Histological Effects of Chronic Administration of Crude Aqueous Extract of Hibiscus Sabdariffa on the Colon of Wistar Rats

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Abstract Hibiscus sabdariffa (Hs) is used in folk medicine for the treatment such as in gastro diseases and infection. The aim of this study was to investigate the effect of chronic administration of crude aqueous extract of Hs calyces on the colon of adult Wistar rats. Rats of both sexes (n = 32), with average weight of 230 g were randomly selected into three treatment (I, II & III) & Control (IV) groups of eight rats each. Groups I, II & III respectively received daily administration of 250, 500 & 1000 mg/kg of body weight of Hs for 31 days through orogastric tube. The control group received tap water through the same route. All rats were feed with growers mash and given water literally. The rats were subjected to light anesthesia (Chloroform) in a urethane saturated chamber and sacrificed at the 32nd day of the experiment and the stomach and the colon were carefully dissected and quickly fixed in 10% formal saline for histological study. The result revealed that the rats in the treated groups showed heavily packed epithelial cells, with significant increase in the level of goblet cells and mucin secretions in the colon mucosa when compared with the control group. This report provides further evidence that the medicinal components of Hs extract has a potential effect as an anti-inflammatory activity on the Colon.

Keywords Anti-Inflammation; Colonic Mucosa; Goblet Cells; Induced Effects

1. Introduction

Medicinal plants have been of great importance to the healthcare needs of individuals and their communities (Okpuzor and Oloyede, 2009). The last decade has witnessed intensive studies on extracts (Collins et al., 1997) and biologically active compounds isolated from plant species used for natural therapies (Nascimento et al., 2000); and the plant, Hibiscus sabdariffa, has been found to possess several health benefits (Dokosi, 1998). Hibiscus sabdariffa belongs to a family of plants called Malvaceae of which over three hundred species have been described (Trease and Evans, 1995). It is an herb that is cultivated for leaf, fleshy calyx, seed or fibre (Dalziel, 1973). The flowers have also been found to contain gossypetin,
anthocyanin and glycoside hibiscin, which contributes to the diuretic and choleretic effects, decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis (Irvin, 1961). Recent scientific research work has established the protective effect of the dried flower extract of Hibiscus sabdariffa as (Tseng et al., 1997), anti-inflammatory activity (Dafallah and Mustapha, 1996), antihypertensive effect of the calyx extract (Adegunloye et al., 1996) and antimutagenic activity (Chewonarin et al., 1999).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain, fever and inflammation (Ozbakis et al., 2007); but some of these drugs have been reported to induce injury (1999; Bessem and Vermeulen, 2001). However, many natural plants have been sourced and used as valuable medicinal agents for many years with proven potentials of treating infectious diseases (Ekpa, 1996) and with lesser side effects compared to synthetic agents (Gbile and Adesina, 1986); and one of these plants is the Hibiscus sabdariffa (Hs).

Given the observations of the potential anti-inflammatory action of Hibiscus sabdariffa (Hs), the objective of this study is to examine the histological effects of chronic administration of the crude aqueous extract of Hs calyces on the microanatomy of the colonic mucosa.

2. Materials and Methods

2.1. Hibiscus Sabdariffa Calyx

Dried samples of Hibiscus sabdariffa calyces were obtained from Central Market, Sokoto, Sokoto State of Nigeria. The plant was identified at the department of Botany, University of Benin, Benin city.

2.2. Test Animals

Thirty-two (32) adult Wistar rats of both sexes with average weight of about 230 g were used. These rats were obtained from the Animal House Section of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Benin. The animals were maintained in standard animal cages in the Animal House of the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State–Nigeria; and were approved by the appropriate authority of the University of Benin.

2.3. Preparation of the Aqueous Extract

The Hibiscus sabdariffa calyces were air – dried for 31 days at room temperature. The air – dried samples were blend with a blender and further macerated to a fine powder. The hibiscus extract was prepared by boiling a 250 g samples of the powdered materials with distilled water for about 30 minutes, after which it is allowed to cool. After extraction, the solution was filtered through a fine mesh muslin cloth to obtain a clear, deeply pink to dark red coloured solution, and the residues discarded. The extract was concentrated by rotary evaporator at 65°C. The resultant extract was stored in capped bottles and kept in the refrigerator until required. A 100 mg/ml and 400 mg/ml solution of the extract was prepared in distilled water before administration to the rats.

2.4. Treatment of Animals

The rats were randomly selected and distributed evenly into four groups I, II, III and IV of eight rats each. Each group was kept in a separate cage. The rats were left to acclimatize to laboratory conditions for two weeks and subsequently employed to testing for three weeks; during which they were fed with commercially formulated rat feed (Growers’ mash) and water was given ad libitum. The animals were exposed to natural room temperature and lighting conditions and handled according to
standard protocols for the use of laboratory animals (National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1978).

2.5. Administration of the Extract

Group IV is the control group, while groups I, II and III are the experimental groups. The Hibiscus extract were administered to the experimental groups orally by orogastric tube, Group IV received normal tap water as the control, while groups I, II, and III received a daily dose of the extract, 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively for 31 days. During this period, they were weighed before and after administration of the extract for each week; and their activity and changes in physical appearances observed.

2.6. Assessment of Morphological Changes and Weighing

The effect of the extract was confirmed by observing the passage of hard dry stools by the test group, which was done visually and by manual palpation of the stool with gloved hands. Moreover, there was a progressive gain in weight from the commencement of the test, although the weight gain was not uniform. The weights were measured with a weighing balance, for every week.

2.7. Histopathological Technique

The rats from the various groups were sacrificed by subjecting them to light anesthesia (with the use of chloroform) in a urethane saturated chamber on the 32nd day of the experiment. The colon were quickly dissected out and fixed in 10% formal saline for routine histological techniques, to obtain sections of the tissues. It was stained with Haematoxylin and Eosin (H & E) as well as Southgate Mucicarmine (special staining to demonstrate mucin in the epithelial cells of the colonic mucosa); and then examined microscopically following standard procedures.

3. Results

The photomicrograph sections (Figure 1) through the upper part of the normal colon intestinal glands, showed packed arrangement of glands in the mucosa with intraepithelial lymphocytes, the lamina propria fills the space between the glands. (x400)

**Figure 1:** Showed a Photomicrograph Section of Group IV (Control) rat colon (H & E X400)
Figure 2: Showed a Photomicrograph of Group III (1000mg/kg Body Weight of Hibiscus sabdariffa-Treated) rat colon (H & E X400)

Transverse sections through the upper part of the colon intestinal glands (Figure 2) from group III showed more closely packed arrangement of glands in the mucosa with intraepithelial lymphocytes (T). The lamina propria fills the space between the glands & also contains lymphocytic cells. (H & E. X400).

Figure 3: Showed a Photomicrograph of Group IV (Control) rat colon (Southgate’s mucicarmine x400)

Transverse sections through the upper part of a normal colon intestinal gland (Figure 3) showed moderate Southgate’s mucicarmine-positive due to surface layer of goblet cell derived mucus. (x400).
4. Discussion

4.1. Effect of Duration on the Epithelial Changes and the Inflammatory Response

At the end of the treatment, there was no mucosal inflammation, but more sheets of lymphocytes present in foci with increased goblet cells (Figure 2). Southgate’s mucicarmine findings showed increased mucin production in the colonic mucosa (Figure 4). This may be due to the increased goblet cell during the treatment with aqueous calyces extract Hibiscus sabdariffa.

The changes showed high progression of epithelial response with lymphocytic infiltration of lamina propria; indicating no inflammation hence, its anti-inflammatory activity (Dafallah & Mustapha, 1996); the mucin production, as demonstrated by Southgate’s mucicarmine remained progressively higher. We observed no inflammation or glandular damage.

4.2. Relationship between Mucin Secretions, Mucosal Changes and Inflammatory Response

It was observed that, there was a progressive increase in the mucin production as the time duration increases. Nevertheless, there is a positive correlation between lymphocytic cells and goblet cells; which in turn create a positive dependent relationship with mucin production. It therefore showed that subchronic administration of the crude aqueous extract of Hibiscus sabdariffa calyces ameliorates the goblet cells, resulting in the thickening of the mucus gel (Figure 4). All these mucosal changes demonstrated that Hibiscus sabdariffa calyx extract exhibited antioxidant bioactivity in intact cells (Zhou et al., 2006).

5. Conclusion

We were able to establish that subchronic administration of the crude aqueous extract calyces Hibiscus sabdariffa caused a strong reaction towards the production of lymphocytic and goblet cells, which in turn increases mucin production. However, the test did not go beyond 21 days in this work, to ascertain whether or not the mucosal changes or the inflammatory response caused by the extract would return to normal or that, it could lead to any uncontrolled increase in the mucosal change.

We therefore recommend further work to ascertain the validation of long-term effect of the extract in the gastrointestinal tracts of rats.
References


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