A Study on the Effects of Acetyl-11-Keto-β-Boswellic Acid Against Dextran Sodium Sulfate Induced Acute and Chronic Colitis in Swiss Albino Mice

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Abstract

Introduction: Inflammatory bowel disease (IBD) is a term used to denote concurrently the two chronic inflammatory conditions of gastrointestinal (GI) tract viz: Crohn’s disease (CD) and ulcerative colitis (UC). This study has aimed to focus on acetyl-11-keto-beta-boswellic acid (AKBA) which is an active phytochemical derivative from the gum resin of the Boswellia serrata in order to investigate its anti-inflammatory potential against dextran sodium sulfate (DSS) induced colitis in Swiss albino mice.

Materials and Methods: The 3% of DSS polymer in drinking water was fed to different mice groups with distinct timeline for both acute (7 days) and chronic colitis induction (3 cycles of 5 days feeding with 15 days gap method). The anti-inflammatory activity of AKBA (50 mg/kg) was evaluated by performing various anti-oxidant assays on tissue homogenate samples (colon, liver and kidney) and further histological studies.

Result: The oral administration of AKBA (50 mg/kg) had managing effects in IBD mice. Results showed that AKBA lowered the inflammation and soreness compared to the DSS administered mice groups. The histopathology of the intestinal wall was performed and clear morphological changes were observed under light microscopy of both acute and chronic colitis group of mice. Furthermore, various anti-oxidant assays were performed on tissue sections of chronic colitis mice. Results from histological studies indicated that the chemo-preventive effect of AKBA was attributed to a collection of activities including anti-proliferation, apoptosis induction, and anti-inflammation.

Conclusions: In accordance to findings, the AKBA active derivative showed anti-inflammatory activity against the DSS induced acute and chronic colitis in mice. However, further clinical studies need to be done to bring AKBA as a potential anti-inflammatory drug candidate for treating IBD.

Keywords: Acetyl-11-Keto-β-Boswellic Acid, Inflammatory Bowel Disease, Inflammation, Dextran Sodium Sulfate


Introduction

Inflammatory bowel disease (IBD) is an inclusive inflammation of both colon and small intestine. The IBD is classified into two types of: specific IBD and non-specific IBD. The genetic overlap, immunological changes or environmental factors or the bowel infections can cause IBD but the cause is not completely understood and hence, called non-specific IBD.¹ When IBD is caused by pathogens like Campylobacter jejuni or some enteropathic Escherichia coli, it is known as specific IBD as the cause is known.²³ This is while, non-specific IBD mainly comprises of Crohn’s disease (CD) and ulcerative colitis (UC). Both CD and UC project similar symptoms and yet they are different disorders. The CD affects the lining of the small intestine and large intestine, it can affect the stomach and even the anus. The UC is a continuous and long-term inflammatory condition of mainly rectum and colon.⁴ In recent years, there has been a rise in the incidence of IBD and has been found in Asian, Middle East and Eastern European countries.⁵ One of the main causes of this is the westernization of the place along with food habits. It is said that UC is similar to CD but it occurs only in the colonial mucosa and cause inflammation and disruption of the cells residing in the colon region. The symptoms of UC are similar to CD which includes bloody stool, weight loss, abdominal pain, fever, anemia etc. Patients with UC have higher chances of developing colorectal cancer when compared to CD and the prevalence of it is high.⁶ The acetyl-11-keto-beta-boswellic acid (AKBA) has the ability to hinder the phosphorylation and degradation of NF-kB gene transcription.⁷ It is known to increase the reactive oxygen species (ROS) concentration as it acts as redox agents. It blocks the formation of leukotriene B4 by inhibiting 5-LOX; does not impair the 12-LOX or COX
Therapeutic effect of Acetyl-11-Keto-β-Boswellic Acid against acute and chronic colitis.

The aim of the present study was to evaluate the anti-inflammatory potential role of AKBA against the dextran sodium sulfate (DSS) induced IBD in Swiss mice for both acute and chronic stages.

Materials and Methods

Mice Care and Maintenance Conditions

Healthy Swiss albino mice (Mus musculus) served as the disease model for the current study. Mice were administered with normal food and water. All mice weighed between 25-30 g. The animals were housed at small mice cages with controlled temperatures and light-dark cycles, fed standard mice food pellets, and access to tap water from bottles were given to mice, and were acclimatized before study was conducted. All experiments were approved by the regional animal study committees. The Animal Ethics Committee, University of Chettinad, approved the experimental protocols (approval number: IAEC 4/Alr. No. 22/12.12.2017) and the experiments were conducted under the supervision of the Animal Welfare Committee, University of Chettinad.

Mice Grouping

Mice were randomized and sub-divided into a total of eight different groups such as two control groups (negative control), 2 AKBA treated groups (positive control), 2 DSS treated groups and 2 AKBA+DSS treated groups for both acute and chronic colitis groups of mice. Each group had 6 mice. The control group were fed with a normal diet and were sacrificed at the 7th and 45th day for the acute and chronic group. The mice of the AKBA group were fed with a normal diet along with 0.2 mL of AKBA which was orally fed per day. The mice of the DSS group were fed with a normal diet along with 3% DSS in their drinking water which was fed daily. For the acute group, 3% of DSS was fed to the mice daily till the seventh day and for the chronic group, 3% of DSS was fed for three cycles. For the chronic colitis group, the cycle started from the first to fifth day that means for 5 days 3% DSS was fed and there was a gap of 14 days which was from day 6th to 19th. In this gap, mice were only fed with water and a normal diet. In the second cycle, 3% of DSS were fed for 5 days followed by 14 days gap. The last cycle continued as mentioned above. Finally, on the 45th day the 3% DSS feeding was stopped. Whereas, in the DSS+AKBA treated group, 3% DSS was mixed in drinking water and was fed daily. After the onset of UC, 0.2 mL of AKBA was fed orally per day to all 6 mice. For the acute group, this dosage started from the fourth day after the DSS induction and for the chronic group, this dosage was given from the 25th day which means after 2 cycles of induction of chronic colitis.

Treatment Method

Induction of IBD

The DSS was supplied to mice orally. For this purpose, DSS was prepared freshly every day. For acute and chronic UC, 1.5 g of DSS was diluted in 50 mL of distilled water.

Treatment of AKBA

For the treatment, 50 mg/kg of AKBA was given orally to both acute and the chronic colitis mice groups. The AKBA was diluted in 5% DMSO (Merck®, India), 50% glycerin and 45% distilled water. Then, mice were sacrificed from all groups with respective time durations as mentioned above for both acute and chronic groups of mice for further studies.

Disease Activity Index

To examine the severity of colitis, water intake, fecal bleeding and weight loss were evaluated daily and averaged for an overall Disease Activity Index (DAI).

Histology

The damage and inflammation were analyzed by histological examination. Sections of the distal colon 1 cm long were cut out longitudinally and fixed in 10% formalin for at least 24 hours and then embedded in paraffin wax for histological analysis. Distal colon was sectioned for 4 μm thick and were stained with Hematoxylin & Eosin stain (Merck®, India) to address the degree of inflammation. Staining of goblet cells and assessment of the collagen deposition were examined by performing periodic acid–Schiff and Masson's trichrome staining.

Estimation of Catalase Activity

Catalase is an antioxidant enzyme that relieves reactive oxygen species and defends from oxidative damage. This enzyme can also break down mutagenic and toxic chemicals like H₂O₂ (Medox®, India) into less harmful products like 2H₂O and O₂. The tissue aliquots were taken with 50 mM of Phosphate buffer for documenting the optical density (OD) readings at 240 nm.

Estimation of Lipid Peroxidation Activity

Lipid peroxidation relates to the oxidative deprivity of lipids. This process occurs in cells by the absorption of electrons from lipids by free radicals, which leads to cell damage by the free radical chain reaction mechanism. For assessment of lipid peroxidation, the colorimetric assay was performed using of TBA method (thiobarbituric acid assay).

Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity in the cell extract was assayed by estimating its capability to hinder the auto-oxidation of pyrogallol. The SOD catalyzes the dismutation of superoxide radical to generate hydrogen peroxide and oxygen. The assay mixture contained 0.05 M sodium phosphate buffer (pH 8.0), 0.01 M EDTA (Orange Biotech Pvt. Ltd., India) and 0.27 mM pyrogallol (Medox®, India). About 420 nm was set to measure OD.

Glutathione (GSH) Activity

The anti-oxidant activity can be determined by the method illustrated by Ellman et al. The tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was taken and added with an equal
volume of 20% trichloroacetic acid (Orange Biotech Pvt. Ltd., India) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 minutes prior to centrifugation at 2000 rpm for 10 minutes. The supernatant (200 μL) was then transferred to a new set of test tubes and added with 1.8 mL of the Ellman’s reagent (5,5'-dithiobis-2-nitrobenzoic acid) (Orange Biotech Pvt. Ltd., India) 0.1 mM prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution. Then all the test tubes containing reaction mixture were made up to the volume of 2 mL. After completion of the total reaction, solutions were measured at 412 nm against suitable blank.16

**Acid Phosphatase Test**

For acid phosphatase tests, the method of Walter and Schutt et al, was considered with minor changes. In order to obtain acid phosphatase activity, 1 ml of acid buffer was added to 0.2 mL tissue aliquot. It was then mixed well and was incubated at 37°C for half an hour. Then, 2 mL of 1N NaOH was added to the mixture. Absorbance was recorded at 405 nm.17

**Alkaline Phosphatase Test**

To obtain alkaline phosphatase activity, the B Klein et al’s method was followed with a slight modification. To about 0.05 mL of tissue supernatant, 2 mL of alkaline buffer was added and mixed thoroughly. It was then incubated at 37°C for half an hour, then 3 mL of 0.05 N NaOH was added. Then the absorbance was taken at 405 nm.18

**Gamma-Glutamyl Transferase Activity (GGT) Assay**

According to Singhal et al, the tissue homogenate sample was added to a substrate solution containing glycylglycine, MgCl₂ and γ-glutamyl-p-nitroanilide (ROBONIK India Private Ltd., India) in 0.05 M Tris (free base), pH 8.2. The mixture was incubated at 37°C for 1 minute and the absorbance was measured at 405 nm at 1 minute interval for 5 minute.19

**Myeloperoxidase Activity**

To obtain the peroxidase activity, 10 μL of the sample was taken to that 80 μL of 0.75 mM H₂O₂ and 110 μL of 3,3',5,5'-Tetramethylbenzidine (Merck®, India) solution (2.9 mM in 145% DMSO and 150 mM phosphate buffer, pH 5.4) were added. Then, they were incubated at 37°C for 5 minutes. The reaction was then inhibited by adding 50 μL of 2 M H₂SO₄. The absorbance was recorded at 450 nm.19

**Results**

**Histology**

In the DSS treated group, there was disruption of mucosal architecture, loss of crypts, depletion of goblet cells, infiltration of inflammatory cells with edema and epithelial cell necrosis. Whereas in DSS+AKBA treated group, they exhibited significant reduced infiltration of inflammatory cells and recovery of goblet cells. Collagen deposition was found in blue/green colour stained (Masson’s trichrome) in the mucosa and sub-mucosal region. It was highly evident in the DSS treated mice group when compared to control group, presumably contributing to the increased rigidity of inflamed region. In contrast, the degree of collagen deposition significantly reduced in both the mucosa and sub-mucosal region of GI tract of DSS+AKBA treated mice group (Figure 1a-1c and Figure 2a-2l).

**Figure 1**

Panel (a) represents the H&E stained colon section of DSS treated group, where in figure, letter A shows the inflammatory cell infiltration. The letter D shows the disruption and loss of normal architecture in the lining of the colon section. Panel (b) illustrates the H&E stained colon section of DSS+AKBA treated group, shows well maintained lining and reduced infiltration of inflammatory cells. Panel (c) demonstrates the liver section of DSS treated group stained with H&E stain, the letter H specifies the hepatocytes, S represents the sinusoidal space, DA represents the degeneration and loss of tissue. Panel (d) shows the weight of mice Panel (g) versus no. of days, it shows that there was decreased in body weight in case of DSS fed mice. Panel (e) shows the water intake of mice (mL) versus no. of days, the graph shows that there is decreased in water intake in case of DSS induced mice.
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Figure 2. (a) Hematoxylin and Eosin stain (H&E), (b) Masson's trichrome (MT), and (c) Periodic acid-Schiff (PA) staining sections of control group colon with normal intact cross section structure. Panel (d) shows the AKBA treated tissue sections of colon H&E stained. Panel (e) colon section with MT stained. Panel (f) shows colon section PA stained with maintained histological structure. In Panel (g) the yellow arrow shows the infiltration of mixed leucocytes in cross section and H&E stained DSS treated colon. (h) DSS treated MT stained colon section with yellow arrow showing the infiltration of inflammatory cells. Panel I shows the DSS treated colon, PA stained specifying the mixed inflammatory cells infiltration sites in the tissue section. Panels j-l show the DSS+AKBA colon sections stained H&E, MT and PA. Yellow arrows represents the reduced infiltration of mixed inflammatory cells and well maintained tissue architecture.

Disease Activity Index
The DAI were analyzed and interpreted for all the four groups of mice. In the control group, the mice were found to be normal with normal body weight, normal water intake, normal stool frequency and consistency. The AKBA treated mice groups, also had normal body weight, water intake, stool frequency and consistency. Whereas in the DSS treated mice group, there was gradual loss of body weight, dropped in water intake, and watery stools was observed. The DSS+AKBA treated mice group was found to gradually gain body weight, with moderately regaining the normal stool consistency. The graph plotted illustrates these observations vividly with respect to distinct groups (Figure 1d-1e).

Catalase Activity
Chronic UC Group
The catalase activity in various tissue samples listed below, belong to both the control and other treatment groups:

Figure 3a which shows the catalase activity, decreased in the DSS treated group and increased in the DSS+AKBA treated group of colon tissue lysate samples, Figure 3b which represents the catalase activity, significantly decreased in the DSS treated group and also significantly increased in the DSS+AKBA treated group of liver tissue lysate samples, Figure 3c reveals that in the kidney tissue lysate, the catalase activity has increased in the DSS+AKBA treated group and has decreased in the DSS treated group.

Lipid Peroxidation
Chronic UC Group
The lipid peroxidase (LPO) activity represented below, has been expressed in both the control and other treatment groups. Figure 3d which shows the LPO activity, increased in the DSS treated group and decreased in the DSS+AKBA treated group of colon tissue lysate samples, Figure 3e which shows LPO activity, increased in the DSS treated group and decreased in the DSS+AKBA treated group of liver tissue lysate samples, Figure 3f represents the LPO activity in kidney tissue lysate samples, which increased in the DSS treated group and decreased in the DSS+AKBA treated group.

Superoxide Dismutase
Chronic UC Group
The SOD activities which belong to both the control and the other treatment groups are listed below:

Figure 4a shows SOD activity which significantly decreased in the DSS treated group and significantly increased in the DSS+AKBA treated group of colon tissue lysate sample, Figure 4b illustrates the SOD activity which decreased in the DSS treated group and increased in the DSS+AKBA treated group of liver tissue lysate samples, Figure 4c which represents SOD activity in the kidney tissue lysate significantly decreased in the DSS treated group and also significantly increased in the DSS+AKBA treated group.
Glutathione Test

**Chronic UC Group**

The GSH activity listed below, belong to the control group and other groups:

Figure 4d which illustrates the GSH activity in the colon tissue lysate sample, significantly increased in the DSS+AKBA treated group and significantly lowered in the DSS treated group. Figure 4e which shows the GSH activity, reduced in the DSS treated group and increased in the DSS+AKBA treated group of liver tissue lysate samples, Figure 4f reveals that in the kidney tissue lysate samples, the GSH activity has significantly increased in the DSS+AKBA treated group and significantly reduced in the DSS treated group.

Acid Phosphatase Test

**Chronic UC Group**

The specific acid phosphatase (ACP) activity in various tissue samples listed below belong to the control and other groups:

Figure 5a which shows the ACP activity, increased in the DSS treated group and decreased in the DSS+AKBA treated group of colon tissue samples, Figure 5b which represents the ACP activity in the liver tissue lysate samples, found to
be expressed high in the DSS treated group and low in the DSS+AKBA treated group, Figure 5c which shows the ACP activity in the kidney tissue lysate, found to have increased in the DSS treated group and decreased in the DSS+AKBA treated group.

Alkaline Phosphatase Test
Chronic UC Group
The specific alkaline phosphatase (ALP) activity in various tissue samples listed below, belong to control and various treatment groups. Figure 5d represents the ALP activity, increase in DSS treated group and no significant reduction in DSS+AKBA group of colon tissue lysate samples. Figure 5e describes ALP activity in liver tissue homogenate, spotted to be expressed high in DSS treated group and lowered in DSS+AKBA group. Figure 5f reveals that in the kidney tissue lysate, the ALP activity has increased in the DSS treated group and on the other hand has decreased in the DSS+AKBA treated group.

Gamma Glutamyl Transferase Test
Chronic UC Group
The GGT activity in various tissue homogenate samples summarized below belong to the control and other groups: Figure 6a shows that the GGT activity decreased in the DSS treated group of kidney tissue homogenate samples. Figure 6b describes the GGT activity in liver tissue homogenate. Figure 6c illustrates a significant increase of the MPO activity in the DSS treated group and a decrease in the DSS+AKBA treated group of colon tissue lysate samples, Figure 6d shows the MPO activity which increased in the DSS treated group and decreased in the DSS+AKBA treated group of colon tissue lysate samples. Figure 6e illustrates a significant increase of the MPO activity in the DSS treated group and a decrease in the DSS+AKBA treated group of liver tissue lysate samples, Figure 6f represents the MPO activity which significantly increased in the DSS treated group and significantly decreased in the DSS+AKBA treated group of kidney tissue homogenate samples.

Discussion
In this study, the AKBA showed anti-inflammatory properties against DSS induced colitis in Swiss albino mice. The performed anti-oxidants assays indicated that AKBA is a potent anti-inflammatory candidate. The AKBA active derivative is known to have medicinal properties since time immemorial and various researchers have reported their findings in numerous studies.

In one a study conducted by Sailer et al, the 5-lipoxygenase (5-LOX) products were produced by the endogenous arachidonic acid and by the stimulation of ionophore peritoneal polymorphonuclear leukocytes and also via exogenous substrate.21 The results supported that the pentacyclic triterpene ring is vital for binding to the active site and also 11-keto functional group is crucial in the inhibition of 5-LOX.21,22 Shamraiz et al. have focused on the synthesis of new boswellic acid derivatives in order to evaluate their anti-proliferative properties. The ring A was modified to prepare AKBA analogs compounds (7, 8, 9 and 10). The compounds 7 & 8 are the pyrazine derivatives of AKBA. The compound aldehyde 9 has an open ring A that is resulted by reacting with ketone in the presence of aniline, the compound 10 is resulted due to reaction with ketone and semicarbazide. The AKBA monomers (11-13), KBA monomers (14-16) as well as heterodimers of KBA and AKBA (17-24) were also prepared.
Here, compounds [11-16] are monomers of boswellic acid. Whereas, compounds [17-24] are ester-linked boswellic acid derivatives. Out of all these modified compounds, the compound 8 exhibited a significant anti-proliferative activity against malignant melanoma (A375) cells with low (2.1 μM) EC50 value. The process of decarboxylation and the cleavage of ring a diminished in their cytotoxic activity. However, the presence of amine enhanced the activity of boswellic acids. The KBA homodimer 20 and KBA-AKBA heterodimer 23 indicated cytotoxic effect. Whereas, other dimers were reported to be inactive. The compound 20 was the most significant compound that exhibited cytotoxic activity against all four human cancer cell lines namely A2780 (ovarian carcinoma), FaDu (pharynx carcinoma), A375 (malignant melanoma) and, HT29 (colon adenocarcinoma) with EC50 value ≤11.0 μM.22

Components such as boswellic, silymarin and curcumin containing dietary formulation showed the anti-cancer property in the animal model with inflammatory intestinal carcinoma. The study suggested the chemo-preventive synergistic effect of the formulation on inherited intestinal polyps.23

In another study by Yi Yuan et al, it was shown that the AKBA inhibited the human colon adenocarcinoma growth via exhibiting the cell cycle arrest in the G0/G1 phase of the cell cycle and also the induction of apoptosis. The study further showed that AKBA administered mice did not show any toxicity and delayed the growth of the HT-29 xenografts. Actually, AKBA prevented the colonic adenocarcinoma by modulating pathways including pentacyclic triterpene ring and the role of 11-keto group. Apart from this, AKBA and its numerous derivatives showed various activities such as induction of apoptosis, cell cycle arrest and reduced polyp formation in intestine. It might be a new choice of treatment for IBD in the coming future.24

Zhang et al. reported in a study that 2 μM α/β-ABA downregulated the production of inflammatory markers such as tumour necrosis factor (TNF-α), interleukin (IL)-6, nitric oxide, IL-10 and IL-1β in RAW264.7 cells. The caerulein induced pancreatitis in mice model showed that intragastrical administration of 100 mg/kg α/β-ABA relieved the inflammatory cell infiltration. It has also diminished the elevation of TNF-α and IL-6 in the mice model. In addition to this, α/β-ABA also downregulated the mitogen-activated protein kinase (MAPK) family. Also, the serum inflammatory factors were reduced by the phosphorylation of p38, JNK and ERK1/2. This study suggested that α/β-ABA can be a potent drug candidate against pancreatitis via modulating MAPK pathways.25

Lv et al. reported a decline of the cell viability in A549, H1299, H460 and BEAS-2B. Accordingly, AKBA exhibited the clone formation in A549 cells by arresting the cell cycle at G0/G1 phase and also induced apoptosis. Also, AKBA suppressed the expressions of LC3A/B-I, LC3A/B-II and Beclin-1 proteins. It also inhibited the signalling pathway PI3K/Akt. The study concluded that the AKBA shows anticancer activity via apoptosis induction, cell cycle arrest and also the autophagy suppression in non-small cell lung cancer cells.26

According to various literature reports, the AKBA exhibits anti-inflammatory properties based on their chemical structure including pentacyclic triterpene ring and the role of 11-keto group. Apart from this, AKBA and its numerous derivatives showed various activities such as induction of apoptosis, cell cycle arrest, autophagy suppression, and decreased level of inflammatory markers. It was also reported that it plays a role in modulating pathways such as MAPK, PI3K/Akt. The AKBA, active derivate from the salai guggal exhibits anti-inflammatory properties, induces apoptosis, cell cycle arrest and reduced polyp formation in intestine. It might be a new choice of treatment for IBD in the coming future.

**Conclusions**

In this study, the biological activity of AKBA in chemically induced acute and chronic UC was obtained by using mice model. The AKBA was orally administrated from the 4th day following after the induction of DSS in case of acute treated group whereas, in the chronic treated group the oral administration initiated from the 25th day onwards. The...
AKBA's efficacy and toxicities were monitored. Histopathology results also showed morphological change after the treatment of the AKBA. There was a regeneration of the damaged crypt and showed unremarkable features in both acute and chronic cases. During this study, no toxicity was observed in the AKBA-treated mice. Various anti-oxidant assays and enzymatic assays were also performed in tissue homogenate (colon, liver and kidney) such as MPO, SOD, LPO, ALP, ACP, Catalase, GSH, GGT tests. All results supported the fact that in chronic conditions in addition to primary target colon some secondary target organs are also affected such as liver and kidney. In conclusion, AKBA might be used as a drug for the betterment of acute and chronic inflammatory conditions of colon, liver and kidney without any significant toxicity in mice. The underlying mechanisms of this effect to AKBA’s action on anti-proliferation, induction of apoptosis have also been shown in some studies.\textsuperscript{3,6,7} However, in order to establish AKBA as a potent drug, more follow up research is required in higher phases of clinical trials. Therefore, AKBA, a naturally occurring component, might be a promising agent in the treatment of human IBD.

Authors’ Contributions
SH, SR, SP, AB conducted the whole experiments, analyzed the results with SP and AB, and wrote the drafts of the manuscript. RK did histology study and interpreted the result. VR interpreted the histology study. GJ helped in the cytotoxicity study, SP, AB conceived the study and participated in its design and coordination and helped to draft the manuscript. All authors reviewed the manuscript.

Conflict of Interest Disclosures
There are no conflicts of interest in the present study.

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