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**Research Article**

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# Inhibitory effect of luteolin on the status of membrane bound ATPases against azoxymethane-induced colorectal cancer

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**ABSTRACT**

*Colon cancer (CRC) is one of the most common malignancies in many regions of the world. Luteolin, a 3′,4′,5,7-tetrahydroxyflavone, consist of many beneficial effects such as antioxidant, anti-proliferative, controls the glycoproteins in malignant and induce apoptosis during AOM-induced colon cancer. In this study, was aimed to analyze the effect of Luteolin on membrane bound ATPases in Azoxymethane (AOM)-induced colon cancer. Male Balb/C mice were divided into four groups: normal control, AOM-induced, AOM induced with Luteolin treated, normal control treated with Luteolin. CRC was induced by administration with AOM (15mg/kg body weight) intraperitoneally (i.p.) once in week for three weeks. The activities of $Na^+$ $K^+$-ATPase $Ca^{2+}$-ATPase and $Mg^{2+}$-ATPase were decreased in AOM-induced group of mice. Upon treatment with Luteolin the activities were increased significantly. In conclusion, Luteolin may be act as a potent chemotherapeutic agent against colon cancer.*

**Key words:** Colon cancer, Luteolon, Chemoprevention, ATPases

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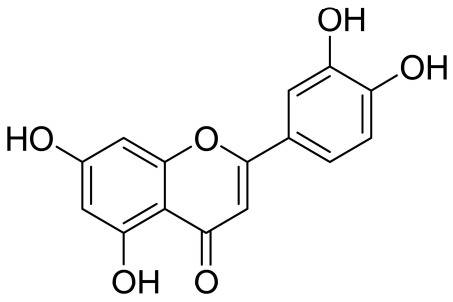
## INTRODUCTION

Colon cancer (CRC) is one of the most common malignancies in many regions of the world [1] and is thought to arise from the accumulation of mutations in a single epithelial cell of the colon and rectum [2]. Numerous genes are altered during the initiation and progression of colon cancer [3]. Azoxymethane (AOM), is potent carcinogen is frequently used to induce colon cancer in rodents [4, 5]. AOM is metabolized in the liver into MAM. This reaction is catalyzed by the enzyme cytochrome $P_{450}$ E1 [6]. Metabolic activation of MAM to a highly reactive electrophile (methyl diazonium ion) occurs in liver and colon, which is known to elicit oxidative stress further adverse effects, leads to development of cancer.

Luteolin, a 3′,4′,5,7-tetrahydroxyflavone, (Figure 1) is usually found in a glycosylated form in celery, green pepper, perilla leaf and seed, chamomile tea, and *Lonicera Japonica*. It consists of many beneficial effects such as antioxidant [7], anti-proliferative [4], controls the glycoproteins in malignant [8] and induces apoptosis during AOM-induced colon cancer [9]. In general antioxidants have the ability to eliminate carcinogens and protect the cell from cancer [10-12]

Fig. 1. Chemical structure of Luteolin




Luteolin

ATPases, membrane bound enzymes present mostly in the basal lateral membrane are responsible for the transport of sodium, potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis [13]. They regulate cellular volume, osmotic pressure and membrane permeability [14, 15]. The integrity of the mitochondrial membrane depends on the proper functioning of ATPases. Detection of ATPase inhibition could prove to be an important index for threshold levels of a large group of environmental contaminants. ATPase enzymes contain sulphydryl group, which are easily inactivated by peroxidation or depletion of glutathione [16].

In this present investigation, the role of Luteolin on the status of membrane bound ATPases such as $Na^+$ $K^+$-ATPase, $Ca^{2+}$-ATPase and $Mg^{2+}$-ATPase were studied.

## EXPERIMENTAL SECTION

### Chemicals

Azoxymethane was procured from (Sigma-Aldrich Chemical Company, St. Louis, USA), Luteolin was purchased from Cayman chemicals, USA. All other chemicals and reagents used were of analytical grade.

### Animals

Male Balb/c mice weighing approximately 25-30 g obtained from the Laboratory Animal Maintenance Unit, Tamilnadu Animal Science and Veterinary University, Madavaram, India and used for this study. The animals were acclimatized to the laboratory conditions for a period of 2 weeks. They were maintained at an ambient temperature of 25±2° C and 12/12 hours of light–dark cycle and given standard rat feed (Hindustan Lever Ltd., Bangalore) and tap water *ad libitum*. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

### Experimental procedure

All mouse were divided into four groups ($n$ = 6 per group). Mice in group 1 served as control animals and received intra peritoneal injections (*i.p.*) of physiological saline. Group 2 mouse were administered AOM (15mg/kg body weight) intraperitoneally (i.p.) once in week for three weeks. Mice in-group 3 (AOM + LUT) were treated with a single dose with1.2 mg/kg body weight of LUT (based on the effective dosage fixation studies) orally until end of the experiment, after AOM administration as mentioned in group 2. Mouse in-group 4 received the same dose of LUT alone.

The experiment was terminated at the end of 17 weeks and all the animals were killed by cervical dislocation after an overnight fast. The tumorous colon tissue were excised out, the tissues were stored at -80° C for various assays.

### Biochemical assays

$Na^+$ $K^+$-ATPase activity was assayed by the method of Bonting, [17]. The activity of $Mg^{2+}$ ATPase was assayed by the method of Ohnishi *et al*., [18]. The activity of $Ca^{2+}$-ATPase was assayed according to the method of Hjerten and Pan, [19].The enzyme activity is expressed as µmoles of inorganic phosphorus liberated / min / mg protein. The phosphorus content of the supernatant was estimated as described earlier by the method Fiske and Subbarow, [20].

**Statistical analysis**

All the data were statistically evaluated with SPSS/10.0 software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference (LSD) test *p<0.05 was considered to indicate statistical significance. All the results were expressed as mean ± S.D.

## RESULTS

**Body weight, Liver weight and relative liver weight**

Mean weights of body and liver (g/ 100 g body weight) in all groups are shown in Table 1. No significant (P< 0.05) changes on comparison with control (Group 1) and LUT treated groups (Group 4) were observed. There were significant (P< 0.05) decrease in mean final body weight and increase in mean liver weight of AOM administered group of animals. Administration of LUT markedly increased mean final body weight and decreased the mean liver weight in group 3.

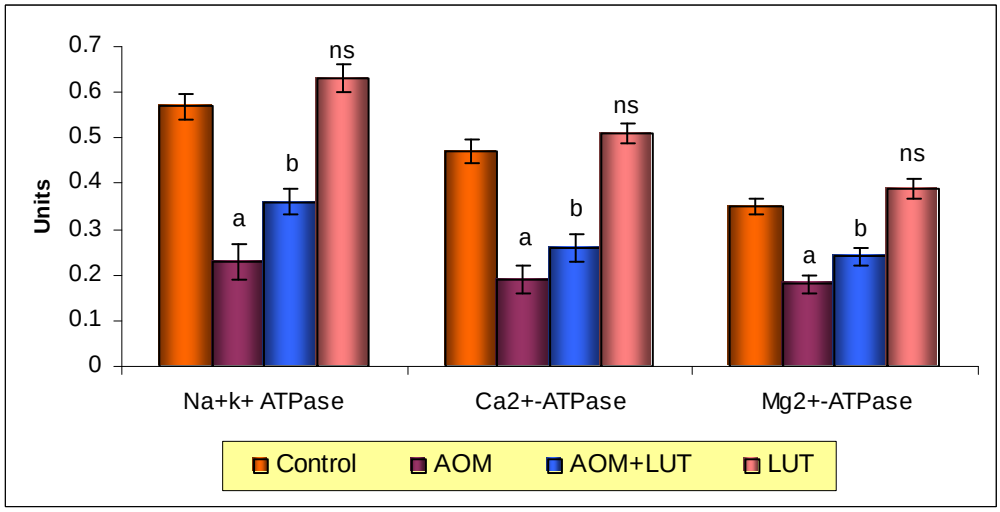
**Table 1. Body, liver and relative liver weights of control and experimental groups of animals**

| Parameters | Control | AOM | AOM + LUT | LUT |
|---|---|---|---|---|
| Initial body weight (g) | 24.75 ± 1.92[a] | 25.22 ± 1.82[b] | 23.92 ± 1.97[c] | 26.18 ± 2.06[ns] |
| Final body weight (g) | 34.48 ± 3.02[a] | 32.29 ± 3.11[b] | 33.71 ± 2.48[c] | 35.57 ± 2.06[ns] |
| Liver weight (g) | 1.45 ± 0.13 | 2.23 ± 0.28 [b] | 1.82 ± 0.38 [c] | 1.51 ± 0.37 [ns] |
| Relative liver weight (g/100 g body weight) | 4.20 ± 0.39 | 6.90 ± 0.77 [b] | 5.39 ± 0.63 [c] | 4.27 ± 0.53[ns] |

*[a] Mean ±S.D. Comparisons: [b]Control Vs AOM, [c]AOM Vs AOM+LUT, ns- non significant. P< 0.05*

Figure 2 represents the activities of $Na^+/K^+$, $Ca^{2+}$ and $Mg^{2+}$ATPases in the colon of control and experimental groups of animals. Activities of ATPases showed significant (p<0.01) decrease in group 2 when compared to control (Group 1) animals. Group 3 (AOM+LUT) had a significant increase in the activities of ATPases when compared to group 2. No significant changes were found between group 1 (Control) and group 4 (LUT).

**Figure 2. Effect of LUT on membrane bound-ATPases in control and experimental groups of animals**




*Units: n moles phosphorus formed/min/mg of protein. Values are expressed as mean ±S.D. for 6 mice in each group. [a]Control Vs AOM, [b]AOM Vs AOM+LUT, ns- non significant, p< 0.05.*

## DISCUSSION

Colon carcinogenesis is a serious health problem leading to morbidity and mortality in developed countries [21]. AOM-induced colon cancer was a reliable model to assess the chemopreventive effect of various drugs [22, 9]. Accumulating evidences from our own laboratory observations as well as others suggest that dietary antioxidants exert significant anti-tumor effects [7, 8].

In malignancy, the cell membrane plays a crucial role in the stimulation and control of cell adhesiveness, mortality and proliferation in a much-damaged condition [23]. The protection of membranes is of potential importance in the treatment of disease processes. The membrane bound enzymes such as $Na^+/K^+$-ATPase, $Mg^{2+}$-ATPase and $Ca^{2+}$-ATPase are responsible for the transport of sodium/potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis [24].

The decreased activities of $Na^+/K^+$ and $Mg^{2+}$-ATPase in AOM-induced colon cancer animals may be due to increased LPO which occur in cancer conditions. $Ca^{2+}$-ATPase, the enzyme responsible for active calcium transport, is extremely sensitive to hydroperoxides and this may lead to its inhibition. The impairment in this enzyme may be due to the peroxidative stress, which may act on the sulphydryl groups present in the active sites of the $Ca^{2+}$-ATPase [25]. The oxygen radical attack results in the disruption of $Ca^{2+}$ homeostasis, which leads to oxidative cell damage [26]. The free radicals produced by the oxidative stress act on the SH groups present in the active sites of $Ca^{2+}$ ATPase resulting in diminished levels of $Ca^{2+}$ ATPase [27].

In the present study, a decrease in the activities of $Na^+/K^+$-ATPase and increase in the activity of $Ca^{2+}$-ATPase were found in colon cancer bearing animals. The restoration of activities of all the three ATPases to near normal values was observed in LUT treated animals. This might be due to the enhancement in the status of GSH by LUT [28].

To conclude that, Luteolin has many beneficial effects in colon cancer, especially it reduces the aberrant crypt foci, tumor number, scavenges the free radicals and enhances the antioxidant enzymes. It also inhibits cell proliferation by inhibiting wnt/β-catenin signaling. Apart from those Luteolin, significantly elevates the membrane bound ATPases in AOM-induced colon carcinogenesis. Hence, Luteolin may be act as a potent chemotherapeutic agent against colon cancer.

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