Assessing the *in-vivo* Effects of Ingesting Tea Fortified Yoghurts in Murine Model

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Abstract

**Background:** Yoghurts were fortified with black, green and purple teas to impart health benefits. The effects of ingesting the fortified yoghurts were investigated using laboratory mice.

**Objectives:** To investigate the effect of ingesting yoghurts fortified with teas using mice model by assaying body weight, packed cell volume and antioxidant biomarkers in blood and liver.

**Methods:** Plain, black, green and purple tea fortified yoghurts were developed with 0, 1, 2 and 4g in 250mL milk. Mice model was used to investigate the effects of their ingestion (Permit Number IRC/13/12) at the National Museums of Kenya- Institute of Primate Research (NMK-IPR). Body weight, blood and liver biomarkers including packed cell volume (PCV), albumin, total protein, alkaline phosphatase (ALP) and glutathione (GHS) were studied. Results were statistically assayed and used for interpretation.

**Results:** Consumption of plain yoghurts registered a significant decrease (P≤0.05) in serum GSH from 23.0 to 19.0 µM, while green and black tea yoghurts registered significantly higher P≤0.05 serum and liver GSH (20.0-33.0µM) implying a boost in antioxidant capacity. Plain and tea fortified yoghurt ingestion increased serum albumin (1.6 µL for negative controls against 2.5-3.0µL for the fortified groups) and liver total protein (2.5 g/dL for negative controls against 2.5-4.0g/dL for the fortified groups), implying that the nutritional benefits conferred by milk proteins in yoghurt were not adversely affected by the tea phenols.

**Conclusion:** Tea can be used for the production of fortified yoghurts rich in antioxidants thus improving the functionality of yoghurts.

**Keywords:** Albumin, Glutathione, Polyphenols, Antioxidants, Tea, Yoghurts, Serum, Liver.

**Key Messages**

- Tea polyphenols can be used to impart antioxidant health benefits to yoghurts.
- At concentrations of 1-4g/250mL tea polyphenols do not suppress the yoghurt microorganisms, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* growth to below the set microbial standard load of between 10⁶and 10⁸cfu/g.
- Anti-oxidant biomarkers in mice where enhanced upon consumption of tea fortified yoghurts implying imparted health benefits to the animals.

What is already known about this subject?

Polyphenols in tea can be used to fortify foods and enhance health benefits. Various food products can be fortified with tea and tea extracts and thus add health aspects desired in health products.

What does the study add?

The study investigates the effects of ingesting tea fortified yoghurts on the antioxidant capacity of mice against controls with no fortification. The results demonstrated the resulting benefits of enhanced body antioxidants from yoghurt fortification with tea.

How might this impact on clinical practice?

Food fortification with tea and other components that boost nutritive and functional status of subjects will be encouraged as a way of enhancing healthy living. This way prevention of diseases will be improved as opposed to curative means of eradicating diseases.

What is purple tea?

Purple tea is a new variety with anthocyanins in the leaves as depicted by the purple...
Introduction

Consumers are becoming more conscious of the quality of the food that they eat and drink. The food and beverage industries have in turn responded to the changes in consumer preferences by enhancing the nutritive value of their products through incorporation of natural health-promoting substances [1-3]. Herbal and botanical agents have elicited considerable interest in food fortification including tea polyphenols [4,5]. In recent years, tea is increasingly being used as a health drink following a growing number of scientific investigations that have associated its polyphenols with important health enhancing properties [6-9]. Biological activities of tea has traditionally and widely been attributed to its anti-oxidant properties and subsequently, its modulative effects against a host of medical conditions sharing oxidative stress as a common denominator [10]. However, drinking tea alone may not provide sufficient levels of polyphenols to achieve the health benefits associated with these biomolecules. Therefore, fortifying food products has been suggested as an alternative way of enhancing intake of tea polyphenols in order to more reliably attain the attributed medicinal value of tea [11]. This research focused on tea fortification with yoghurts as an alternative format for incorporating tea in food and imparting its inherent health benefits.

Yoghurt is a popular dairy product all over the world. It is prepared by bacterial fermentation of milk [1]. Its health effects range from improvements in lactose intolerance and gastrointestinal functions, immunostimulatory, hypcholesterolemic and weight loss effects [12]. The effects accrue from original yoghurt nutrients and the beneficial effects of starter cultures. Recent research has demonstrated that yoghurts with enhanced antioxidants confer added health benefits to consumers when compared to the ordinary yoghurts [13,14]. Notably, yoghurts fortified with tea displayed enhanced antioxidant capacities in-vitro as well as a higher level of starter culture during cold storage [15]. This notwithstanding, it remains uncertain whether this promising antioxidant capacity from in-vitro studies can be sustained in-vivo. Moreover, given the known interactions of milk proteins with polyphenols, it remains to be determined whether the nutritional benefits conferred by the milk proteins in yoghurt are adversely affected from such an interaction in-vivo [16,17]. To test this hypothesis, the present investigation attempted to produce yoghurts fortified with tea and assess their antioxidant and nutritive capacities using a mouse model.

Material and Methods

Tea Samples

The raw materials used in the manufacture of the teas for yoghurt fortification were obtained from the Kenya Agricultural and Livestock Research Organization, Tea Research Institute (KALRO-TRI), Timbilib Estate in Kericho (latitude 0°22S, longitude 35°21E, altitude 2180 m a.m.s.l.). Non-aerated green and aerated black teas were processed from the tea cultivar TRFK 6/8, while the non-aerated purple tea was processed from the purple leaf coloured cultivar TRFK 306. The purple coloured cultivar TRFK 306 is a putative interspecific natural hybrid between Camellia irrawadiensis (variety TRFK 91/1) and an unknown tea cultivar (Camellia sinensis) and is characterized by high levels of anthocyanins [14,18].

Processing of Tea for Yoghurt Fortification

Freshly harvested young tender shoots comprising of two leaves and a bud were used to process the non-aerated and aerated teas. For the manufacture of non-aerated tea, leaves were steamed (Philips HD9120, China) at 90°C for 1 min, macerated using a Cut, Tear and Curl (CTC) machine (Williamson Tea, UK) and dried at 120°C (Tea Craft Fluid Bed Drier, England) for 30 min to attain a moisture content (MC) of 3-4% [19]. Aerated black tea processing involved withering (two leaves and a bud) for 18 h to a moisture content of 50 and 65%MC, maceration using CTC machine, aeration for 90 min at 22°C (Aeration cabinet, Tea Craft, Bedford, UK) and drying at 120°C (Tea Craft Fluid Bed Drier, England) to 3-4% MC [20].

Yoghurt Development

Raw Materials: The ingredients used for the plain and tea fortified yoghurts included aerated black tea and non-aerated green and purple teas processed from Kenyan tea varieties (TRFK 6/8 and TRFK 306) incorporated at 0g (0% w/v), 1g (0.4% w/v), 2g (0.8% w/v), and 4g (1.6% w/v) per 250 mL of milk. Milled white cane sugar (Munias Sugar Company Limited, Kenya) at 6.5% w/v, whole milk (250mL Gold crown Pasteurized Fresh milk from Kenya Cooperative Creameries (KCC) Ltd, Nairobi, Kenya), yoghurt starter culture (2.0% w/v) Streptococcus salivarius ssp. thermophilus and Lactobacillus delbrueckii ssp. bulgaricus mixed at a ratio of 2:1 (S. salivarius subsp. thermophilus and L. delbrueckii subsp. bulgaricus Thermophilic Yoghurt Culture-YoFlex®, Freeze-dried Lactic Culture for Direct Vat Set (DVS) YF-L812 Freeze – dried 500u, CHR hansen, (Québec) CANADA) and skimmed milk powder (Kenya Cooperative Creameries (KCC) Ltd, Nairobi, Kenya- skimmed milk powder) at 2.5% w/v.

Development and Storage of Yoghurts: Yoghurt development was carried out aseptically at the food processing and value addition laboratory unit of KALRO-TRI, as described elsewhere [21-23]. Fresh pasteurized milk was homogenized by heating and stirring to 90°C for 3 min before the incorporation of teas and other ingredients. A further boiling for 10min was done after the addition of aerated black and non-aerated green and purple teas, sugar and skimmed milk powder. Thereafter the mixture was filtered, cooled to 45°C and inoculated with the mixed yoghurt starter culture. Incubation was subsequently done for 4-9h at 45°C. The end products were cooled and stored at 4°C. Each set of yoghurt had a control without tea and was prepared in triplicate.
Experimental Animals

All experimental procedures and protocols involving mice strictly adhered to protocols approved by Institutional Animal Care and Use Committee (IACUC) of the National Museums of Kenya-Institute of Primate Research (NMK-IPR). A permit was acquired (Permit number ICR/13/12) from NMK-IPR Karen, Kenya, prior to the start of the research. A total of 55, eight weeks old female and male adult Swiss white mice weighing between 26-36g were obtained from IPR rodent breeding colony and used in the research. The animals were housed in groups of 5 (separated according to sex) under conventional animal housing conditions within standard mice cages at a temperature range of 21-28°C. They were provided ad libitum access to water and standard mice cubes obtained from Unga Feeds Ltd Kenya. Sterile wood-chippings were provided as bedding material. As a precautionary measure all mice were treated with 0.02mL Ivermectin (Ivermectin®, Anupco, Suffolk, England) injected subcutaneously for protection against internal parasites. Carbon dioxide (CO₂) gas was used to euthanize the animals at the end of the experiment as described by Close et al. [24].

Experimental Design

Test Animals: Prior to the start of the experiment, the study was blinded by random selection and allocation into groups of mice of similar sex, where each mouse served as a replicate in a completely randomized design (CRD).

Test products: A total of 55 mice were subdivided into 11 groups of 5 mice each including: (1) Group 1- controls on mice cubes and water only (the negative control group). (2) Group 2- controls fed on mice cubes, water and plain yoghurt (the positive control group). (3) Group 3- fed on mice cubes, water and black tea fortified yoghurt at 1g/250mL. (4) Group 4- fed on mice cubes, water and black tea fortified yoghurt at 2g/250mL. (5) Group 5- fed on mice cubes, water and black tea fortified yoghurt at 4g/250mL. (6) Group 6- fed on mice cubes, water and green tea fortified yoghurt at 1g/250mL. (7) Group 7- fed on mice cubes, water and green tea fortified yoghurt at 2g/250mL. (8) Group 8- fed on mice cubes, water and green tea fortified yoghurt at 4g/250mL. (9) Group 9- fed on mice cubes, water and purple tea fortified yoghurt at 1g/250mL. (10) Group 10- fed on mice cubes, water and purple tea fortified yoghurt at 2g/250mL. (11) Group 11- fed on mice cubes, water and purple tea fortified yoghurt at 4g/250mL.

Administration of Test Products: Mice cubes and water were administered ad libitum to the test mice during the experimental period. The test products included plain and tea fortified yoghurts (black, green and purple teas at 1g, 2g, and 4g/250mL) were administered orally at a dosage of 1mL per mouse after every second day using a gavage needle. The administration time was around 9.30AM in the morning at every dosage session. Administration of the test products was carried out for 28 days during which mice were monitored for changes in body weight (bwt) and packed cell volume (PCV). The experiment was terminated through euthanasia 24 h post the last dosage. Liver samples were obtained and whole blood drawn via cardiac puncture, serum separated and stored at -80°C until required for analysis.

Determination of Food Value, Antioxidant Capacities and Polyphenol Contents of Yoghurts

Proximate composition of yoghurts including carbohydrates [25], moisture [26], fats [26], proteins [26], ash [26] and fibre [26] were assayed. Water soluble Vitamin B1 and fat soluble Vitamin A were assayed according to AOAC methods [26]. Antioxidants were assayed by the method described by Yen and Duh [27] while polyphenols were determined by the method described by ISO [28].

Packed Cell Volume (PCV) and Body Weight (bwt)

At one week interval, PCV was determined as per the method by Hoff [29]. Body weight of each mouse was determined every 2 days using an electronic analytical balance (Mettler PM2000 balance, Ohio, USA) as described by Rashid et al., [8].

Liver and Blood Sample Preparation

Frozen whole livers were homogenized at 4°C (in ice) in a buffer containing 0.25M sucrose, 5mM Hepes-Tris (pH 7.4), 1mM ethylenediaminetetraacetic acid (EDTA) with protease inhibitor cocktail to a final concentration of 10% (w/v) using a tissue homogenizer (Stuart homogenizer SHM2/UK, Bibby Scientific Limited, USA) as per the method by Tripathi and Srivastav [30]. The homogenate was aliquoted and stored at -80°C until required for analysis. Approximately 1mL of whole blood drawn in falcon tubes (1mL) was left to stand for 1 h at room temperature in an upright position for clotting to occur. The crude serum sample was then transferred to 1.5mL microfuge tubes and centrifuged (Heraeus Labofuge 400R, Hanau, Germany) for 15min at a speed of 10000g. Serum was then aliquoted into 1.5mL microfuge tubes and immediately stored at -80°C until required for biochemical analysis [31].

Glutathione (GSH) Assay

Glutathione assay was performed as described by Rahman et al., [32] with slight modifications. A volume of 200μm/L of GSH standard solution was prepared in 0.5% sulphasalicylic acid (SSA) and serial dilutions made using the same solution (0.5% SSA) to final concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78μm/L. Ellman’s reagent (5,5’-Dithiobis 2-nitrobenzoic acid (DTNB)) was prepared by dissolving in 0.1M potassium phosphate buffer with 5mM EDTA disodium salt, pH 7.5 (KPE buffer) to final concentration of 0.6mg/mL. A volume of 50μL of liver homogenate or serum was mixed with 50μL solution containing 5% SSA and 0.25mM EDTA. This mixture was centrifuged at 8000g at 4°C for 10 min and 25μL of the supernatant loaded on a 96-well microtitre plate in triplicate. Approximately 25μL of each standard and a blank were loaded to the remaining wells. To each well, 100μL of freshly prepared DTNB was then added and the absorbance measured at 405nm in intervals of 30sec using a multi-detection microtitre plate reader (DYNEX MRX, Vancouver, USA). Measurements from the series of GSH standard dilutions (100-0.78μm/mL) prepared earlier were used to construct a standard curve from which experimental values were interpolated.

Biochemical Analyses

A clinical biochemical analyzer (Humalyzer 2000, Wiesbaden, Germany) was used to analyze serum samples and liver homogenates for the estimation of total proteins, albumin...
and alkaline phosphatase using commercial reagent kits (Human Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions [31].

Data Analysis

Data was analyzed using Prism Graph-pad version 5.0 and P≤0.05 considered as being statistically significant. Significant differences between means for glutathione (GSH), total proteins, albumin and liver alkaline phosphatase levels was determined by one way ANOVA and Turkey post hoc test were performed to evaluate differences among group means. Graphs were plotted to show the trend of the various response variables [33]. The data was expressed as mean ± SEM (standard error of the mean).

Results

Food Value Antioxidant and Polyphenol Values of the Developed Yoghurts

Food value results for the developed yoghurts including carbohydrates, proteins, ash, minerals, fats among others have been given in Table 1.

The food values of the yoghurts were not significantly different (P≤0.05) between plain and fortified yoghurts from black, green and purple teas. However significant differences (P≤0.05) were registered on their polyphenol and antioxidant values as recorded in Table 2.

Effects of Tea Fortified Yoghurts on Packed Cell Volume (PCV) and Body Weight (bwt) of Mice

Packed cell volume and body weights measured prior to the start of the experiment were used as base line data. Mice supplemented with yoghurts fortified with black tea at, 4g/250mL recorded the greatest decrease in PCV which dropped from 62.8% to 56.6% ten days post start of yoghurt administration (Figure 1A). There was a slight increase at 7 days thereafter reaching 59.0% which was however followed by a drop to 53.6% at the end of the experiment period. The group at 2g/250mL recorded a steady increase in PCV immediately after administration and progressed until the third day rising from 56.2% to 63.4%, followed by a drop to 55.8% by day 10. A marginal increase of 57.0% was recorded before a fall to 53.0% by the end of the experiment. At 1g/250mL an increase of 62.0% was reached by the third day of administration from 57.2% four days prior to start of yoghurt supplementation.

Decrease in PCV was evident with green tea yoghurts at 4 and 1g/250mL till the end of the experiment (Figure 1B). Animals administered green tea fortified yoghurts at 2g/250mL recorded an initial decline in PCV from 64.6% to 54.8% by the third day followed by a steady rise at day 7 before another drop until the end of the experiment. The PCV of mice supplemented with yoghurts fortified with purple tea are presented in Figure 1C. At 4g/250mL, mice registered a progressive drop in PCV from the start to the end of the experiment. Groups of 2 and 1g/250mL recorded an initial drop in PCV from 63.6% and 63.8% to 54.6% and 53.6% respectively by the day 10 post start of yoghurt administration. Both groups recorded steady increments at day 7 before recording progressive drops until the end of the experiment.

All groups supplemented with plain or tea fortified yoghurts registered a drop in body weight (bwt), with only two exceptions of green tea and purple tea yoghurts at 3 and 4g/250mL respectively. Animals supplemented with black tea yoghurts lost bwt from the start to the end of the experiment with periodic fluctuations (Figure 2A). Mice supplemented with green tea yoghurts at 2g /250mL recorded a steady decrease from the

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<th>Table 1: Proximate composition of tea fortified yoghurts made from black green and purple tea.</th>
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<td><strong>Yoghurt Type</strong></td>
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Value= Mean ± S.D on dry weight basis. Each value is a mean of 3 replicates. Means of the same parameter followed by the same letter are not significantly different (P=0.05). S.D=Standard deviation. LSD= Least significant difference (P≤0.05). CV =Coefficient of variation (%). Vit.A=Vitamin A. Vit.B=Vitamin B. CHOs=Carbohydrates
start to day 11, dropping from 31.1g to 26.4g. This was followed by a progressive increase reaching 29.6g translating to a 5.0% drop by end of the experiment. Green tea at 4g and 1g/250mL groups registered a drop of 1.2% and an increase of 6.6% in bwt respectively by the end of the experiment (Figure 2B). The purple tea yoghurts group at 1g and 2g/250mL resulted in marginal decreases in bwt. A different scenario was observed in the 4g/250mL group which recorded a 5.5% increase in bwt by the end of the experiment period (Figure 2C).

Effects of Tea Fortified Yoghurts on Total Proteins in Serum and Liver

Total serum proteins were comparable between mice supplemented with both plain and tea fortified yoghurts and water only for the placebo group (Figure 3A). However, there was a significant reduction (P≤0.05) in total serum protein in mice supplemented with tea fortified yoghurts with green and purple teas at 2g and 1g/250mL rates respectively. No significant differences (P≤0.05) were observed between the plain and placebo yoghurts.

Completely different trends in total proteins were observed in liver homogenates when compared to the serum samples (Figure 3B). Both plain and tea fortified yoghurts; with the exception of black tea fortified yoghurt (4g/250mL) caused a general increase in liver total proteins though not significantly (P≤0.05). However, purple tea fortified yoghurts (4g/250mL) caused a significant (P≤0.01) increase in liver total proteins compared with the water only group. No significant differences (P≤0.05) were recorded between plain and other types of tea fortified yoghurts.

Effects of Tea Fortified Yoghurts on Albumin in Serum

Both plain and tea fortified yoghurts induced significant (P≤0.05) increase in serum albumin in the plain yoghurt groups compared to animals supplemented with water only (negative control) (Figure 4). Serum albumin levels were also significantly higher (P≤0.05) in animals supplemented with yoghurts fortified with black and purple teas at 1g/250mL compared to the water only group (negative controls).

Effects of Tea Fortified Yoghurts on Liver Alkaline Phosphatase (ALP)

There were no significant differences (P≤0.05) in liver alkaline phosphatase (ALP) levels between animals supplemented with plain and tea fortified yoghurts and the placebo group on water only (Figure 5). Moreover, no significant differences (P≤0.05) were observed between animals administered plain yoghurts and those administered black, green or purple tea fortified yoghurts. However, experimental animals supplemented with either plain or tea fortified yoghurts recorded slight but insignificant increments in liver ALP levels vis-à-vis the placebo group (P≤0.05).

Effects of Tea Fortified Yoghurts on Glutathione (GSH)

Serum GSH were significantly reduced (P≤0.05) in mice supplemented with plain yoghurts when compared to the water only control group (Figures 6A and 6B). However, fortification of yoghurts with tea restored serum GSH levels to within the range recorded for healthy control animals on water only in the respective groups. Experimental animals supplemented with green tea yoghurts at all the concentrations (0g, 1g, 2g and 4g/
250mL) recorded significantly higher (P≤0.001) GSH levels when compared to both the plain and the placebo groups. Black tea yoghurts group at 4g/250mL recorded significantly (P≤0.001) higher serum GSH compared to plain and water only groups. Yoghurts fortified with black tea at 2g/250mL significantly raised liver GSH (Figure 6B) when compared to mice supplemented with plain yoghurts (P≤0.01) or the placebo group (P≤0.001).

Discussion

The study revealed that the consumption of plain yoghurts resulted in a significant decrease in serum GSH when compared to the water only group. These results differed with previous studies which reported the ability of yoghurts and fresh milk to up-regulate cellular GSH levels [34-36]. However, from the findings, it can be noted that there could be some interaction of milk moieties with thiols in blood making them unavailable. Consistent with this, an earlier study had established that during milk pasteurization, micro-organisms and enzymes are denatured leading to an increase in yoghurt viscosity. Conformational changes expose the reactive thiol group on β-lactoglobulin of milk whey protein to form disulphide links with other thiol groups [37]. The data also revealed that animals supplemented with tea fortified yoghurts recorded higher serum GSH compared to plain yoghurt groups. Indeed, animals supplemented with green tea yoghurts at all concentrations and black tea at 4g/250mL resulted in a significant increase (P≤0.05) in serum GSH compared to plain yoghurts or water only groups, implying a boost in serum antioxidant capacity.

These findings agree with investigations of the effects of tea polyphenols from Kenyan tea cultivars which reported the ability of tea phytochemicals to boost GSH in the brain [8] and kidneys [8] of mice. Moreover, green tea has also been shown to enhance the nutritive value of other foods. Fortification of bread with green tea for example has been reported to sufficiently ameliorate oxidative stress against renal failure in rats [39]. The main bioactive molecules of tea are the polyphenols [powerful
antioxidants) which when consumed, may act as free radical scavengers squelching endogenous free radicals generated through metabolism [40,41]. However, the importance of tea in enhancing protection against oxidative stress goes beyond simply free radical scavenging and has also been attributed to its ability to amplify the activity of most detoxifying enzymes such as glutathione peroxidase and glutathione reductase [6].

Results from this study also indicate that both plain and tea fortified yoghurts increased serum albumin which corroborates findings of earlier studies [42-43] which demonstrated that fermented milk fortified with probiotics improved nutritional status among the elderly as had been demonstrated by an increase in serum albumin. Low serum albumin is as a marker of malnutrition and is associated with higher mortality, morbidity and poor outcome in a range of medical situations [44]. These findings therefore suggest an improvement in the nutritional status of experimental mice supplemented with tea fortified yoghurts. The results also indicate that animals supplemented with either plain and tea fortified probiotic yoghurts had slightly higher liver total protein levels compared to animals supplied with water only. Oral intake of yoghurt and milk proteins in general exposes the liver to high levels of amino acids. Considering that protein synthesis across the splanichic bed is highly sensitive to amino acid availability [45], there is a likelihood that this phenomenon could have stimulated hepatic protein synthesis and hence resulted in higher liver protein levels. The results further indicated that liver ALP levels were comparable between animals supplemented with both tea fortified and plain yoghurts and water only. Alkaline phosphatase is a useful marker in assessing liver functionality; increased levels indicating a higher burden on
the liver in terms of detoxifying consumed toxic substances or even acute liver damage. Therefore these results can imply that the tea fortified yoghurts were well tolerated by the mice and no signs of hepatotoxicity were apparent.

Conclusions and Recommendations

The findings of this study suggest that consumption of black, green or purple tea fortified yoghurts compared to a non-fortified equivalent food boosts innate anti-oxidant capacity in mice. A confirmation of the findings using higher mammals such as non-human primates, the use of tea to produce novel "functional" yoghurts and other dairy products could be justified.

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Contributorship Statement

Ochanda SO- Was responsible for the overall content of the article, he participated in developing the research work and design. The development of the tea fortified products, conducting of animal research work and writing of the research article.

Rashid K- Participated in the development of the contents of the article and conducted the animal model studies.

Wanyoko JK- Participated in the development of the research project, planning of the work and proofreading the content of the work.

Faraj AK- Participated in the development of the research project and responsible for seeking funds and proofreading the contents of the article.

Onyango CA- Participated in the development of the research project, was responsible for the development of content and proofreading of the article.

Ngotho M- Was responsible for seeking of the permit of the work on mice model, provision of laboratory facilities for conducting the animal research work and proofreading the content.

Maranga DN- Was responsible for conducting the mice research work, reporting and proofreading of the research article.

Wachira FN- Was responsible of overall coordination of the work between the various institutions involved in the project and proofreading the article.

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