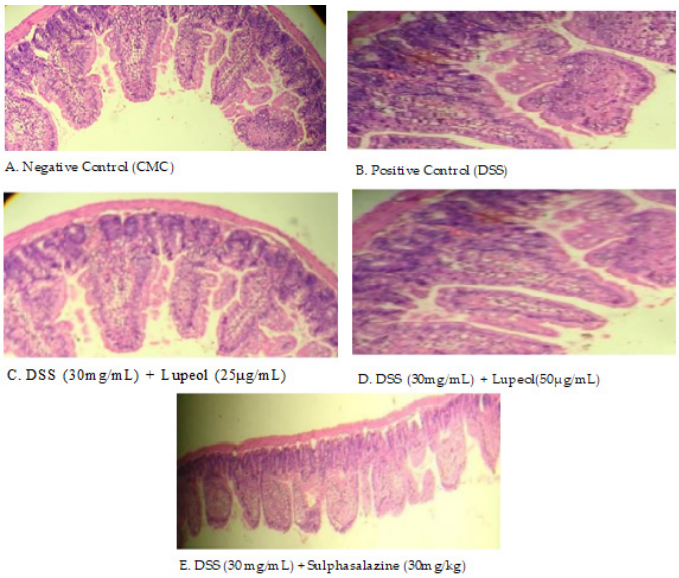

Figure 2: Photomicrographs showing Hematoxylin and Eosin-stained segments of rat colons in DSS-induced rat colitis model.

as a reduction in time spent. Both frequencies of inputs and hrs invested in the open arm have been recorded for all categories, and the frequency of entry and hours invested in different parts of the EPM model was used to quantify anxiety by the EPM model.

Open Field Test

The open field test was developed to investigate exploratory behaviour. Throughout this study, lupeol at doses of 50 and 25 µg/mL enhanced the choice for the central portion, increasing the frequency of crossings, the time that has been spent in the central part, and rearing in the OFT apparatus. A rise in the aforementioned measures is a symptom of anti-anxiety properties.

The OFT revealed that central area crossing was presented in both the doses of lupeol (50 µg/mL and 25 µg/mL) and diazepam (1 mg/kg b.w.) respectively (**) $p < 0.01$, and (***) $p < 0.001$) as compared with the control and standard group. In central area rearing there was an increased response by lupeol at a dose (50µg/mL and 25 µg/mL) and Diazepam (1 mg/ kg b.w) with significant *p*-value (***p <0.001) (Table 4; Figure 4).

DISCUSSION

Several Complementary Alternative Medications (CAM) treatments have been widely used for the successful management of FGID patients with Inflammatory Bowel Disease (Ulcerative Colitis). So, in this regard, the present work is focused towards the bioactive constituents from the herbal source. Lupeol is a triterpene also called Fagarsterol found in numerous fruits and it is pharmacologically useful in curing a variety of illnesses in animal models in pre-clinical scenarios with a special focus on its anti-inflammatory activity. The remarkable chemical lupeol is now focused on screening its therapeutic applications in the treatment of a wide range of illnesses.

In this research study, two different experimental models of colitis TNBS and DSS were used for anti-inflammatory activity. For the anxiolytic activity EPM and OFT were used. The favourable effect was exerted by lupeol when administered after the induction of colonic damage in TNBS and DSS models. In both situations, the findings suggested that encouraging the rehabilitation of inflamed tissues is dose-dependent. Hence, lupeol can be considered a good candidate in human Inflammatory Bowel Disease therapy because its low toxicity is supported by prolonged therapy in traditional medicines.[14,15] The TNBS and DSS models of colitis on experimental are the most commonly used models for experimental colitis. On the other hand, the TNBS model of rat colitis causes a transmural lesion with clinical features comparable to Crohn's disease. Inflammation restricted to the intestinal epithelium occurs in the DSS model in rats which is more precisely matched to human ulcerative colitis.[16]

The CNS activity was also performed by using Swiss albino mice to correlate with the already established potential of lupeol on GI disorder with the Brain (ENS-CNS) correlation in common terminology FGID's. It was necessary to screen the lupeol after colitis model on different models of anxiety. The anxiolytic activity was followed to check the gross behavioural changes in mice. The test drug lupeol at both the selected doses was found to significantly change the stereotyped behaviour i.e. by increasing the rearing, sniffing and grooming activities of treated mice. The behaviour alteration patterns shown by lupeol at a dose of 50 µg/mL and 25 µg/mL after performing the gross behavioural activities the experimental animals were selected for EPM and OFT models to reflect psychopharmacological changes by anxiolytic effect. The behavioural alterations induced by the lupeol (25 µg/mL and 50 µg/mL) in the EPM model significantly

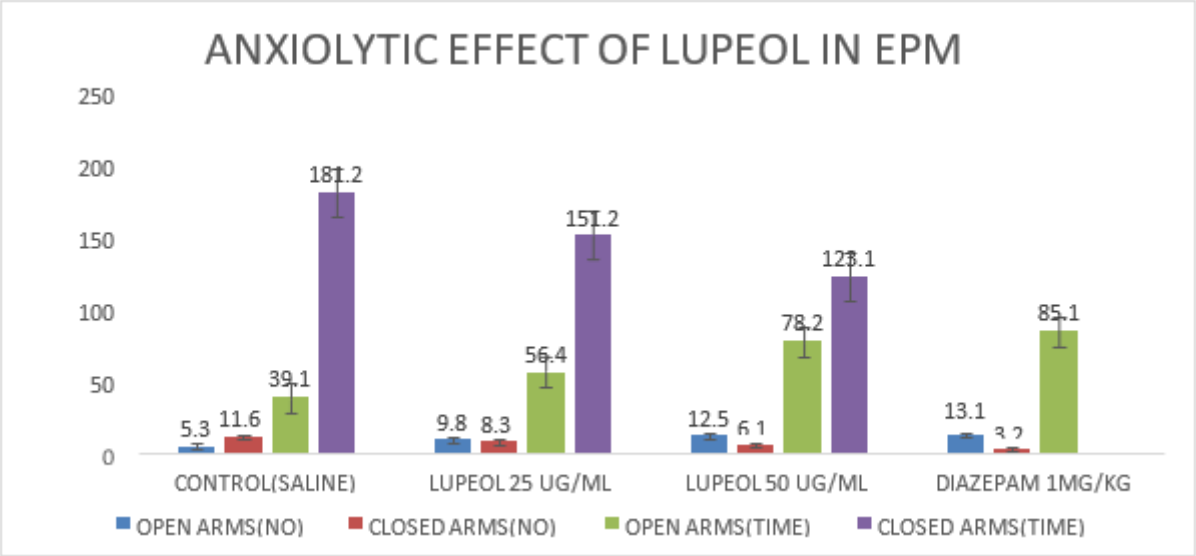

Figure 3: Anxiolytic Effect of Lupeol in EPM.

Time spent and open arm enteries were quantified in the control group ($n = 6$), which received CMC application. Test drug groups received the Lupeol at a dose of 50µg/mL and 25µg/mL via i.p. Data were expressed as mean ± SEM (*) $p< 0.05$, (**) $p< 0.01$, and (***) $p< 0.001$ vs. control vs. standard group (One-way ANOVA followed by "Tukey's multiple comparison *post hoc* tests).

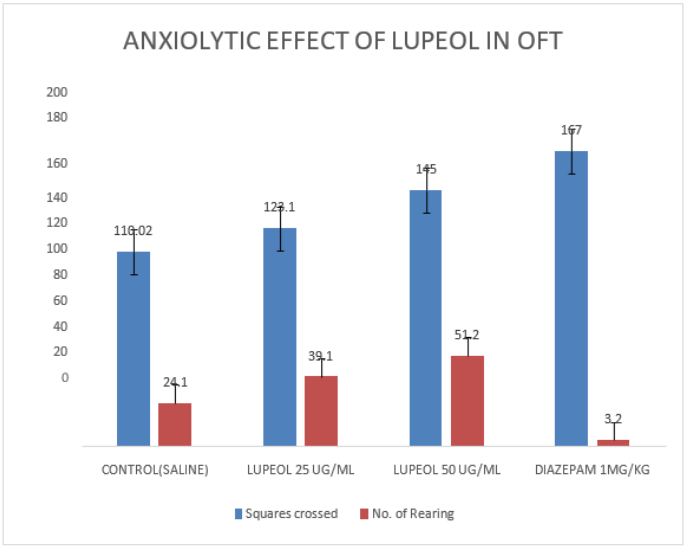

Figure 4: Anxiolytic Effect of Lupeol in OFT.

No. of squares crossed in central area and no. of rearing in the central area were quantified in the control group, which received saline application. Test drug groups ($n = 6$) received the Lupeol at a dose of 50µg/mL and 25µg/mL via i.p. Data were expressed as mean ± SEM (**) $p< 0.01$, and (***) $p< 0.001$ vs. control vs. standard group (One-way ANOVA followed by "Tukey's multiple comparison *post hoc* tests).

increased the arm entries in open arms and decreased the time spent and arm entries in the closed arm in a similar fashion. Diazepam increased the time spent and arm entries in the open arms. Lupeol showed a significant increase in the entries in the open and closed arm and decreased the time spent when compared with control group mice. The OFT model revealed that central area crossing was presented in both the doses of lupeol (25µg/mL and 50µg/mL) and diazepam (1mg/kg) respectively as compared with the control and standard group. In central area rearing there was an increased response by lupeol at a dose of 25µg/mL and 50µg/mL along with Diazepam (1mg/kg) with a significant p-value (***$p<0.001$).

As expected from the results obtained from the IBD and anti-anxiety screening models the present study revealed that colonic inflammation was associated with increased free radicals in the CNS and ENS. Moreover, the results obtained revealed that lupeol was able to ameliorate the experimental colitis and anxiety symptoms, which are the important phenomenon described in the FGID. Drossman and Ringel also suggested that the psychological disturbances in the FGID patient as an important component of the neuropsychological illness that ameliorate the clinical expressions of Inflammatory Bowel Disease (UC).[17]

CONCLUSION

In the present scenario, the prevalence of IBD is increasing day by day due to changed lifestyles and food habits. There is an utmost requirement to screen the alternative option for the successful management of ulcerative colitis that combines significant efficacy and show an adequate safety profile. So, keeping in mind the use of bioactive lupeol for the treatment of IBD as well as in anxiety. Both the screening procedures for colitis were linked to the up-regulation of pro-inflammatory enzymes in inflamed mucosal tissues in the current investigation, indicating that it has considerable anti-inflammatory impacts in

the intestine. Additionally, lupeol's potential to control the free radical scavenging action was strengthened by its amazing results in reversing colitis in both experimental models. The findings of this study enhance the rationale for additional research into the medicinal benefits of lupeol in the management of human IBD. Anxiety, sleeplessness, and psychosis are all common in today's fast-paced world, which is filled with a variety of high-stress situations. According to the findings, the bioactive phytoconstituent triterpenoid lupeol had a substantial antianxiety impact in mice.

The current study found that colonic inflammation was linked to increased free radicals in the CNS and ENS, as predicted by the results of the IBD and anti-anxiety screening models. Furthermore, the findings demonstrated that lupeol was able to alleviate experimental colitis and anxiety symptoms, both of which are important FGID phenomena.

ACKNOWLEDGEMENT

Authors acknowledge expressing our sincere gratitude to the management of the Noida Institute of Engineering and Technology, (Pharmacy Institute) for continuous support, motivation, enthusiasm, and immense knowledge.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of Variance; **CAM:** Complementary and Alternative Medicine; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **CNS:** Central Nervous System; **ENS:** Enteric Nervous System; **UC:** Ulcerative Colitis; **FGIDs:** Gastro Instestinal Diseases; **IBS:** Irritable Bowel Syndrome; **IBD:** Inflammatory Bowel Disease; **TNBS:** Tri nitro benzene sulphonic acid; **DSS:** Dextran sodium sulfate; **GI:** Gastro Instestinal; **QOL:** Quality of Life; **FD:** Functional dyspepsia; **OFT:** Open Field Test; **EPM:** Elevated pus mazel; **NFκB:** Nuclear factor kappa-B; **PI3K:** Phosphatidylinositol-3-kinase; **SEM:** Standard Error Mean.

SUMMARY

Several Complementary and Alternative Medicine (CAM) treatments have been widely used for the successful management of FGID patients with Inflammatory Bowel Disease (Ulcerative Colitis). So, in this regard, the present work is focused towards the bioactive constituents from the herbal source.

In this research study, two different experimental models of colitis TNBS and DSS were used for anti-inflammatory activity. For the anxiolytic activity EPM and OFT were used. The favourable effect was exerted by lupeol when administered after the induction of colonic damage in TNBS and DSS models. In both situations, the findings were suggested that encouraging the rehabilitation of inflamed tissues is dose dependent. Hence, lupeol can be considered as a good candidate as in human Inflammatory Bowel Disease as well as in anxiety management. Hence, lupeol can be suggested as a better therapeutic approach for the patients with FGIDs.

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Cite this article: Das S, Shafiq N, Das MK, Kumar V, Rajangam J. Lupeol in Functional Gastrointestinal Disorders: An Evidence-based Preclinical Study. Indian J of Pharmaceutical Education and Research. 2023;57(3):773-81.