

Antioxidant activity of *Caryota urens* L. (Kithul palm) treacle

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In Sri Lanka, traditionally, treacle made from *Caryota urens* L. (Family: Arecaceae) sap is used as a natural sweetener and in Sri Lankan ethnomedicine it is claimed to possess anti-ageing and antidiabetic potentials. These claims suggest that *C. urens* treacle may have antioxidant activity. However, this has not been scientifically tested and this study evaluates antioxidant potential of *C. urens* treacle (n=12) *in vitro* [using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺), ferric reducing antioxidant power (FRAP) and ferrous ion chelating assays with five different treacle concentrations (ranged from 0.2-18.0 mg/l)] and *in vivo* using rats. Two groups of rats (8/group) were selected and one group was fed daily with standard diet containing 56% sucrose and the other with standard diet containing 56% treacle for 28 days. Blood was collected from tail, serum separated on day 1 (post treatment) and antioxidant activity was determined by ABTS⁺ assay using five serum concentrations (0.06, 1.25, 2.50, 5.00 and 10.0 µl/ml).

The *in vitro* results showed DPPH radical scavenging activity (0.15 ± 0.01 mmoles Trolox equivalent antioxidant activity (TE)/100 g of treacle), ABTS⁺ radical scavenging activity (0.57 ± 0.07 mmoles TE/100 g of treacle), ferric reducing antioxidant activity (2.65 ± 0.22 mmoles TE /100 g of treacle) and ferrous ion chelating activity (IC₅₀; 19.6 ± 3.9 mg/ml). *In vivo* results showed significant increase in serum antioxidant capacity of treacle fed group compared to control (IC₅₀; control vs treatment: 5.74 ± 0.13 vs 4.63 ± 0.18 µl/ml and Trolox equivalent antioxidant concentration; control vs treatment: 2.96 ± 0.06 vs 3.70 ± 0.13 mM TE).

This is the first report of both *in vitro* and *in vivo* antioxidant capacity of any treacle and it is concluded that Sri Lankan *C. urens* treacle has moderate antioxidant activity both *in vitro* and *in vivo* and has the potential to be used as health promoting natural sweetener.

Diuretic activity of hot water infusion of BOPF grade Sri Lankan black tea (*Camellia sinensis* L.) in rats

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Oral diuretic activity of black tea was investigated in rats using hot water infusion (HWI) of BOPF grade Sri Lankan black tea, manufactured from buds and tender leaves of *Camellia sinensis* L (O) Kuntze (Family: Theaceae) plant. Tea samples were drawn randomly from three major agro-climatic elevations: high (above 1200 m, average mean sea level), mid (1200 – 600 m) and low grown (below 600 m). Different concentrations of

HWI (300, 600, 2400 mg/kg, body weight), furosemide (positive control) and water (negative control) were orally administered to previously starved (18 h) hydrated male rats (n=9/group). Acute (6 h) and chronic (28 d) diuretic activities were assessed by measuring the cumulative urine output at hourly intervals up to 6 h. Electrolyte levels (Na^+ , K^+ , Ca^{2+} , H^+ , Cl^- , HCO_3^-), pH, osmolarity of urine and glomerular filtration rate (GFR) of treated rats were determined. Administration of HWI induced a significant ($p < 0.05$) and dose-dependent diuretic activity which varied with agro-climatic elevations. Diuretic activity had a rapid onset (1st h), peaked at 2nd h and maintained up to 4th h (except the low dose). Further, there was a dose-dependent increase in micturation frequency which peaked at 2nd h. A close association between phyto-chemicals (especially caffeine) and diuretic activity was evident. HWI induced diuresis was accompanied with an increased urine volume, urinary Na^+ and GFR. The diuresis is mediated via multiple mechanisms: inhibition of aldosteron secretion (in terms of increased Na^+ / K^+ ratio), inhibition of carbonic anhydrase (in terms of decreased $\text{Cl}^- / (\text{Na}^+ + \text{K}^+)$ ratio) and via thiazide type of diuretic action (evaluated in terms of increased $\text{Na}^+ / \text{Cl}^-$ ratio). It is concluded that, the Sri Lankan BOPF grade black tea possesses mild oral diuretic activity whose efficacy differs with the agro-climatic elevations.

An assessment of the contents of some heavy metals in Sri Lankan black tea (*Camellia sinensis* L.) and effect of oral administration of tea infusion in rats.

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Hot water infusions (HWI) of black tea manufactured using fresh bud and tender leaves of *Camellia sinensis* L. (O) Kuntze (Family: Theaceae) plant is a popular daily consumed beverage in Sri Lanka. Some heavy metals are inherently present in black tea. Ingestion of excessive amounts of these metals could cause health risk. Therefore, in this study, Copper, Mercury, Lead and Arsenic content of BOPF grade black tea, sampled from major agro-climatic elevations: low, mid and high grown in Sri Lanka and their HWI were determined using Atomic Absorption Spectrophotometer. The serum metal contents were assessed (using AAS) in rats orally administered with different doses of black tea HWI of high grown (human equivalent of 1.5, 3 and 12 cups per day), high dose (equivalent of 12 cups) of mid and low grown or water (control), thrice a day for consecutive 90 days. During the treatment, rats were daily observed for overt signs of toxicity and at the end of the treatment on chronic toxicity (renal and hepato-toxicity) were assessed. The total contents of heavy metals in black tea differ according to the agro-climatic elevations: Cu, 27.3 – 75.6 mg/kg; Hg, 0.01 – 0.026 mg/kg; Pb, 0.02 – 0.065 mg/kg and As, 0.003 – 0.006 mg/kg. The percent releases from black tea to their infusions were: Cu, 86 ± 8 %; Hg, 78 ± 6 %; Pb, 42 ± 3 % and As, 56 ± 9 %. Compared to control, blood serum metal contents were significantly ($p < 0.05$) and dose-dependently increased by black tea tested with high grown: Cu, 1.14 – 1.93 $\mu\text{g}/\text{dl}$; Hg, 0.10 – 0.19 $\mu\text{g}/\text{dl}$ and Pb, 3.78 – 6.84 $\mu\text{g}/\text{dl}$. In contrast, As content was not altered (0.05 – 0.07 $\mu\text{g}/\text{dl}$). All metals tested were below the permissible limit prescribed by FAO. Further, there were no signs of toxicity. Therefore, daily consumption of even 12 cups of Sri Lankan black tea may not produce any health risk.