ABSTRACT

_Averrhoa bilimbi_ L. belongs to family Oxalidaceae. Among different _Averrhoa_ species, _Averrhoa bilimbi_ L. and _Averrhoa carambola_ are the only two plants which give fruits (edible to eat) with biological activity. Traditionally, people use these plants (root, bark, leaves and fruits) for treating several illnesses including itches, boils, syphilis, whooping cough, hypertension, fever and inflammation. _Averrhoa bilimbi_ L. was chosen for our studies, because it is well used by tribal people for medicine in the Kerala region. Certain research studies on _Averrhoa bilimbi_ L. fruit extract have also reported the anti-inflammatory, antioxidant and anti-cancer effects. As this fruit is rich in biologically active phytochemicals with antioxidant potency and regulatory function, we were interested to analyse the phytochemicals and to study its treatment effects on Ulcerative Colitis, lymphoma and colon cancer along with its antioxidant potency.

Inflammatory Bowel Disease (IBD) circumscribes with ulcerative colitis (UC) and inflammation in chronic part (Chrons diseases). The etiology for UC is not clearly mentioned and the genetic factors, environmental factors and free radicals are the possible reasons for UC. Ulcerative Colitis (UC) is a lingering type of Inflammatory Bowel Disease (IBD) which affects the colon mucosa.
Ulcerative colitis is majorly associated with oxidative stress and inflammation in colon tissue leading to damage. The inflammation mediators, prostaglandins, leukotriene’s and Reactive Oxygen Species (ROS) are involved in the induction and development of these diseases. The combination of both ulceration and inflammation causes abdominal discomfort and frequent emptying of the colon lymphoid and a macrophage cell which produces local mediator cells called cytokines. Accumulation of free radicals and oxidative stress plays a significant role in the initiation and progression of UC. TNF-α, iNOS, COX-2 and Interleukins like IL1, IL1β and IL6 are the major molecules involved in inflammation and ulceration. The available treatments for the ulceration results in side effects and decreases the quality of human life. The best way to treat ulcerative colitis and other major diseases can be the use of plant products which is rich source of phytochemicals and antioxidants.

Acute ulceration and inflammation leads to uncontrolled division of cells in which cells undergo proliferation, differentiation and down regulation of apoptosis (cell death). This uncontrolled division of cells without apoptosis leads to the glitch of an organism and this disturbance in the body develop into colon cancer. In 17th century itself the cancer was documented and according to the World Health Organisation (WHO 2015) 8.2 million deaths were recorded all over the world due to cancer. Metastasis is the major problem of the cancer and available treatment for the cancer also leads to adverse side effects. The alterations and
active involvement of immune system for chronic inflammatory and ulcerative condition of UC may have the chance of developing lymphoma.

The phytochemicals present in the *Averrhoa bilimbi* L. fruit methanolic extract was examined by the preliminary tests, GC-MS analysis and the total phenolic content was estimated and expressed as gallic acid equivalent in milligram per gram (GAE mg/g) of methanolic fruit extract. The presence of phenolic content was further analysed by the High Pressure Thin Layer Chromatography (HPTLC). Preliminary phytochemical test on the *Averrhoa bilimbi* L. revealed the presence of alkaloids, carbohydrates, phenols, flavonoids, saponins and tannins. The presence of high level of phenols were further confirmed by the HPTLC and the total phenolic content in 250 µg/mL sample was found to be 209.25 GAE mg/g methanolic fruit extract. The active biologically useful compounds such as Hexadecanoic acid ethyl ester (Antioxidant, hypcholesterolemicnematicide, pesticide, antiandrogenic flavour, hemolytic, 5-alpha reductase inhibitor) Squalene (Chemo-preventive against colon cancer), Erucic acid (X-linked adrenoleukodystrophy), Oleic acid (Reduce blood pressure), Chimanine D (Antileishmanial), 5-Hydroxymethyl furfural (Against sickle cell anaemia), Mannitol (used for acute traumatic brain injury), Desulphosinigrin (Antibacterial) and Methyl Pyroglutamate (Antibiotic preparation) were identified by the GC-MS analysis in our study.
The UC was induced in the colon of Wistar rats by acetic acid and the dissected colonic specimens were morphologically analysed for the level of ulceration to see the treatment effect of *Averrhoa bilimbi* L. fruit extract. Then the histological changes were analysed in the blocked colon tissue using H & E staining. The anti-inflammatory activity of the *Averrhoa bilimbi* L. was analysed by checking the levels of expression of inflammatory marker enzyme i-NOS and COX-2 in the histology by Immune histochemistry analysis. The levels of cytokines TNF-α, IL6 and IL1b, as well as iNOS and COX-2 also were analysed in the colon tissue homogenate by ELISA. The anti-inflammatory and cytokine levels were even compared with the standard drug (Sulfasalazine). The abnormal increase observed in the inflammation mediator cytokines in control rats, i.e IL-1b, IL-6, TNF-α levels were decreased significantly (**p ≤ 0.01**) in the *Averrhoa bilimbi* L. fruit extract treated groups. The increase in weights of the colon tissue and spleen of the control rats were found to be reduced in treated groups. The levels of inflammatory markers iNOS and COX-2 were also decreased in treated group significantly (**p ≤ 0.01**) when compared with the control. These results demonstrate the effective anti-ulcerative colitis activity of the *Averrhoa bilimbi* L. fruit extract in experimental Wistar rats.

For Anti-lymphoma study, DAL cell line was injected via intraperitoneal to Swiss albino mice and were treated with *Averrhoa bilimbi* L. fruit extract for 15 days. Then, the animals were sacrificed on 15th day to test haematological
parameters, body weight and tumor volume. The histopathology of the liver was analysed for the cellular change assessment by Haematoxylin and Eosin staining. The *in vitro* growth inhibition effect of *Averrhoa bilimbi* L. fruit extract on DAL cell line was also studied using MTT assay. The *Averrhoa bilimbi* L. Fruit extract showed a promising anti-lymphoma activity in Swiss albino mice. Treatment with *Averrhoa bilimbi* L. normalized the haematological parameters significantly (**P ≤ 0.01**) by decreasing the high level of white blood cell and by increasing the levels of red blood cells and haemoglobin count when compared with DAL control mice. The treated mice group with *Averrhoa bilimbi* L. fruit extract resulted in significant (**P ≤ 0.01**) decrease in body weight when compared with the DAL control. The MTT assay also has shown a significant growth inhibition percent (97.96%) on DAL cell lines for the fruit extract treatment at highest dose used. The liver of control mice showed necrosis with surrounding fibrosis and indication of inflammation. Whereas the normal lobular architecture was observed in the normal group with intact central vein and sinusoids, normal portal tracts and preserved hepatocytes. The current study thus revealed a significant anti-lymphoma activity of the *Averrhoa bilimbi* L. fruit extract against DAL cell line induced lymphoma.

The *Averrhoa bilimbi* L. fruits were also evaluated for its treatment effects against COLO-205 cells (human colon cancer cell line) in our study through
trypan blue assay for cytotoxicity and MTT assay for cell growth inhibition. The DNA fragmentation level in the treated samples were also tested for its separation in agarose gel as well as its UV absorption at 260nm in UV spectrophotometric analysis. The evaluation gave a rough assessment for the apoptosis of COLO-205 cells upon treatment. Then the apoptosis status of the COLO-205 cells upon treatment was observed by performing the most reliable TUNEL assay. The MTT IC50 concentration of the fruit extract (126.26µg) has shown the better genomic DNA fragmentation in the treated samples which is also an indication of apoptosis induction. To assess the level of metastasis, the cell migration for COLO-205 cells was analysed. The migration of the cells was inhibited in treated cells when compared with the cell migration level observed in control sample.

In vitro, the fruit extract was tested for its free radical scavenging activity by the (DPPH) 2,2-diphenyl-1-picrylhydrazyl and Nitric oxide (NO) assays. In vivo model used for the study was the UC developed in wistar rats. The supernatant of colon tissue homogenate obtained was subjected for the measurement of the level of NO, SOD and GSH. Ascorbic acid was used as a standard and the IC50 value of Averrhoa bilimbi L. fruit extract and standard Ascorbic acid on nitic oxide scavenging were found to be 108.10µg and 85.01 µg respectively. The IC50 values of the Averrhoa bilimbi L. fruit extract on DPPH scavenging was 116.07 µg and that of for standard ascorbic acid was 44.975 µg. Furthermore, the treatment with Averrhoa bilimbi L. fruit extract has shown a
significant antioxidant activity in the UC condition by reducing the levels of NO and enhancing the levels of antioxidants SOD and GSH in the colon tissue.

In conclusion the pre-administration of *Averrhoa bilimbi* L. fruit extract has reduced the inflammation in acetic acid induced UC in rats. The cytokines TNF-α, IL6, and IL1b levels also found to be reduced by the fruit extract. The effects were even better than that of the standard drug sulfasalazine. The phytochemicals which we have reported in *Averrhoa bilimbi* L. such as squalene have been reported to have antioxidant role and chemopreventive role in cancer through their effects on regulating signal transduction in cell proliferation. Thus *Averrhoa bilimbi* L. could be used as an efficient drug from natural source for ulcerative colitis and cancer therapy. It is clear that the compounds present in *Averrhoa bilimbi* L. fruit methanolic extract are significant and can be isolated for further study at molecular level in the treatment of Ulcerative Colitis and cancer. This will help to develop an effective alternate drug from natural source in place of existing chemical based drugs.