

## Antispasmodic Effects of Rooibos Tea (*Aspalathus linearis*) is Mediated Predominantly through K<sup>+</sup>-Channel Activation

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**Abstract:** Rooibos tea has been widely used for abdominal spasm and diarrhoea. The aim of the present study was to explore the possible mechanism for its use in such ailments. Its aqueous extract (RT) at 0.3–10 mg/ml produced relaxation of spontaneous and low K<sup>+</sup> (25 mM)-induced contractions of rabbit jejunum, with weak effect on high K<sup>+</sup> (80 mM)-induced contractions. In the presence of glibenclamide, relaxation of low K<sup>+</sup>-induced contractions was prevented. Cromakalim inhibited contractions induced by low K<sup>+</sup>, but not high K<sup>+</sup>, while verapamil did not differentiate in its inhibitory effect on contractions produced by the two concentrations of K<sup>+</sup>. RT also exhibited antidiarrhoeal and antisecretory activities in mice. The spasmolytic effect was concentrated in organic fractions. Its constituents, chrysoeriol, orientin and vitexin showed a similar pattern of spasmolytic effects to the extract, while rutin was more like verapamil. So Rooibos tea possesses a combination of dominant K<sub>ATP</sub> channel activation and weak Ca<sup>++</sup> antagonist mechanisms and hence justifies its use in hyperactive gastrointestinal disorders.

*Aspalathus linearis* (Fabaceae) commonly known as Rooibos (syn. red bush), is an indigenous South African plant of 0.5–2 m height, with bright green needle-shaped leaves and small yellow typically pea-shaped flowers. Local people harvest the wild plants in summer and produce a tea by cutting leaves and twigs, bruising them with wooden hammers, fermenting the product in heaps and finally drying it, which results in caffeine-free, low tannin herbal tea popular in South Africa (Morton 1983; Wyk *et al.* 2002).

Due to its proven antioxidant properties, Rooibos tea is considered as a useful natural product for human health and is attracting much popularity in the international market, as evident by its 5 times increased sale in 2001 compared to the previous years (Joubert *et al.* 2004). Rooibos tea is commonly used in the traditional medical system for treating abdominal colic, diarrhoea, anaemia, cardiopathy, eczema (Duke *et al.* 2002), asthma (Brown 1995), inflammation, malignancies (Na *et al.* 2004), nervous tension and allergies (Bramati *et al.* 2002).

Its active principles are aspalathin, nothofagin, orientin, isoorientin, vitexin, isovitexin, quercetin, isoquercetin, chrysoeriol, caffeic acid, rutin, p-coumaric acid, p-hydroxybenzoic acid, ferulic acid, protocatechuic acid, syringic acid, vanillic acid (Duke *et al.* 2002), cinnamic acid and gallic acid (Rabe *et al.* 1994). The volatile components of Rooibos tea include acetic acid, 4-butanolide, dihydroactinidiolide, geranylacetone, guaiacol, hexanoic acid, methyl-ethylmaleimide, 2-methoxy-2-buten-4-olide, 3-methylbutanoic acid

and 2-phenylethanol (Kawakami *et al.* 1993). Minerals in tea include iron, potassium, calcium, copper, zinc, magnesium, fluoride, manganese and sodium (Kamen 2000).

Rooibos tea has been known to possess antiaging (Inanami *et al.* 1995), anticancer (Marnewick *et al.* 2005), antihaemolytic (Simon *et al.* 2000), antiinflammatory (Na *et al.* 2004), antimutagenic (Standley *et al.* 2001), hepatoprotective (Kucharska *et al.* 2004), hydrogen peroxide, superoxide anion and  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl radical scavenging (Pazdzioch-Czochra & Widenska 2002; Joubert *et al.* 2004) and radioprotective (Shimoi *et al.* 1996) properties. The polysaccharides isolated from Rooibos tea have strong anti-HIV activity (Nakano *et al.* 1997).

However, the tea has not been widely studied to rationalize its use in disorders resulting from hyperactive gut states, such as spasms and diarrhoea. In this investigation we report that the aqueous extract of Rooibos tea exhibits spasmolytic effect mediated possibly through a combination of dominant K<sup>+</sup> channel opening and weak Ca<sup>++</sup> antagonist actions, and that it also showed antidiarrhoeal and antisecretory activities in experimental animals (mice). The resultant fractions and some of the commercially available known principles of Rooibos were also tested in isolated jejunum preparations. The spasmolytic effect was found concentrated in the organic fractions. Among the constituents studied (fig. 1), chrysoeriol, orientin and vitexin were found to possess K<sub>ATP</sub> channel activating while rutin showed Ca<sup>++</sup> antagonist-type mechanism.

### Materials and Methods

**Chemicals.** Acetylcholine perchlorate, caffeic acid, gallic acid, loperamide hydrochloride, rutin, vanillic acid, verapamil hydrochloride

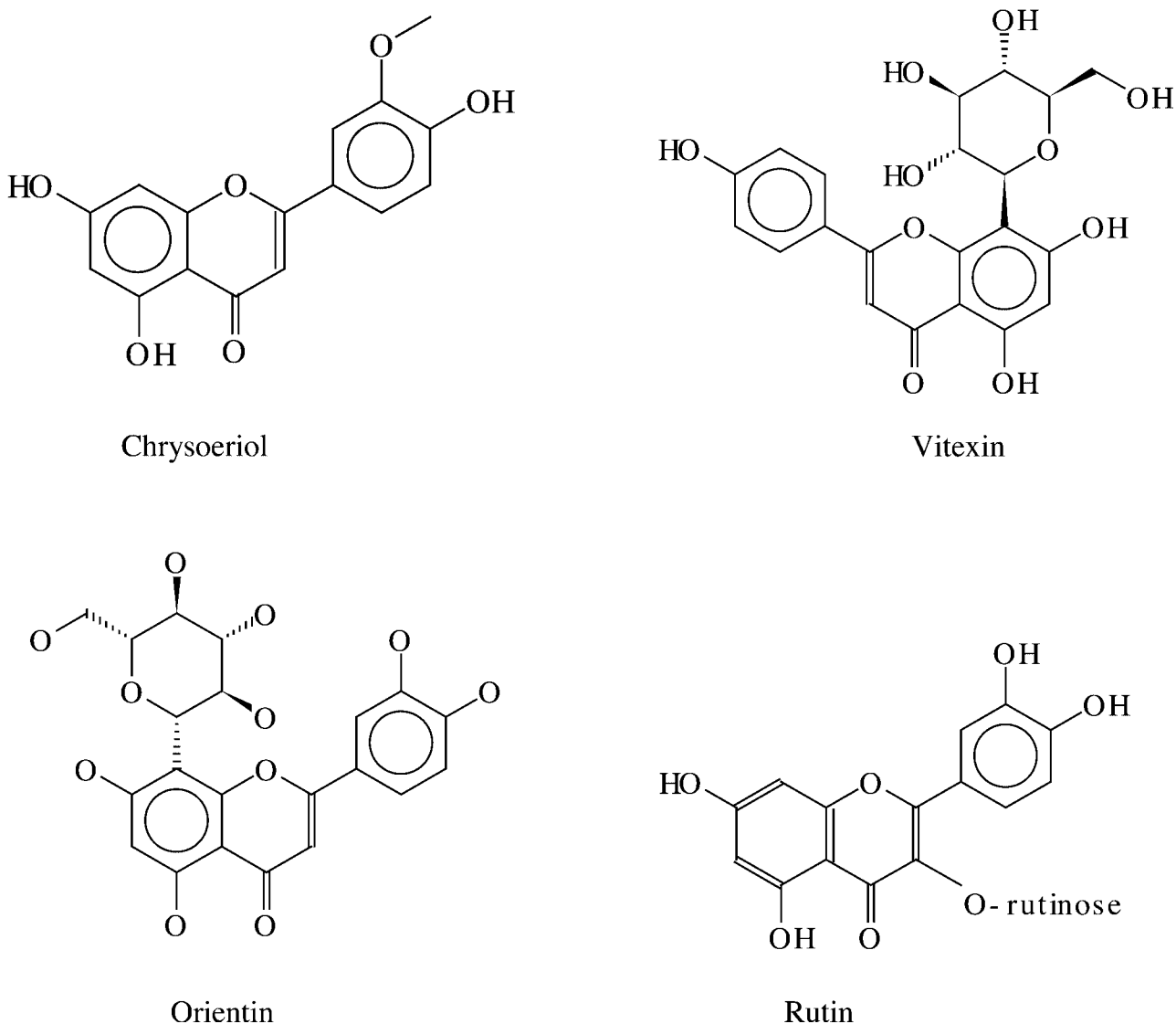


Fig. 1. Chemical structures of the Rooibos (*Aspalathus linearis*) constituents, chrysoeriol, orientin, vitexin and rutin.

were obtained from Sigma Chemicals Co., St. Louis, MO, USA. Cromakalim, glibenclamide and castor oil respectively from Tocris, Ellisville, MO, USA, RBI Chemicals Co., Natick, MA, USA and KCI Pharma, Karachi, Pakistan respectively. Pure compounds: chrysoeriol, orientin and vitexin were purchased from ChromaDex, Santa Ana, CA, USA. Chemicals used for making Tyrode's solution were: potassium chloride (Sigma Chemicals Co., St. Louis, MO, USA), calcium chloride, glucose, magnesium chloride, sodium bicarbonate, sodium dihydrogen phosphate (Merck, Darmstadt, Germany) and sodium chloride from BDH Laboratory supplies, Poole, England. All chemicals used were of the highest grade available and solubilized in distilled water/saline except cromakalim, glibenclamide, chrysoeriol, orientin and vitexin which were dissolved in 10% DMSO. The respective vehicle used for solubilization of drugs had no effect in the control experiments.

**Animals.** Animals used in this study such as adult rabbits (1.2–1.5 kg) and Balb-C mice (20–25 g) of either sex and local breed were housed at the Animal House of the Aga Khan University, maintained at 23–25° and were given a standard diet and tap water. Rabbits had free access to water, but food was withdrawn 24 hr prior to the experiment and killed by a blow on the back of the

head. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and were approved by the Ethical Committee of the Aga Khan University.

**Plant material.** Commercially best quality Rooibos tea was bought from a herbal market near Khayelitsha, Western Cape Province, South Africa, in June 2001, and a sample voucher (AL-LF-07-01-33) has been submitted to the herbarium of Department of Biological and Biomedical Sciences, Aga Khan University, Karachi.

**Preparation of crude extract and fractions.** Aqueous extract of the tea was prepared by boiling 150 g dry tea in 1 liter distilled water for 10 min. with subsequent standing for 20 min. and cooling down to room temperature. The resultant tea concentrate was filtered through Whatman qualitative grade 1 filter paper and then evaporated in a Buchi rotary evaporator yielding a thick, brown colored extract (RT) weighing 30.5 g (yield 20.4% w/w). RT was completely solubilized both in distilled water and saline for use in *in vitro* and *in vivo* experiments.

Activity-guided fractionation of the extract was carried out by standard phytochemical procedures using solvents of increasing po-

larity (Williamson *et al.* 1998). Approximately 4 g of RT was dissolved in about 150 ml of distilled water. Petroleum spirit was added to it and shaken vigorously in a separating funnel. The mixture was allowed to separate in two layers. The petroleum spirit layer (upper) was removed. The extraction with petroleum spirit was repeated twice more. All of the petroleum spirit layers were combined and evaporated in a rotary evaporator to give the petroleum spirit fraction (RT.Pet). The other separated layer (lower) was taken in a separating funnel; chloroform was added to it and separated as above. The chloroform layers (lower in this case) were collected thrice and evaporated on a rotary evaporator to give the chloroform fraction (RT.CHCl<sub>3</sub>). The other layer (upper in this case) was again taken into a separating funnel, ethyl acetate was added into it, separated and was also evaporated in a rotary evaporator to give the ethyl acetate fraction (RT.EtAc). The remaining lower layer was collected and evaporated to obtain the aqueous fraction (RT.Aq).

**Phytochemical analysis.** Phytochemical screening was done for the presence of flavonoids, tannins, saponins, alkaloids, anthraquinones, coumarins and sterols according to Evans (1996) and Edeoga *et al.* (2005) with some modifications. To measure the total amount of phenolic compounds, 1 ml of Folin-Ciocalteu Reagent was added to the extract solution adjusted to 46 ml by addition of distilled water (Singleton *et al.* 1999). After 3 min., 3 ml of Na<sub>2</sub>CO<sub>3</sub> (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 hr at room temperature and then absorbance was noted at 760 nm. Phenolic content was expressed as mg of quercetin equivalent/g of the extract. Total flavonoid content was determined by the method previously described by Huang *et al.* (2004). Briefly, 1.5 ml of the extract solution was added to an equal volume of solution of 2% AlCl<sub>3</sub>.6H<sub>2</sub>O in methanol. The mixture was vigorously shaken and absorbance was read at 367 nm after 10 min. of incubation. Flavonoid content was expressed as mg of quercetin equivalent/g of the extract. Absorption of the sample was measured by using a DU-70 Spectrophotometer (Beckman Instruments Inc, Palo Alto, CA, USA).

**Isolated rabbit jejunum preparations.** The spasmolytic activity of the plant materials was studied by using isolated rabbit jejunum preparations (Gilani *et al.* 2005a). Respective segments of 2 cm in length were suspended individually in 10 ml tissue baths containing Tyrode's solution, maintained at 37° and aerated with a mixture of 95% oxygen and 5% carbon dioxide (carbogen). The composition of the

Tyrode's solution in mM was: KCl 2.68, NaCl 136.9, MgCl<sub>2</sub> 1.05, NaHCO<sub>3</sub> 11.90, NaH<sub>2</sub>PO<sub>4</sub> 0.42, CaCl<sub>2</sub> 1.8 and glucose 5.55 (pH 7.4). Intestinal responses were recorded isotonicly using Bioscience transducers and an oscillograph. Each tissue was allowed to equilibrate for at least 30 min. before the addition of any drug and then stabilized with a submaximal concentration of acetylcholine (0.3 μM) and the bath fluid was subsequently replaced with normal Tyrode solution before starting the experiment. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing of the relaxant (spasmolytic) activity directly without the use of any agonist.

For elucidation of mechanism of spasmolytic activity, high K<sup>+</sup> (80 mM) and low K<sup>+</sup> (25 mM) concentrations were used to depolarize the isolated tissues which in turn produced sustained contractions. The plant material was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Van-Rossum 1963). The relaxation of isolated tissue preparations was expressed as percent of the control response mediated by added low and high K<sup>+</sup> concentrations.

**In vivo experiments.** These experiments were carried out by methods employed previously in our laboratory (Gilani *et al.* 2005b).

**Castor oil-induced diarrhoea.** Mice were fasted for 24 hr before the experiment. The animals were housed in individual cages and divided in four equal groups. The first group received saline as vehicle control (10 ml/kg, orally) and thus acted as negative control. A group of mice was treated with loperamide (10 mg/kg), as positive control. One hour after the treatment, each animal received 10 ml/kg of castor oil orally through a feeding needle. Afterwards, the cages were inspected for the presence of the typical diarrhoeal droppings; their absence was noted as a positive result, indicating protection from diarrhoea at that time.

**Intestinal fluid accumulation.** Different groups of overnight fasted mice were treated with increasing doses of RT intraperitoneally, 60 min. before the oral administration of castor oil (10 ml/kg). The mice were sacrificed 30 min. later by cervical dislocation and the whole intestine was isolated out and weighed with care, not allowing any intestinal fluid to leak out. The results were expressed as (Pi/Pm)×1000 where Pi is the weight (g) of the intestine while Pm is the weight of the animal.

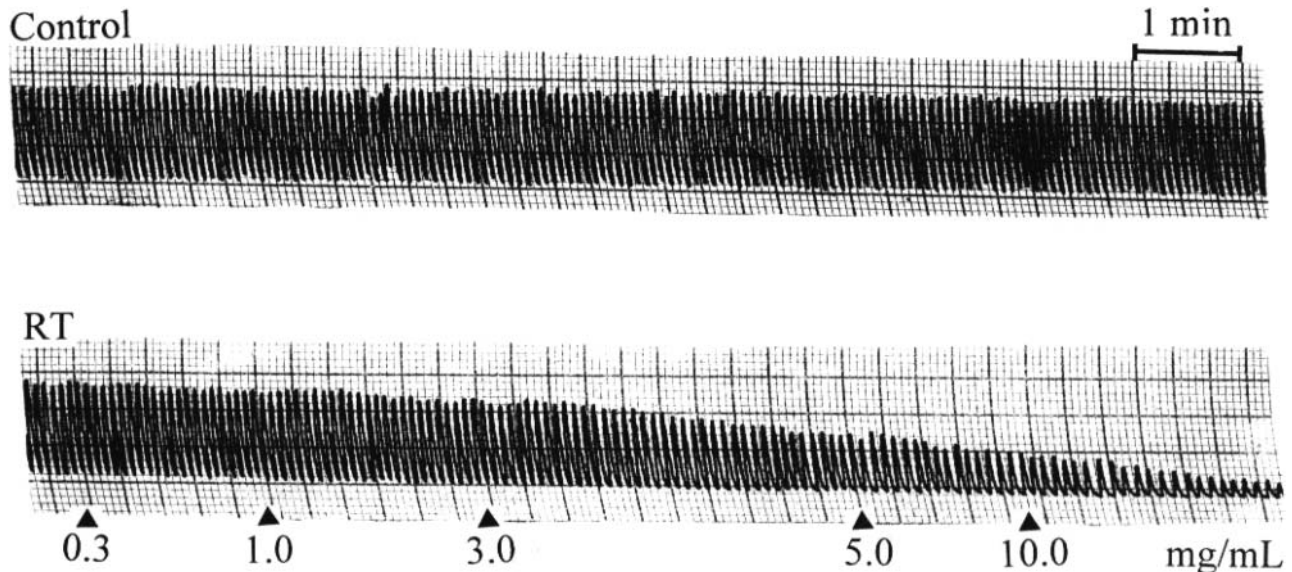


Fig. 2. Tracing showing the spasmolytic effect of Rooibos tea extract (RT) on spontaneously contracting isolated rabbit jejunum preparation.

**Acute toxicity study.** Animals were divided in groups of five mice each. The test was performed using increasing doses of the plant extract, given orally, in 10 ml/kg volume to different groups serving as test groups. Another group of mice was administered saline (10 ml/kg) as negative control. The mice were allowed food *ad libitum* and kept under regular observation for 6 hr while the lethality was recorded after 24 hr.

**Statistical analysis.** All the data expressed are mean  $\pm$  standard error of the mean (S.E.M.,  $n$ =number of experiments) and the median effective concentrations ( $EC_{50}$  values) with 95% confidence intervals (CI). The statistical parameter applied is the Student's *t*-test except in the castor oil-induced diarrhoea test where Chi-square-test was used.  $P < 0.05$  was noted as significantly different. Concentration-response curves (CRCs) were analyzed by non-linear regression using GraphPad program (GraphPAD, San Diego, CA, USA).

## Results

### Phytochemical screening.

RT was found to contain flavonoids, tannins and saponins, while it tested negative for the presence of the rest of the classes of compounds. Total phenolic and flavonoid contents were respectively  $120.0 \pm 1.6$  and  $199.98 \pm 1.93$  mg of quercetin equivalent/g of the extract.

### Effect on rabbit jejunum.

RT caused concentration-dependent (0.3–10 mg/ml) relaxation of spontaneous contractions of isolated rabbit jejunum preparations (fig. 2) with  $EC_{50}$  value of 4.7 mg/ml (3.4–6.6, 95% CI,  $n=5$ ). When tested against high  $K^+$  (80 mM)-induced contractions, RT produced a weak inhibitory effect, while it caused complete relaxation of the contractions induced by low  $K^+$  (25 mM) with  $EC_{50}$  value of 2.48 mg/ml (1.62–3.79). In the presence of glibenclamide (3  $\mu$ M), relaxation of low  $K^+$  (25 mM)-induced contractions was prevented (fig. 3A). Similarly, cromakalim also caused selective and glibenclamide-sensitive relaxation of the contractions induced by low  $K^+$  (25 mM) with  $EC_{50}$  value of 0.05  $\mu$ M (0.02–0.11), without any effect on high  $K^+$  (80 mM)-induced contractions (fig. 3B), whereas verapamil inhibited low  $K^+$  (25 mM) and high  $K^+$  (80 mM)-induced contractions at a similar concentration range, with  $EC_{50}$  values of 0.05 (0.02–0.11) and 0.05  $\mu$ M (0.03–0.1) respectively (fig. 3C).

### Effect on castor oil-induced diarrhoea.

RT exhibited a dose-dependent (500–1000 mg/kg) antidiarrhoeal effect against castor oil-induced diarrhoea in mice (table 1). The negative control group (saline-treated) did not show diarrhoea in any of the animals, while all mice in the castor oil-treated group showed diarrhoea. When animals were pretreated with RT before castor oil treatment, the

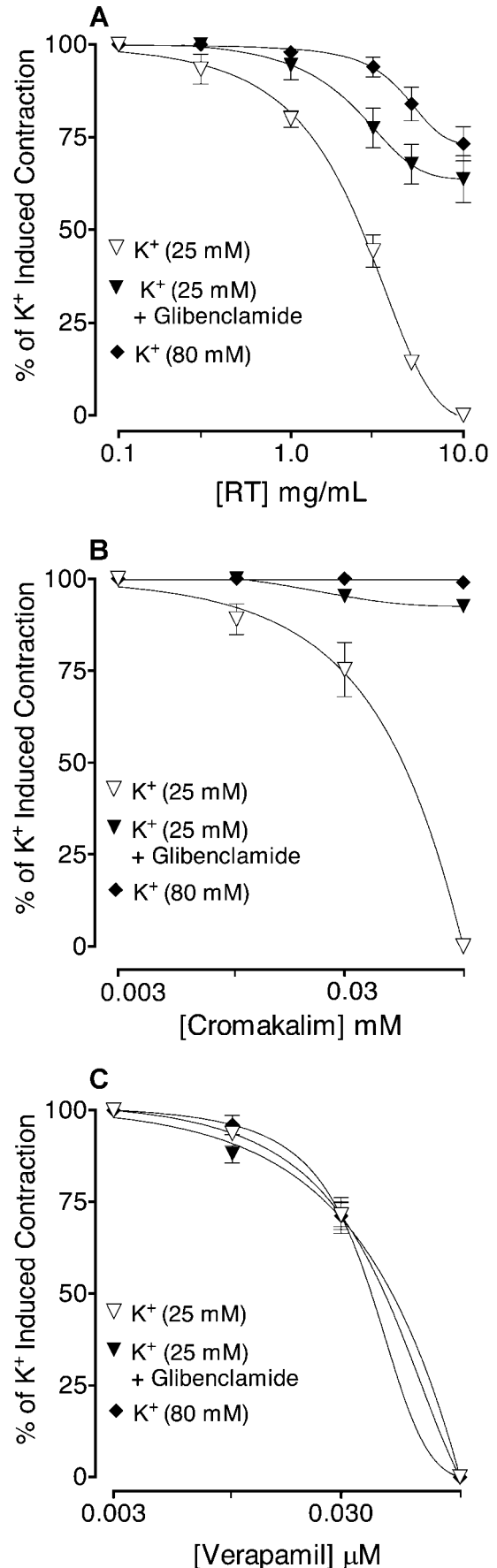


Fig. 3. Concentration response curves showing the comparison of (A) Rooibos tea extract (RT), (B) cromakalim and (C) verapamil for the inhibitory effect against low  $K^+$  (25 mM), in the absence ( $\nabla$ ) and presence ( $\blacktriangledown$ ) of glibenclamide (3  $\mu$ M) and high  $K^+$  (80 mM)-induced contractions ( $\blacklozenge$ ) in isolated rabbit jejunum preparations. Symbols represent mean  $\pm$  S.E.M.,  $n=3-6$ .

Table 1.

Effect of Rooibos tea extract (RT) on castor oil-induced diarrhoea in mice.

Treatment (p.o.)	No. of mice/ 5 with diarrhoea	% Protection
Control (saline, 10 ml/kg)	0	–
Castor oil (10 ml/kg)	5	0
+RT (500 mg/kg)	4	20
+RT (1000 mg/kg)	2*	60
Loperamide (10 mg/kg)	0*	100

\*P<0.05, compared to castor oil group, Chi-square test.

extract showed 20% protection from diarrhoea at a dose of 500 mg/kg and 60% protection at 1000 mg/kg (P<0.05 versus castor oil). Loperamide (10 mg/kg) showed complete protection from diarrhoea in the positive control group.

#### Effect on fluid accumulation.

RT dose-dependently (500–1000 mg/kg) exhibited an antisecretory effect (fig. 4). Intestinal fluid accumulation in the saline-treated group was 102.8±0.86 g, while with castor oil it was 155.0±6.55 g (P<0.001 versus saline). RT at the doses of 500 and 1000 mg/kg reduced the castor oil induced fluid accumulation to 127.4±8.14 g (P<0.05 versus castor oil) and 122.1±4.34 g (P<0.01 versus castor oil) respectively.

#### Acute toxicity test.

When the tea extract was tested up to a dose of 5 g/kg, it was found to be safe, as no mortality was observed in the animals.

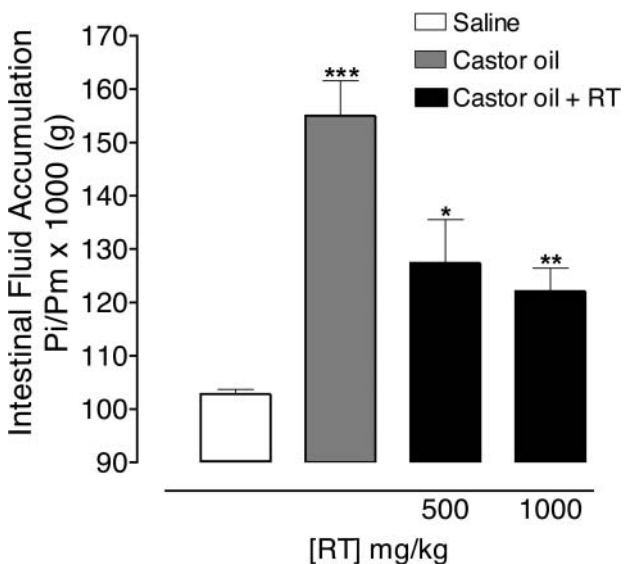


Fig. 4. Effect of increasing doses of Rooibos tea extract (RT) on castor oil-stimulated fluid accumulation in small intestine of mice. Results shown are mean±S.E.M. of 5 animals for each experimental group. Intestinal fluid accumulation is expressed as Pi/Pm×1000 (g) where Pi is the weight of the small intestine and Pm is the weight of the mouse. \*\*\*P<0.001 versus saline; \*P<0.05, \*\*P<0.01 versus castor oil, Student's t-test.

#### Effect of fractions on rabbit jejunum.

The organic fractions of Rooibos extract, i.e., RT.Pet, RT.CHCl<sub>3</sub> and RT.EtAc caused concentration-dependent relaxation of spontaneous and low K<sup>+</sup> (25 mM)-induced contractions with respective EC<sub>50</sub> values of 2.20 (0.93–5.03) and 1.34, 3.34 (2.15–8.78) and 2.46 (1.39–4.33), 5.69 (2.46–13.8) and 3.26 mg/ml (1.81–5.88). In the presence of glibenclamide (3 μM), relaxation of low K<sup>+</sup> (25 mM)-induced contractions was prevented. RT.Pet and RT.EtAc had no effect on high K<sup>+</sup> (80 mM)-induced contractions, while RT.CHCl<sub>3</sub> exhibited a weak inhibitory effect. The aqueous fraction (RT.Aq) was found to possess a negligible spasmolytic effect (fig. 5).

#### Effect of pure compounds on rabbit jejunum.

Some known constituents of Rooibos tea namely chrysoeriol, orientin, vitexin, rutin, caffeic acid, gallic acid and vanillic acid were also tested in rabbit jejunum preparations. Chrysoeriol, orientin and vitexin caused concentration-dependent relaxation of spontaneous and low K<sup>+</sup> (25 mM)-induced contractions with respective EC<sub>50</sub> values of 32 (15.5–66.6) and 37.8 (22.9–68.3), 32.1 (17.8–57.4) and 12.3 (8.68–17.4), 162.3 and 170.6 μg/ml. In the presence of glibenclamide (3 μM), relaxation of low K<sup>+</sup> (25 mM)-induced contractions was prevented. All the three constituents exerted no affect on high K<sup>+</sup> (80 mM)-induced contractions (fig. 6). These observations were based on one or two experiments of each, due to their limited supply. Rutin caused inhibition of spontaneous, low K<sup>+</sup> (25 mM) and high K<sup>+</sup> (80 mM)-induced contractions with respective EC<sub>50</sub> values of 204.4 (107.4–388.9), 492.7 (245.1–861.2) and 84.6 μg/ml (58.79–121.8) as shown in fig. 6D. Glibenclamide had no effect on the relaxant effect of rutin against low K<sup>+</sup>. Caffeic acid, gallic acid and vanillic acid failed to relax spontaneous, low K<sup>+</sup> (25 mM) and high K<sup>+</sup> (80 mM)-induced contractions up to the dose of 10 mg/ml.

## Discussion

The results of our preliminary phytochemical analysis reveal that the aqueous extract of Rooibos tea contains flavonoids, tannins and saponins with high phenolic and flavonoid contents, which is in accordance with the previous findings (Ferreira *et al.* 1995), except that the presence of saponins is reported for the first time. Due to the folkloric reputation as a gastrointestinal relaxant, the tea extract was tested for its possible spasmolytic effect in spontaneously contracting isolated rabbit jejunum preparations, where it inhibited spontaneous contractions, thus showing an antispasmodic action.

In our earlier studies, we have observed that the spasmolytic effect of medicinal plants is usually mediated through Ca<sup>++</sup> channel blockade or K<sup>+</sup> channel opening (Gilani *et al.* 2005a, b & c). To see whether the spasmolytic effect of the Rooibos tea extract is also mediated via similar mechan-

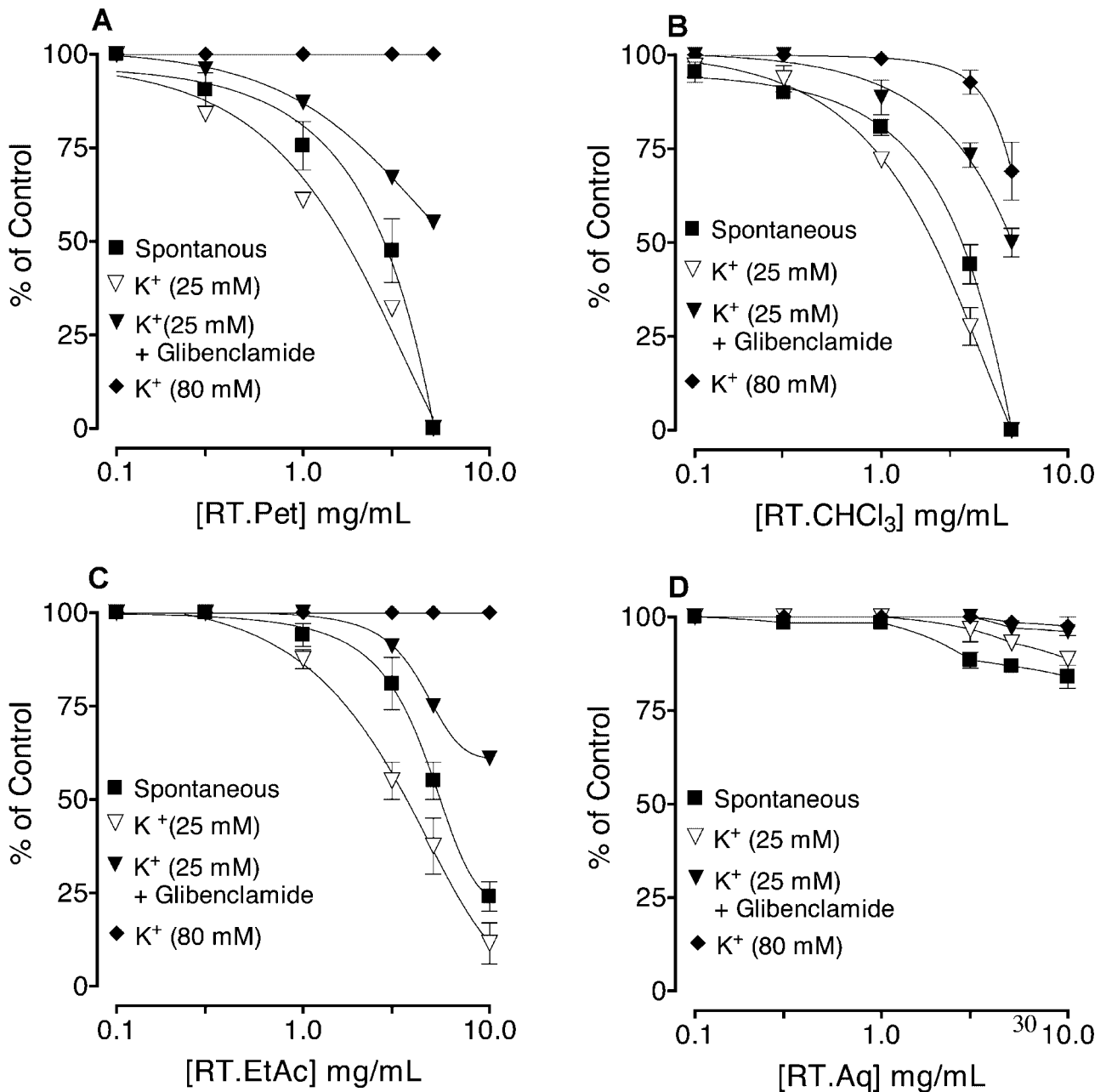


Fig. 5. Concentration response curves showing the effect of Rooibos tea extract (RT) fractions: (A) petroleum spirit (RT.Pet), (B) chloroform (RT.CHCl<sub>3</sub>), (C) ethylacetate (RT.EtAc) and (D) aqueous (RT.Aq) on spontaneous, low K<sup>+</sup> (25 mM), in the absence (▽) and presence (▼) of glibenclamide (3 μM), and high K<sup>+</sup> (80 mM)-induced contractions (◆) in isolated rabbit jejunum preparations. Symbols represent mean ± S.E.M., n=1–5.

isms, the tea extract was tested on high and low K<sup>+</sup>-induced contractions. It caused weak inhibitory effect against high K<sup>+</sup> while completely inhibited the low K<sup>+</sup>-induced contractions. The substance that selectively relaxes the contractions induced by low K<sup>+</sup> is considered a potassium channel opener, while Ca<sup>++</sup> antagonists inhibit both low and high K<sup>+</sup>-induced contractions equally, and these experiments allow distinguishing K<sup>+</sup> channel opening from Ca<sup>++</sup> channel blocking activities (Hamilton *et al.* 1986; Kishii *et al.* 1992; Gilani *et al.* 2005c). The K<sup>+</sup> channel opening effect

was confirmed, when the inhibition of low K<sup>+</sup>-induced contractions was prevented in the presence of glibenclamide, a specific blocker of the ATP-dependent K<sup>+</sup> channels (Frank *et al.* 1994). Cromakalim, a prototypical K<sub>ATP</sub> channel opener (Escande *et al.* 1988) produced similar results to that of the tea extract, except that it produced no effect on high K<sup>+</sup>-induced contractions, while verapamil, a standard calcium channel blocker (Fleckenstein 1977) inhibited low and high K<sup>+</sup>-induced contractions at similar concentrations. These results clearly indicate that the spasmolytic effect of

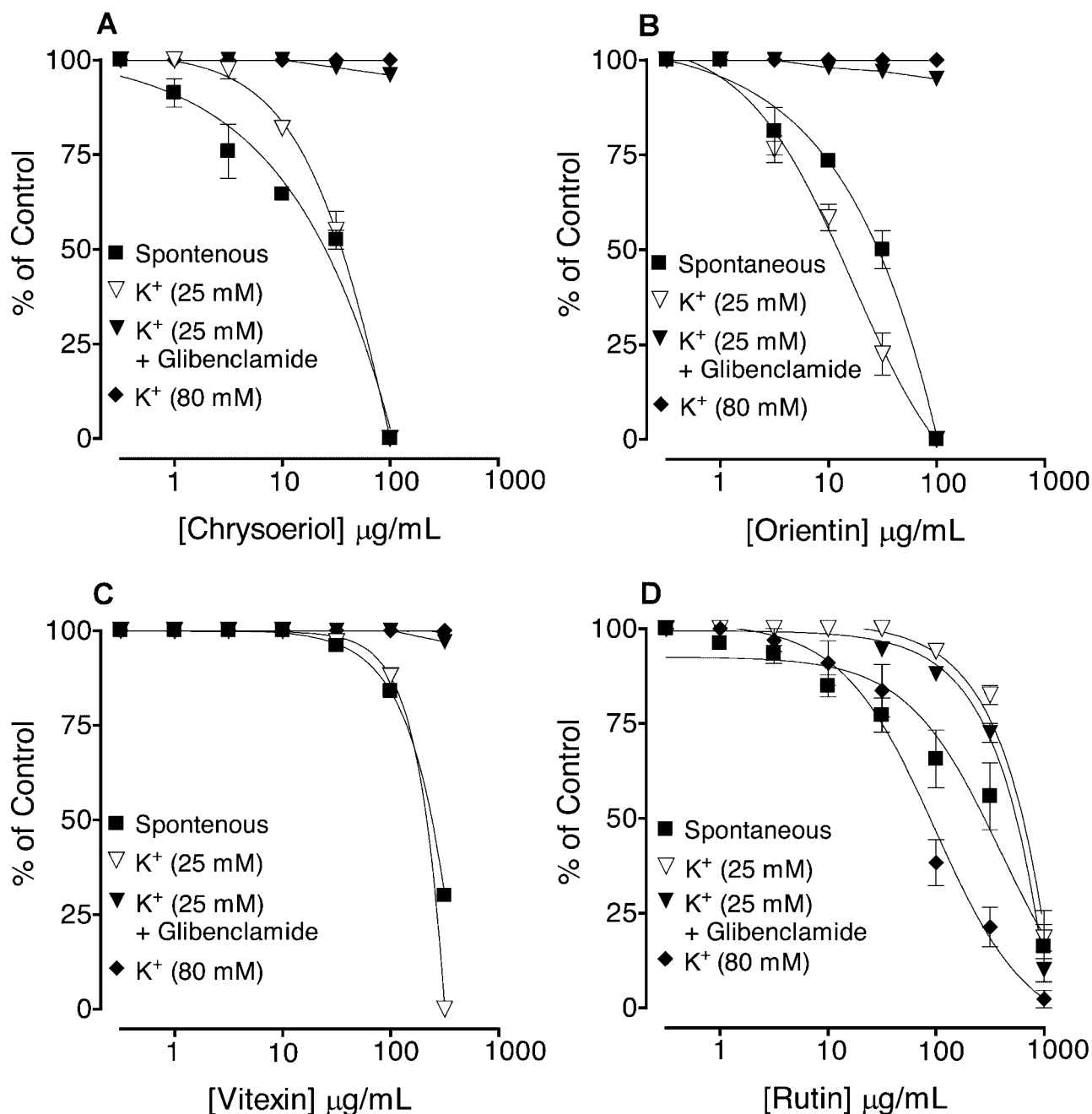


Fig. 6. Concentration response curves showing the effect of the Rooibos active ingredients: (A) chrysoeriol, (B) orientin (C) vitexin and (D) rutin on spontaneous, low K<sup>+</sup> (25 mM), in the absence (▽) and presence (▼) of glibenclamide (3  $\mu\text{M}$ ) and high K<sup>+</sup> (80 mM)-induced contractions (◆) in isolated rabbit jejunum preparations. Symbols represent mean  $\pm$  S.E.M., n=1-4.

the Rooibos extract is mediated possibly through a combination of dominant ATP-dependent K<sup>+</sup> channel activation and weak Ca<sup>++</sup> entry blocking mechanisms.

Both K<sup>+</sup> channel activators and Ca<sup>++</sup> antagonists produce smooth muscle relaxation via a decrease in intracellular free Ca<sup>++</sup> through respective mechanisms of membrane hyperpolarization via increase in K<sup>+</sup> efflux by opening of K<sup>+</sup> channels (Quest & Cook 1989; Weston & Edwards 1992) and inhibition of Ca<sup>++</sup> ingress through blockade of calcium channels (Karaki *et al.* 1997).

The resultant fractions were obtained in the following order of potency for their spasmolytic effect: petroleum spirit > chloroform > ethylacetate, while the aqueous fractions showed negligible spasmolytic effect. The petroleum ether and ethylacetate fractions, like cromakalim caused glibenclamide-sensitive relaxation of the low K<sup>+</sup>-induced contractions, but showed no effect on the contractions induced by high K<sup>+</sup>, indicating the presence of K<sub>ATP</sub> channel opening constituents, while the chloroform fraction also caused partial inhibition of high K<sup>+</sup>-induced contractions, thus sug-

gesting the existence of a combination of  $K^+$  channel opening and  $Ca^{++}$  antagonist mechanisms.

Some of the known constituents of Rooibos tea, such as chrysoeriol, orientin and vitexin showed a cromakalim-like inhibitory pattern, while rutin like verapamil caused relaxation of high  $K^+$ -induced contractions, thus showing a  $Ca^{++}$  channel blocker-type of action (Bolton 1979). Chrysoeriol and orientin were found around 100, vitexin 25 and rutin 15 times more potent than the parent extract. Although orientin and chrysoeriol are found equipotent in their spasmolytic effect, the former compound being present in a relatively higher concentration (Bramati *et al.* 2002), is likely to contribute more towards the therapeutic success of the parent extract. Quercetin and luteolin (known plant constituents) are previously reported to act as  $Ca^{++}$  antagonists (Sanchez de Rojas *et al.* 1996; Revuelta *et al.* 1997). Thus, Rooibos extract contains a mixture of  $K_{ATP}$  channel opening and  $Ca^{++}$  antagonist constituents likely to be responsible for its antispasmodic effect.

The property of the Rooibos tea to influence diarrhoea was further determined by its protective effect on the castor oil-induced diarrhoea in mice. The induction of diarrhoea with castor oil results from the action of ricinoleic acid formed in the hydrolysis of the oil (Iwao & Terada 1962), which produces changes in the transport of electrolytes and water resulting in the generation of giant contractions of the transverse and distal colon (Crocì *et al.* 1997). Thus a potential antidiarrhoeal agent may exhibit its antidiarrhoeal effect by inhibiting bowel contractions. This is in accordance with the expectation, as  $K^+$  channel openers and  $Ca^{++}$  antagonists were reported to possess antidiarrhoeal properties (Reynolds *et al.* 1984; Poggioli *et al.* 1995). Considering the 20.4% yield of the Rooibos tea extract, and a 13 times smaller equivalent human dose compared to mice (IRIS 1992), the antidiarrhoeal dose of the herbal tea for a person of 70 kg is approximately 25 g, i.e. around 1.5 table spoon. Rooibos tea extract also protected the mice against castor oil-induced secretions. Secretory functions in the gastrointestinal glands have been shown to be dependent to a certain extent on the intracellular  $Ca^{++}$  level (Scratcherd & Grundy 1984), hence the extract by virtue of possessing the spasmolytic activity, might influence the gastric acids and intestinal fluid release. In addition, the absence of caffeine, a stimulant of gastrointestinal secretions (Mercadante 1995) makes Rooibos tea a useful gastroprotective herbal beverage.

The flavonoids are well known for their antispasmodic activity (Pietta 1998) and the presence of these type of compounds such as quercetin, rutin, orientin, vitexin, chrysoeriol and luteolin in Rooibos (exhibiting different modes of spasmolytic action) are likely to contribute in its gastrointestinal effects, though the likely role of tannins present in the plant cannot be ignored as the tannins are known to have a beneficial role in diarrhoea (Heinrich *et al.* 1992). In acute toxicity testing, the extract was found safe up to the maximum dose (5 g/kg) tested, which is in accordance with the wide therapeutic and nutritional use of Rooibos tea.

The present results show that the aqueous extract of Rooibos tea exhibits a combination of dominant  $K_{ATP}$  channel activating and weak  $Ca^{++}$  antagonist effects, owing to which it shows spasmolytic, antidiarrhoeal and antisecretory activities. The spasmolytic effect is concentrated in the organic fractions. Some of its constituents such as chrysoeriol, orientin and vitexin were found to exhibit their spasmolytic effects through  $K^+$  channel activation while that of rutin through  $Ca^{++}$  channel blocker like mechanism. Thus, this study provides a scientific basis for the medicinal use of Rooibos tea in hyperactive gut disorders.

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