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POLYPHENOL RICH PASSION FRUIT INHIBITS FENTON'S REAGENT INDUCED LIPID PEROXIDATION IN RABBIT COLON

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Abstract

The present study was aimed to estimate the phenol content of passion fruit (*Passiflora edulis*) fruit and to study its antioxidant property in rabbit colon. Cold extraction of fruit was performed with 50% ethanol and fractionation was done using chloroform in a separating funnel. The phenol content was estimated by Gallic acid curve method. Oxidation was induced by incubating rabbit colon tissue substrate with Fenton's reagent and the formed thiobarbituric acid reactive species was estimated using spectrophotometer at 535nm.The total phenolic content of fruit was found to be 102.7mg/gm. There is a gradual increase in antioxidant potency with increase in concentration from 20µg/ml. The maximum prevention (58.40%) was shown by the fruit at 50 µg/ml which is found to more effective than standard drug diclomine at the same dose (57.33 %). Imbalanced ROS production and defense mechanism by various factors may ultimately results in lipid peroxidation in colon cells which may leads to inflammatory bowel disoreders, ulcers and colitis etc,. Thiobarbituric acid- reactive substances are the byproducts of lipid peroxidation which is considered to be the clinical bio marker for of ulcerative colitis. In our study the fruit has the ability to protect from lipid peroxidation which may prevents the complications associated with IBD.

Keywords: Fenton's reagent, IBD, lipid peroxidation, *P. edulis*

Introduction

Aerobic cells upon oxidative metabolism during cellular respiration generate some vulnerable byproduct called the Reactive oxygen species (ROS). Apart from ageing process, these ROS are also involved in many pathological conditions like major cardiac disorders, diabetes as well as many functional GIT disorders [1]. They can directly attack

the PUFA (polyunsaturated fatty acids) of the lipid bilayer of the cell membrane and initiates peroxidation. This ended up in the formation of Malondialdehyde (MDA) which can further leads to tissue damage. In case of several intestinal disorders colon part of GIT is more prone to oxidative stress, since lack in mitochondrial oxidative defense system [2]. Polyphenols are the phyto-compounds synthesized by plants to overcome and adopt themselves in various climatic stress conditions like water, cold and also even in certain infections [3]. This leads the hypothesis for the researchers in working on polyphenols for various human ailments. Inflammatory bowel disorders (IBD) like Ulcerative colitis and Chron's disease are common colonic diseases which may come out with increase ROS in the large intestine. The antioxidant potency of herbs and drugs can be price out by methods like DPPH radical scavenging ability, lipid peroxidation assay, H_2O_2 scavenging activity, Fenton's reagent induced lipid peroxidation assay etc [4]. *Passiflora edulis* is a woody creeper, widely grown in several countries and were used for many medicinal purpose like lowering the blood pressure, treating bronchial asthma, etc. It has been reported that the rich content of polyphenols like flavanoids in its fruit has good antioxidant property [5]. Hence the present study was undertaken to figure out the potency of *P.edulis* in preventing the oxidative tissue damage in IBD using isolated rabbit colon tissue substrate.

Materials and Methods

Preparation of extract [6]:

Fruit pulp of *P. edulis* was separated from seed and homogenized with 50% ethanol and then fractionated with chloroform in a separating funnel. Chloroform fraction of *P. edulis* (CPE) thus obtained was collected, evaporated and used for further experimental studies.

Estimation of Total Phenol Content (Gallic acid method) [7]:

1 ml of sample containing 100 mg of CPE in methanol was incubated with Folin Ciocalteau reagent and sodium carbonate (7.5%) for 2 hrs. The absorbance of the mixture was measured at 765nm. The obtained values were interpolated against the Gallic acid calibration curve.

Total phenol was calculated by

$$\text{Total Phenol} = \frac{\text{Conc. of Gallic acid x Volume of the extract}}{\text{Weight of pure plant extract}}$$

Fenton's reagent induced lipid peroxidation in rabbit colon:

Preparation of tissue substrate:

The colon part of healthy adult rabbit was harvested and the intestinal cells were scrapped out. It is then homogenized in ice cold saline (1/10 w/v) with 10 strokes at approximately 1200 R/min in a Teflon glass homogenizer and centrifuged for 10 minutes at 3000 × g. The sediment pellet was discarded and the low speed supernatant was collected, which was kept for the assay [3].

Estimation of thiobarbituric acid reactive species:

Plant extracts of five different concentrations (10, 20, 30, 40 and 50µg/ml) were added to 100µl of tissue substrate. 30µl of Fenton's reagent (500µl $FeSO_4$ and 250µl H_2O_2) was added to the above mixture to induce oxidation. The reaction mixture was incubated for 2 h at 50ºC. After 2 hour the supernatant was separated and 1mL of Thiobarbituric acid (0.67%) and 1mL of Trichloro acetic acid (10%) were added and kept in boiling water bath for 30 min and cooled. After cooling 3mL butanol was added to the test tubes and the pink color in butanol layer was measured at 532 nm. IC50 values were calculated using mean values of triplicates [5]. Dicyclomine HCl was used as standard.

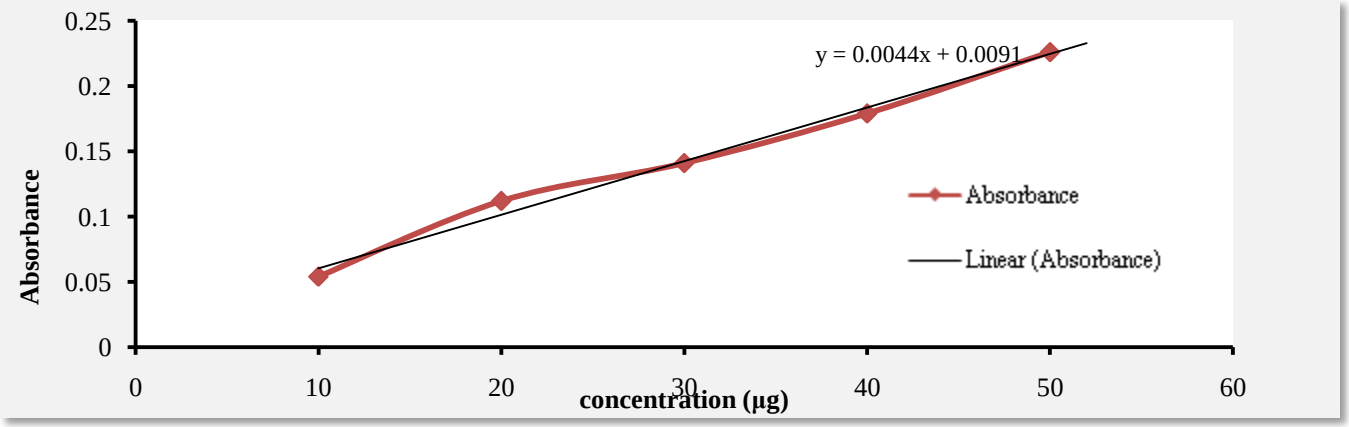

Figure 1. Standard Gallic acid curve for total phenol estimation.

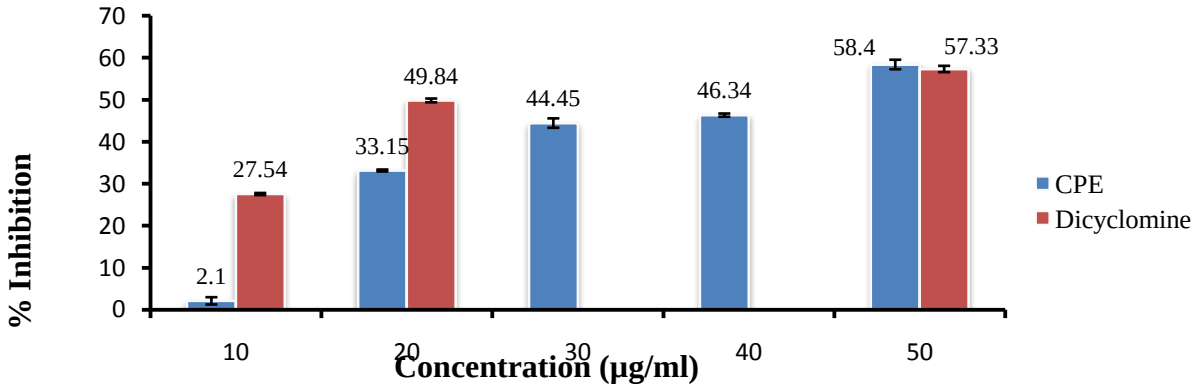

Figure.2: Effect of Passion fruit on Fenton's reagent induced lipid peroxidation.

Results and Discussion

Dietary polyphenols are found to be rich in passiflora species in the form of flavanoids. The total phenolic content estimation of fruit in CPE using Gallic acid as reference standard was found to be 102.7mg/gm (Gallic acid equivalent =51.35µg/ml). The standard gallic acid curve is shown in figure 1. As mentioned by Pillai CK and Pillai KS 2006, clinically the lipid peroxidation can be measured by estimating the diene conjugates and thiobarbituric acid- reactive substances (TBARS) human tissues and body fluids [8]. The antioxidant potency of CPE is determined by measuring TBARS in metal ion and H_2O_2 induced oxidative damage. Adding Fenton's reagent into the tissue substrates induces the generation of free radical and later causes degradation of PUFA, which results in the formation of Malondialdehyde (MDA). It reacts with thiobarbituric acid and forms pink color malondialdehyde thibarbituric acid (MDA-TBA) complex which can be determined colorimetrically at 532 nm. The percentage inhibition of CPE was found to be less promising at the concentration of 10µg, but there is a gradual increase in potency with increase in concentration. As shown in the figure 1, the percentage oxidative inhibition of CPE is found to be 33.15±0.23, 44.45 ± 1.12, 46.34 ± 0.38 and 58.40 ± 1.13 at 20, 40 and 50µg/ml respectively which is almost similar to 10, 20 and 50µg/ml of std drug dicylomine (27.54 ± 0.26, 49.84 ± 0.45 57.33 ± 0.75 respectively). Intestinal disorders like IBD, Ulcers, Diversion colitis also involves in the formation of reactive oxygen species (ROS) [9].The stress caused due to this ROS is most common in the colonic region of the intestine because of its continuous exposure to oxidizing substance and antigens which ultimately results in the imbalance between ROS production and antioxidant defense [10]. The production of hydroperoxyl radical and hydrogen peroxide (H_2O_2) accelerates in the presence of certain metals like iron known as lipid peroxidation induced by Fenton's reaction [9,11]. The triggering conditions like oxidative stress and ischeamic situations in intestinal disorders, iron are released from the metalloprotein [12]. In a study conducted by Carrier J, et al., 2001 that oral iron supplementation for colitis induced rats increases complication of disease by formation of reactive oxygen species [13].As passiflora species consist of enormous amount of polyphenols in the form of flavanoids it has the ability to protect from lipid peroxidation caused by various oxidative stress. Previous studies have proved about the ability of polyphenols rich food or beverages to prevent certain chronic diseases like cancer and cardiovascular disease [14]. Some of naturally occurring polyphenols are Quercetin, Myricetin, Kaempferol etc., found to be have free radical scavenging properties and inhibition lipid peroxidation, reduction of hydroperoxide formation and metal chelating properties [15].

Passiflora edulis have very good antioxidant properties in leaves and stems that have been already proved from its anticancer and antiasthmatic effect. From our studies it is proved that the poly phenol rich edible fruit also possesses a good antioxidant effect in colonic inflammation induced by metal ions. Further studies are under process in the laboratory for exploring the effects of the fruit in inflammatory bowel disorders.

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