# SOME NEW PHARMACOLOGICAL PROPERTIES OF BLACK TEA POLYPHENOLS

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BLACK tea has been known to possess important physiological properties and these were originally attributed to the presence of caffeine in large amounts. Later Vitamin P properties of polyphenols and their glycosides were also found to be exhibited by tea extracts[1]. In view of our recent finding that the oligomeric polyphenols (biflavonoids) possess very desirable pharmacological properties[2], it was considered necessary to study the pharmacological properties of black tea polyphenols. During black tea manufacture catechins which are $C_{15}$ compounds are transformed into $C_{29}$ and $C_{30}$ or further multiples of $C_{15}$ units known as theaflavins[3] and thearubigins[4] respectively. These compounds may be treated as the structural variants of biflavonoids. Some results of their study are given below.

*Bradykinin Antagonism.*—Bradykinin, a nonapeptide is liberated in the body from the pseudoglobulin portion of plasma by proteolysis. Four principal actions of this peptide have been recognized : (*i*) effect on smooth muscle, (*ii*) effect on blood-vessels or circulation, (*iii*) effect on capillary permeability and (*iv*) local effects on tissues thereby causing pain. At present it appears that bradykinin might play a part in the regulation of blood supply to certain glands and also that it might be implicated in shock, inflammation, asthma, rheumatic disease and painful states[5].

A careful pharmacological screening on guinea-pig ileum preparations revealed that the bradykinin antagonism of the theaflavins and thearubigins was really pronounced. In order to understand the structure-activity relationship related compounds were also studied for this effect. The compounds studied and the results obtained are given in Table I. Theaflavin represents a tropolone system having the basic structure of (–)epicatechin. The thearubigins originate from (–)epicatechin, (–)epigallocatechin and gallic acid and resemble polymeric proanthocyanidins in their structure having also the *o*-quinone system. The structures of these compounds also have catechol, pyrogallol, phloroglucinol and gallic acid units in different parts of the molecules. A careful study of all these compounds revealed that the tropolone and *o*-quinone systems are important for the activity. The activity was enhanced by the oligomeric size of the compounds.

TABLE I

*Bradykinin antagonism in guinea-pig ileum preparations*

| | Compound | | Bradykinin antagonism |
|---|---|---|---|
| 1 | Thearubigins | .. | 75% |
| 2 | Theaflavins | .. | 75% |
| 3 | Purpurogallin | .. | 50% |
| 4 | Purpurocatechol (tropolone) obtained by mixed oxidation of catechol + pyrogallol | | 20% |
| 5 | (–) epicatechin | .. | nil |
| 6 | (–) epicatechin gallate | .. | Feebly active in higher doses |
| 7 | (–) epigallocatechin gallate | | |
| 8 | Pyrogallol | .. | nil |
| 9 | Catechol | .. | do. |
| 10 | Phloroglucinol | .. | do. |
| 11 | Gallic acid | .. | do. |

*Anti-Spasmogenic Action Against Prostaglandins $PGE_1$ and $PGF_{1\alpha}$.*—The prostaglandins which are distributed in different parts of our system have very important biological role. Their functions are related to the reproductive organs, intestines cardiovascular system, central nervous system and also capillaries in regard to permeability[6].

During the present work the prostaglandin antagonism (towards $PGE_1$) of the compounds mentioned above were studied in guinea-pig ileum preparations. It was found that here also the thearubigins and theaflavins exhibit high degree of activity. The same activity was found when the study was carried out with $PGF_{1\alpha}$. In both the cases the activity of caffeine was also tested and it did not respond in either case.

TABLE II

*Prostaglandin antagonism in guinea pig ileum preparations ($PGE_1$ and $PGF_{1\alpha}$)*

| | Compound | | Antagonism |
|---|---|---|---|
| 1 | Thearubigin | .. | 75% |
| 2 | Theaflavin | .. | 75% |
| 3 | Purpurogallin | .. | 50% |
| 4 | Purpurocatechol | .. | 50% |
| 5 | (−) epigallocatechin gallate | .. | feebly active in higher doses |
| 6 | (−) epicatechin gallate | .. | nil |
| 7 | (−) epicatechin | .. | do. |
| 8 | Catechol | .. | do. |
| 9 | Pyrogallol | .. | do. |
| 10 | Phloroglucinol | .. | do. |
| 11 | Gallic acid | .. | do. |

These properties have been observed for the first time and the findings may be highly useful particularly in assessing the advantages of the use of tea as a beverage.

## EXPERIMENTAL

Green tea (800 gm) was extracted with ethyl acetate (2 lit) continuously after exhaustive pre-extraction with benzene. The ethyl acetate extract after concentrating to 400 ml. on standing deposited a white solid which was found to contain (−)epigallocatechin gallate as the major entity with small amounts of (−) epicatechin gallate and (−)epigallocatechin. On repeated crystallisation pure (−)epigallocatechin gallate was obtained. The mother liquor of the ethyl acetate extract yielded another crop of white solid which was found to be a mixture of (−)epigallocatechin gallate and (−)epicatechin gallate. Their authenticities was confirmed by their $R_f$ values in paper chromatography[7]. The solvent systems used for T.L.C. studies were, (*i*) Ethyl acetate, (*ii*) Toluene : Pyridine : Acetic acid (4 : 1 : 1) ; (2 : 1 : 1); (1 : 1 : 1), (*iii*) Pyridine : Acetic acid (1 : 1).

For testing the activity pure (−)epigallocatechin gallate and the mixture of (−)epicatechin gallate and (−)epigallocatechin gallate were used.

*Preparation of Purpurogallin*[8] *and Purpurocatechol*[9].—Pure samples of purpurogallin and purpurocatechol were obtained by modified procedures.

A cold aqueous solution of potassium iodate (8%, 100 ml) was slowly added dropwise to stirred solution of pyrogallol (10 gm) dissolved in minimum amount of cold water. The reaction proceeded smoothly with the precipitation of purpurogallin (7·6 g). The crude compound was adsorbed on silica gel (10 g) (BDH : 60–120 mesh size). This was put on a dry column of silica gel and eluted with ethyl acetate-benzene (1 : 10). The eluate contained pure purpurogallin which could be obtained as reddish orange needles from acetone, m.p. 275-77° (decomp).

A cold aqueous solution of potassium iodate (8%, 100 ml) was slowly added dropwise to a stirred solution of pyrogallol (5 g) and catechol (4·4 g) dissolved in minimum amount of cold water. The reaction proceeded smoothly with the precipitation of a brown solid. On T.L.C. [Toluene : Pyridine : Acetic acid (10 : 1 : 1)] this was found to contain pyrogallol ($R_f$ 0·4), purpurogallin ($R_f$ 0·55), catechol ($R_f$ 0·6) and purpurocatechol ($R_f$ 0·7). This mixture was subjected to dry column chromatography over silica gel. On elution with a mixture of pet. ether-benzene (1 : 1) purpurocatechol could be separated. This crystallised as red needles from benzene, m.p. 197–98°.

Theaflavins and thearubigins (mixtures) were isolated from Black tea using Roberts isolation procedure[10]. Care was taken to remove even traces of caffeine by repeated treatment with chloroform.

The purity of pyrogallol, catechol, phloroglucinol and gallic acid were confirmed by T. L. C. and melting points.

*Pharmacology*[11,12].—Isolated segments of the guinea-pig ileum preparations were suspended in 10 ml of Tyrode solution at 37°, gassed with oxygen. The contractions induced were recorded on a smoked drum with a frontal writing lever. In the beginning of the assay, bradykinin was tested repeatedly to obtain uniform response. During the experiment, black tea polyphenols and other compounds were added in such a way that they would be in contact with the tissue for 5 mt. and then bradykinin or $PGE_1$ or $PGF_{1\alpha}$ was added to elicit contractions. Dose range varied from 10 ng to 100 ng per ml and higher concentrations were used when needed. They were dissolved in propylene glycol and further dilutions were done with normal saline. After each contraction, the preparation was rested for 30 mt A total of six experiments for each dosage range were carried out.

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## EPOXY BASED ANION EXCHANGE MEMBRANE*

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THE use of ion exchange resin for electrolytic processes is relatively becoming more popular day by day. The more important of these processes use ion exchange membranes (*e.g.*, electrodialysis, preparation of chemicals, etc.). These membranes act as selective barriers and to some extent as "ionic sieves". Commercial ion exchange membranes are either of the homogeneous or heterogeneous type. Heterogeneous membranes consist of finely powdered ion exchange resin, held together by inert plastic binding materials. A large number of binding materials may be used in the preparation of the membranes. Polystyrene, polythylene, phenolic resins, methyl methacrylate, selectron, synthetic and natural rubber, and many other binders have been used[1]. The present work deals with the preparation of anion exchange membrane with the use of epoxy resin as a binder to the anion exchange resin, the membrane being cast during the polymerisation of epoxy resin brought about by a crosslinking agent. Similar type of epoxy based cation exchange membrane has been reported[2]. Out of several epoxy resins (adhesives and the hardeners) tried, it was found that the mixture of Araldite (AY 103) and Lancast (both products of CIBA Ltd.) used as binding agent for the exchange resin powder gave satisfactory membranes as far as flexibility, toughness and other mechanical properties are concerned.

### EXPERIMENTAL

*Preparation of Membrane*

(*a*) About 200 g Amberlite IRA 400, strongly basic anion exchange resin was treated in the conventional way till it was completely in chloride form. The dried resin was ground till the desired mesh size was obtained (− 240, + 400).

(*b*) *Preparing Binding Mixture.*—(Six different mixtures having different ratios of epoxy resin (Araldite AY 103) and the crosslinking agent (Lancast) were prepared. These were used as the binding agent for the anion exchange resin powder.

(*c*) To 10 g of the dried resin powder was added the mixture of (*b*) dropwise till on proper mixing the mass was transformed into a dough like form. The dough was passed through rubber rollers several times till a sufficiently tough sheet was obtained.

(*d*) The sheet so obtained was cured at 50–60° C in an oven for one hour, rolled again, and was left in a glass cabinet at room temperature for 5-6 days for complete curing.

The well cured membranes were immersed in distilled water for several hours and then dried between filter-paper folds. This process of hydration and drying was repeated six

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