Effect of camel milk and pistachio-based product on cancer-induced sarcopenia in Sprague Dawley rats

Asna ZAHID1, Tahir ZAHOOR1, Aysha SAMEEN2*, Muhammad Naeem FAISAL2

Abstract
In the current study, we investigated the role of camel milk and pistachio in the muscle growth of rats with cancer-induced sarcopenia. Female Sprague Dawley rats weighing 180-200 grams were selected for a bioefficacy trial for 13 weeks and divided into four groups: G1 (negative control), G2 (positive control), G3 (normal diet + camel milk), and G4 (normal diet + camel milk and pistachio based product). Tumors were induced in rats by administering 50 mg/kg of 7,12-dimethylbenz(a)anthracene via gastrogavage followed by chemotherapy in G2, G3, and G4. Histopathological analysis, muscle strength analysis, muscle fiber histology and renal function tests were performed. The administration of camel milk and pistachio-based product (CMP) significantly (P < 0.01) improved muscle strength in the treatment group. Muscle mass analysis of the extensor digitorum longus (EDL) and soleus muscle showed significant (P < 0.01) improvement in the treatment group. Muscle fiber diameter also significantly (P < 0.01) increased in the treatment group. Camel milk and pistachio-based product (CMP) have shown promising effects against cancer-induced sarcopenia in rat modelling by enhancing muscle function and strength.

Keywords: cancer; sarcopenia; muscle mass; camel milk; pistachio.

Practical Application: In the current study, a product comprising camel milk and pistachio was developed to ameliorate cancer-induced sarcopenia. The product improved indicators of sarcopenia including muscle strength, muscle mass and muscle fiber diameter in rats. It can be used as an effective strategy to prevent and treat sarcopenia in cancer patients. Further research can be conducted to improve indicators of sarcopenia and explore the mechanisms involved in its action.

1 Introduction
Sarcopenia is the loss of skeletal muscle mass, low physical performance and decreased muscle strength (Cao et al., 2022). In sarcopenia, muscles depict a reduced number of satellite cells (stem cells) and myofibers are terminally differentiated. Fibrotic tissues replace normal muscle fibers, resulting in increased muscle deterioration (Li et al., 2022). Sarcopenia can occur under various conditions, including diabetes, obesity, growth hormone deficiency, cirrhosis, congestive heart failure, rheumatoid arthritis, cancer, and certain cancer medications (Petermann-Rocha et al., 2022).

Sarcopenia is predominantly observed in cancer patients. Tolerance to surgery, radiation, and chemotherapy decrease because of compromised muscle mass. At cancer diagnosis up to 46-49% of patients suffer from sarcopenia (Bentahila et al., 2023). Variability exists in the prevalence of sarcopenia among patients with cancer depending on the type of cancer, metastasis (if present), disease stage, and diet quality. In the early stages of cancer, sarcopenia depends on the type of cancer, the type of treatment, and whether the patient underwent surgery (Zhu et al., 2022). Studies suggest that food and food-related factors act as therapeutic agents to treat sarcopenia by controlling protein synthesis and degradation (Pötsch et al., 2020).

Milk has a great dietary and nutritional characteristic (Mohamad et al., 2020, 2021; Ahmad et al., 2019) while camel milk is rich in protein and it contains a high concentration of branched-chain amino acids, i.e., leucine, isoleucine, and valine (Alhaj, 2020; Shakeel et al., 2022). Consumption of branched-chain amino acids increases muscle protein synthesis by enhancing the recycling efficiency of amino acids. Moreover, they suppress protein degradation possessing a positive effect on muscle regeneration (Wolfe, 2017).

Pistachio (Pistacia vera L.) is a nutrient-dense dry fruit, a good source of Mn, Cu, Mg, and vitamins A, B and C; contains 20% of plant protein and in particular branched-chain amino acids (Bulló et al., 2015; Hassan et al., 2022). Pistachio contains a high amount of potassium (1025 mg/100 g) (Mateos et al., 2022). Potassium has an anabolic effect on skeletal muscle mass. It lowers inflammation and insulin resistance. Insulin resistance and inflammation cause proteolysis, leading to muscle atrophy. Thus, potassium causes the regeneration of muscles reducing muscle atrophy (Lee et al., 2020).

Adverse effects of the disease itself and its treatment make the quality of life vulnerable. Sarcopenia affects a wide range of cancer patients making it a challenge for the person to recover adequately. In the current research, camel milk and pistachio owing to their composition was studied for cancer-induced sarcopenia. Camel milk due to its amino acid composition and...
pistachio due to its high potassium content were chosen and a product was prepared. Camel milk alone and in combination with pistachio was given to tumor-bearing rats to analyze the status of sarcopenia. It is hypothesized that camel milk and pistachio-based product (CMP) possesses positive effect on those suffering from cancer-induced sarcopenia.

2 Materials and methods

2.1 Procurement of raw material

Camel milk from "dromedary" camels was procured from a private dairy farm in Pattoki, Pakistan. Milk was collected in sterile bottles and transported to the laboratory in cool boxes. Non-roasted pistachios (P. vera L.) were purchased from the local market in Faisalabad, Pakistan. All chemicals required were supplied by Sigma and Merck, Germany. Sprague Dawley rats for the bio efficacy trials were acquired from the University of Veterinary and Animal Sciences, Lahore, Pakistan and kept in the animal room of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan.

2.2 Preparation of product

Raw pistachio kernels were ground into powder, and camel milk was pasteurized at 65 °C for 40 min. Camel milk and ground pistachio nuts were combined to form a product (CMP). Considering the amino acid and mineral requirements of rats as described by Benevenga et al. (1995), 80% camel milk and 20% pistachio powder were combined (Seleet et al., 2016).

2.3 Compositional analysis of camel milk and CMP and caloric count of CMP

Physicochemical analyses of milk were performed following the method of Association of Official Analytical Chemists International (2006). It included the analyses for pH, total solid, non-fat solid, protein, fat, and lactose. All tests were performed in triplicate. Proximate analysis of CMP including crude protein, moisture, crude fiber, crude fat, and carbohydrate was analyzed following the methods described by American Association of Cereal Chemists (2011). All tests were performed in triplicate. Caloric count/energy was calculated following the method described by Hunt et al. (1987) using the following Equation 1:

\[
\text{Energy (kcal)} = (\text{protein (g) x 4}) + \\
(\text{total carbohydrate (g) x 1.1 x 3.75}) + \text{fat (g) x 9}
\]  

\[1\]

2.4 Amino acid analysis

Amino acid analysis of the camel milk, CMP and feed was performed following the method described by Azam et al. (2019). The sample was estimated on a dry-matter basis. For this purpose, 50 mL of each sample was frozen at –80 °C for 24 h. The sample was then freeze-dried for 24 h to obtain a dry powder. The amino acid analyzer used was Biochrom 30+ (Biochrom Limited, Cambridge, UK). Five hundred microliters of each freeze-dried sample were oxidized using performic acid. The samples were hydrolyzed using 6 M hydrochloric acid/phenol for 24 h, and the pH of the samples was maintained at 2.2. The samples were filtered and poured into vials to determine the amino acid content using Biochrom 30+ using ion exchange chromatography.

2.5 Mineral content

The mineral contents of camel milk and CMP were determined following the method of Association of Official Analytical Chemists International (2006). Samples (0.5 mL) were digested using 7:3 HNO₃ and perchloric acid on a hot plate until a light green solution was obtained. For mineral analysis, the digested samples were diluted to 100 mL. Calcium, potassium, and sodium levels were calculated using the Flame Photometer 410 (Sherwood Scientific Ltd., Cambridge, UK), whereas copper, iron, and zinc levels were quantified using an atomic absorption spectrophotometer (Varian AA240, Australia).

2.6 Bioefficacy trial

A bioefficacy study was conducted after approval from the Institutional Biosafety and Bioethics Committee (certificate number: 4980/ORIC), and all bioethics protocols were followed during the study. Thirty-two Sprague Dawley rats weighing 180–200 g was kept in aluminium cages (4 rats/cage) in the animal room of the National Institute of Food Science and Technology, UAF, at room temperature (27 ± 2 °C) in a 12/12 dark to light cycle. The rats were acclimatized for 1 week before the experiment. The study duration was 13 weeks (91 days).

2.7 Tumor induction

A single dose (50 mg/kg body weight of 7,12-Dimethylbenz (a)anthracene (DMBA) was used to induce tumors in the mammary glands of rats. DMBA was mixed with 1 mL of soy oil and intragastrically administered to animals via gavage, following the method described by Barros et al. (2004). Histopathological analysis was performed to assess tumor onset.

2.8 Experimental protocol

Rats were randomly divided into four groups comprising eight rats per group according to the following criteria:

- **Group one (G1):** Positive control group containing normal rats fed a normal diet orally [corn oil (10%), flour (82%), mineral mix (1%), vitamin mix (3%), and casein (4%) and tap water].
- **Group two (G2):** Negative control group containing tumour-bearing rats + administered chemotherapy along with normal diet and tap water orally.
- **Group three (G3):** Tumor-bearing rats + treated with chemotherapy and fed 2-mL camel milk daily in addition to a normal diet (orally) and tap water. The dose (camel milk) was administered according to the standard operating procedures described by Jones (2015) with gastrogavage (4.5 cm in length and 2.5 mm in diameter).
• Group four (G4): Tumor-bearing rats + treated with chemotherapy and fed 2-mL CMP daily in addition to normal diet (orally) and tap water. The dose (CMP) was administered according to the standard operating procedures described by Jones (2015) with gastrogavage (4.5 cm in length and 2.5 mm in diameter).

2.9 Chemotherapy

Rats received an intravenous injection of doxorubicin (2 mg/kg body weight) mixed with cyclophosphamide (20 mg/kg body weight) via the tail vein. Four cycles of (once a week) chemotherapy were administered from the 10th week of the study after completing the DMBA 9-week cycle (Fan et al., 2017).

2.10 Feed and water intakes and body weight

The total feed intake of rats was measured by subtracting the given feed from the remaining feed. In the same manner, the water intake of rats was measured daily by subtracting a given amount from the amount remaining in the bottles by following the method of Patel et al. (2013).

The weight of each rat was measured on a weekly basis using a weight scale following the method described by Stewart et al. (2019).

2.11 Histopathological analysis of mammary gland

After 9 weeks of DMBA administration, one rat from each group was decapitated to perform histopathology of the mammary gland using the method described by Feng et al. (2015). At the end of the trial (after 13 weeks), all rats were subjected to the histopathological analysis of their mammary glands. For this purpose, 10% chloral hydrate was administered intraperitoneally to induce anesthesia. The rats were decapitated and the mammary gland was resected. Samples of rat mammary glands were fixed in 10% formaldehyde for 15 days. Subsequently, the mammary glands were dehydrated using ethanol. The dehydrated mammary glands were embedded in paraffin and cut into 5-µm-thick sections. The sections were stained using hematoxylin-eosin stain, and observed under a light microscope.

2.12 Survival rate

Based on the number of deaths in each group, the survival rate was calculated from the 10th week of the study when deaths started to occur (Akhouri et al., 2020).

2.13 Muscle strength

Forelimb grip strength was measured using the method described by Bonetto et al. (2015). A commercially available grip strength meter (dynamometer) was used. Rats could hang on a wire mesh using their forelimbs and were pulled against the mesh by their tails. The peak resistance when the rat lost its grip on the wire was recorded using a force meter (expressed in g). The grip strength of each rat was measured in triplicate, and the average value was recorded. Before each measurement, two-min rest was provided to each rat.

The four-limb hanging strength test was performed according to the method described by Bonetto et al. (2015). In this test, rats were allowed to hang on a mesh, and their ability to hold their body weight as opposed to limb tension was measured. The timing of the hanging was checked using a stopwatch for each rat. The grid was maintained at a height of 35 cm, and the foam was placed underneath to protect rats from any injury. The test was conducted three times, and the longest duration was recorded. Two-minute rest was given before each repetition.

2.14 Muscle mass assessment

Muscle mass assessment of the rats was performed following the method described by Kemmochi et al. (2018). Overnight-fasted rats were decapitated, and their hind limbs were removed carefully without the skin to extract muscles. To ensure viability, the limbs were placed in a salt solution. The soleus and extensor digitorum longus (EDL) muscles were removed from the hind limbs, and tendons were cut by hanging the rat with a burette clamp. Subsequently, the removed muscles were weighed for muscle mass analysis.

2.15 Histological section of muscle fiber

Histological sections of the muscle fibers were prepared following the method described by Nemoto & Goyagi (2021). Rat EDL and soleus muscles were extracted. The muscles were kept in 10% formalin for 48 h and fixed in paraffin. A small cross-section of 5 µm was cut, stained with hematoxylin-eosin stain, and observed under a light microscope.

2.16 Biochemical parameters

Biochemical parameters were measured at the end of the study after rat decapitation and compared to those of the control.

Renal function tests

Serum and urinary creatinine levels were analyzed using the method described by Thammitiyagodage et al. (2020). For serum creatinine analysis, Jaffe solution (equal amounts of NaOH and picric acid) was prepared. Serum samples (1 mL) and standards (50 µL) were mixed with 1,000 µL of Jaffe solution and incubated at 25 °C for 30 s. The serum creatinine levels were also measured. Urine was collected in sterile plastic bottles and urinary creatinine was measured. The urine was mixed with distilled water at a ratio of 1:50. The resulting solution was mixed with Jaffe solution. The absorption of the reaction mix was measured using an auto-biochemical analyzer.

Liver function tests

Blood collected in vials were allowed to clot at room temperature for 10 min. Subsequently, the blood was centrifuged at 2,500 rpm for 20 min, and the obtained serum was used to estimate alanine aminotransferases (ALT), aspartate aminotransferases (AST), and alkaline phosphatase (ALP) levels, following the method described by El-Baz et al. (2014).
2.17 Statistical analysis

After the data were acquired for all parameters, statistical analyses were conducted to determine the level of significance. A completely randomized design (CRD) was used for the study using “Statistix 8.1” Statistical Package. The level of significance (P < 0.05 and P < 0.01) was determined by applying a two-factor factorial CRD to the bio-efficacy study using the method described by Montgomery (2017). Post-hoc Tukey’s honest significant difference test was used to compare the ranges of significance.

3 Results and discussion

3.1 Compositional analysis of camel milk and CMP

The mean values for the physicochemical analysis of camel milk and the proximate composition of CMP are listed in Table 1. Mean values for the physicochemical analysis of camel milk and proximate composition of the CMP were in synchronization with the results of Ismaili et al. (2019). According to them, raw camel milk had a pH (6.47 ± 0.08) whilst fats, proteins and total solids were 2.72 ± 0.64%, 2.55 ± 0.27% and 10.42 ± 1.04% respectively. Whereas Kola et al. (2018) found that pistachios contain 96.06% dry matter, 3.94% moisture, 3.13% ash, 27.45% crude protein and 7.63% dietary fiber.

The mean values for amino acid analysis of feed (normal diet), camel milk, and CMP are shown in Table 2. The mean value of leucine, isoleucine and valine in the product was 0.31 ± 0.021%, 0.14 ± 0.010% and 0.29 ± 0.008%, respectively. Branched-chain amino acids in camel milk investigated by He et al. (2019) showed similar results i.e., isoleucine 0.21 ± 0.05%, leucine 0.40 ± 0.09% and valine 0.25 ± 0.05%.

Table 3 presents the mean values for the mineral content of camel milk and CMP. The mean value of Potassium in CMP was 314.85 ± 2.12 mg/100 mg. The value of potassium in pistachio was investigated by 1025 mg/100 g. In the current study, CMP comprised 20 g/100 g pistachio and 80 mL/100 mL camel milk thus results were according to the values.

Table 1. Mean values for physicochemical analysis of camel milk and proximate composition of CMP.

<table>
<thead>
<tr>
<th>Compositional analysis of camel milk</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (%)</td>
<td>3.46 ± 0.127</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>2.98 ± 0.095</td>
</tr>
<tr>
<td>TS (%)</td>
<td>12.07 ± 0.158</td>
</tr>
<tr>
<td>SNF (%)</td>
<td>8.62 ± 0.104</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.82 ± 0.089</td>
</tr>
<tr>
<td>pH</td>
<td>6.58 ± 0.119</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compositional analysis of CMP</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/100 g)</td>
<td>167.88 ± 2.055</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>70.63 ± 0.47</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>10.87 ± 0.35</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>6.09 ± 0.23</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.33 ± 0.068</td>
</tr>
<tr>
<td>pH</td>
<td>6.78 ± 0.12</td>
</tr>
</tbody>
</table>

Values are taken as the average of three observations. TS: total solids, SNF: solid not fat.

3.2 Feed and water intake of rats

Feed and water intake both increased initially for all groups but later declined from the 8th week, except for the normal rats (G1) (Figure 1a-1b). The highest feed intake was seen in G1, which increased from 15.51 ± 0.668 g/day to 20.53 ± 0.796 g/day. Contrarily, feed intake decreased in G2, G3 and G4 over time by 60.7%, 42.2% and 31.3%, respectively. The decrease among all groups was highly significant (P < 0.01) from G1. Among treatment groups, G4 has increased feed intake from G2 by 30.21% (P < 0.01) and from G3 by 44.03% (P < 0.01). The difference between G3 and G4 was 19.79% (P < 0.05).

Water intake increased in G1 from 19.36 ± 0.873 mL/day to 24.66 ± 8.36 mL/day whereas it decreased in tumor-bearing rats.

Table 2. Mean values for the amino acid composition of the normal diet (feed), camel milk and CMP.

<table>
<thead>
<tr>
<th>Amino acids (DM basis %)</th>
<th>Feed</th>
<th>Camel Milk</th>
<th>CMP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential amino acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.031 ± 0.001</td>
<td>0.06 ± 0.003</td>
<td>0.06 ± 0.002</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.074 ± 0.001</td>
<td>0.53 ± 0.015</td>
<td>0.56 ± 0.010</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.38 ± 0.020</td>
<td>0.15 ± 0.010</td>
<td>0.11 ± 0.010</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.26 ± 0.04</td>
<td>0.12 ± 0.015</td>
<td>0.05 ± 0.005</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.065 ± 0.003</td>
<td>0.07 ± 0.008</td>
<td>0.05 ± 0.002</td>
</tr>
<tr>
<td><strong>Non-essential amino acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.80 ± 0.40</td>
<td>0.09 ± 0.009</td>
<td>0.16 ± 0.010</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.74 ± 1.02</td>
<td>0.08 ± 0.004</td>
<td>0.21 ± 0.010</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.4 ± 0.07</td>
<td>0.25 ± 0.021</td>
<td>0.27 ± 0.006</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.21 ± 0.04</td>
<td>0.04 ± 0.005</td>
<td>0.05 ± 0.003</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4.59 ± 0.240</td>
<td>0.66 ± 0.031</td>
<td>0.60 ± 0.010</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.98 ± 0.08</td>
<td>0.04 ± 0.003</td>
<td>0.08 ± 0.002</td>
</tr>
<tr>
<td>Proline</td>
<td>1.86 ± 0.2</td>
<td>0.68 ± 0.015</td>
<td>0.29 ± 0.010</td>
</tr>
<tr>
<td>Serine</td>
<td>0.60 ± 0.02</td>
<td>0.23 ± 0.010</td>
<td>0.18 ± 0.006</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.34 ± 0.05</td>
<td>0.05 ± 0.003</td>
<td>0.19 ± 0.005</td>
</tr>
<tr>
<td><strong>BCCA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.13 ± 0.004</td>
<td>0.28 ± 0.006</td>
<td>0.31 ± 0.021</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.1 ± 0.05</td>
<td>0.11 ± 0.005</td>
<td>0.14 ± 0.010</td>
</tr>
<tr>
<td>Valine</td>
<td>0.15 ± 0.02</td>
<td>0.23 ± 0.010</td>
<td>0.29 ± 0.008</td>
</tr>
</tbody>
</table>

Values are taken as the average of three observations. BCAA: Branched-chain amino acids; CMP: Camel milk and pistachio based product.

Table 3. Mean values for mineral analysis of camel milk and CMP.

<table>
<thead>
<tr>
<th>Minerals (mg/100 g)</th>
<th>Means ± S.D Camel milk</th>
<th>Means ± S.D CMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>106.67 ± 1.52</td>
<td>104.52 ± 1.13</td>
</tr>
<tr>
<td>Potassium</td>
<td>169.67 ± 4.5</td>
<td>314.85 ± 2.12</td>
</tr>
<tr>
<td>Sodium</td>
<td>63.33 ± 2.08</td>
<td>50.87 ± 1.66</td>
</tr>
<tr>
<td>Copper</td>
<td>0.17 ± 0.01</td>
<td>0.40 ± 0.009</td>
</tr>
<tr>
<td>Iron</td>
<td>0.24 ± 0.025</td>
<td>0.88 ± 0.023</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.34 ± 0.03</td>
<td>0.62 ± 0.022</td>
</tr>
</tbody>
</table>

Values are taken as the average of three observations.
rats G2, G3 and G4 from the 7\textsuperscript{th} week. The decrease in G2 was 32.84\%, in G3 the decrease was 23.56\% whilst in G4 the decrease was 17.06\%. The decrease among all groups was highly significant (P < 0.01) from G1. Among treatment groups, G4 has increased water intake by 11.11\% from G3 (P < 0.05) and 21.1\% from G2 (P < 0.01).

Decrease in feed and water intake resulted from cancer and chemotherapy-induced appetite suppression (Patel et al., 2013). The decrease in feed and drink intake showed a similar pattern and was the highest in G2 (control) whereas the lowest was in group given CMP. As suggested by Lueders et al. (2022) branched-chain amino acids regulate hunger by protein leveraging effect. Thus, adequate intake of branched-chain amino acids leads to less appetite suppression as compared to the control group.

### 3.3 Body weight of rats

The effects of cancer and treatment with camel milk and CMP on the body weight of rats are shown in Figure 1c. The body weight of G1 increased from $187.85 \pm 4.83$ g to $243.42 \pm 6.43$ g up to week 13. In tumor-bearing rats, body weight increased initially and later declined. In treatment groups, body weight declined by 2.3\% (G4) and 3.65\% (G3). G2 showed a drastic early decline in body weight from the 8\textsuperscript{th} week by 10.89\%. Results showed a significant decline (P < 0.01) in the weight of G2, G3 and G4 as compared to G1. Among treatment groups, the difference between the weight of G3 and G4 was 3.48\% (P < 0.05).

A less drastic decline was seen in G3 and G4 as compared to G2. Among tumor-bearing rats, a decline in body weight was seen due to disease-associated malabsorption and hypermetabolism (Chindapasirt, 2015). The less drastic decline in treatment groups was due to the presence of high Leucine content and insulin-like growth factor. Both these mediate growth-promoting effects on growth hormones causing weight gain (Kaskous & Pfaffl, 2017).

### 3.4 Histopathological analysis of mammary gland

Histopathological analysis of the mammary glands of rats in each group is shown in Figure 2. Histopathology showed cancer-related changes in the mammary glands in groups given DMBA i.e., G2, G3 and G4. Whereas in G1 normal mammary gland was visualized. Our findings were in accordance with Cahill et al. (2018) where malignant changes in the breast were seen with glandular structure infiltrates.
3.5 Survival rate

Survival rate was directly related to cancer induction and chemotherapy, as shown in Figure 1d. Mortalities started in the 10th week of the study, and data were collected from this point until the termination of the study. G1 showed a 100% survival rate. The minimum survival rate was 43% at the end of trial G2. The survival rate in G3 and G4 was 71% at the end of the study. There were no accidental deaths, and all fatalities were due to tumor-related complications.

Sarcopenia is directly associated with an increased death rate from cancer. Anjanappa et al. (2020) studied the effect of cancer and chemotherapy-induced sarcopenia on the overall survival rate of patients. Sarcopenia lowered the survival rate of patients (65.3%) in comparison to non-sarcopenic patients (79.9%) over three years.

3.6 Muscle strength

Forelimb grip strength reduced as the disease progressed in all tumour-bearing rats (Figure 3a). The highest forelimb grip strength was reported in G1 (393.71 ± 10.516 g) whereas G2 showed a significant reduction (P < 0.01) in grip strength (239.88 ± 12.616 g). Grip strength in G2 decreased by 31.46% (P < 0.01), G3 by 21.7% (P < 0.01) and G4 by 14.08% (P < 0.01). The highest decrease in grip strength was seen in G2 whereas the lowest decrease was seen in G4. A non-significant (P > 0.05) difference existed between G3 and G4.

The four-limb hanging strength test showed a pattern similar to that of forelimb grip strength (Figure 3b). The four-limb hanging strength was the highest in G1 as 320.43 ± 14.513 seconds and the lowest in G2 at 66.67 ± 7.506 seconds. Reduction in G2 was highly significant (P < 0.01) by 78.32%. Hanging strength also decreased in treatment groups G3 and G4 by 71.19% and 68.2%, respectively, but the decrease was less drastic as compared to G2. A non-significant (P > 0.05) difference existed between G3 and G4.

Muscle strength is a strong predictor of muscle mass, as a decline in muscle strength indicates a loss of lean mass with increased mortality (Cooper et al., 2013). In the current study,
muscle strength showed a decline in treatment groups due to progressing sarcopenia. Better outcomes were seen in the group given CMP and camel milk. Better outcomes can be attributed to increased muscle protein synthesis owing to the presence of branched-chain amino acids (Ko et al., 2020). Toledo et al. (2011) reported a significant reduction in forelimb grip strength of tumour-bearing rats compared to normal rats. Milk has a high protein content with minerals that stimulate muscle protein synthesis. Supplementation with 500 mL of whole milk per day provides 20 g of proteins essential for muscle protein synthesis and could improve grip strength in humans over 6 weeks (Granic et al., 2019).

3.7 Muscle mass assessment of EDL and soleus muscles

Skeletal muscle mass assessment is imperative for the diagnosis of sarcopenia. A reduced muscle mass indicates muscle atrophy (sarcopenia). In the EDL muscle, the highest muscle mass was seen in G1 as 89.23 ± 5.830 mg whilst the lowest muscle mass was observed in G2 as 58.42 ± 2.537 mg (Figure 4). G3 contained tumor-bearing rats with chemotherapy given camel milk along with a normal diet whilst group four (G4) contained tumor-bearing rats with chemotherapy given CMP. Two-way ANOVA followed by Tukey’s HSD multiple comparison tests indicated a highly significant difference (P < 0.01) among groups.

In the soleus muscle, muscle mass was the highest in G1 at 78.68 ± 1.615 mg whereas the lowest in G2 was 61.13 ± 1.775 mg. Among tumor-bearing rats, G3 and G4 showed increased (P < 0.01) muscle mass at 67.91 ± 5.376 mg and 72.64 ± 6.146 mg, respectively as compared to G2.

The muscle mass analysis showed an overall decline in muscle mass in the tumor-bearing rats. Rats receiving the CMP showed improvement in muscle mass as compared to other groups. Supplementation with protein-containing higher amounts of branched-chain amino acids shifted the net protein balance of the body from negative to positive, exceeding the rate of protein synthesis and improving muscle mass, strength, and function (Blomstrand et al., 2006).

3.8 Histological section of muscle fiber

Histological sections of the muscle fibers are shown in Figure 5. G1: parallel-running muscle fibers with normal diameters as 34.14 ± 1.528 μm. G2: Muscle fibers did not run in parallel. The reduction in muscle fiber diameter is seen as 22.33 ± 1.528 μm. Reduction in muscle fiber diameter is highly significant (P < 0.01). G3: The muscle fiber diameter of 27.60 ± 1.140 μm was slightly smaller than normal. G4: Less variation in muscle fiber diameter. The muscle fiber diameter was close to normal as 28.20 ± 1.924 μm. A non-significant (P > 0.05) variation was seen among G3 and G4.

Results depicted that the group given CMP has muscle fiber diameter closest to that of normal rats whereas in the control group drastic decrease in the diameter of muscle fiber was seen. The size of muscle fibers and spacing between fibers are among the foremost determinants of muscle mass and function. In sarcopenia, a reduction in the diameter of myofibers occurs.

Figure 3. (a) Forelimb grip strength of rats expressed in grams. (b) Four-limb hanging strength test of rats expressed in seconds. Group one (G1) consisted of normal rats with a normal diet. Group two (G2) comprised tumor-bearing rats with chemotherapy-fed normal diet. Group three (G3) encompassed tumor-bearing rats with chemotherapy given camel milk along with a normal diet whilst group four (G4) contained tumor-bearing rats with chemotherapy given CMP. Two-way ANOVA followed by Tukey’s HSD multiple comparison tests indicated a highly significant difference (P < 0.01) among groups.

Figure 4. EDL and soleus muscle mass of rats expressed in mg. Group one (G1) consisted of normal rats with a normal diet. Group two (G2) comprised tumor-bearing rats with chemotherapy-fed normal diet. Group three (G3) encompassed tumor-bearing rats with chemotherapy given camel milk along with normal diet whilst group four (G4) contained tumor-bearing rats with chemotherapy given CMP. Two-way ANOVA followed by Tukey’s HSD multiple comparison tests indicated a highly significant difference (P < 0.01) among groups.
with increasing variation in the size of myofibers in a single section. As muscle atrophy progresses, muscle fiber diameter decreases. Some fibers can be normal in a single section, whereas others can be affected (Schmidt, 2014).

### 3.9 Renal function test

The serum creatinine levels of the rats were measured to assess muscle function. Figure 6a shows that the serum creatinine level was the highest in G1 as $42.18 \pm 0.782$ µmol/L. In G2, the level of serum creatinine declined drastically ($P < 0.01$) to $37.29 \pm 0.979$ µmol/L. The value of serum creatinine in G3 and G4 was $38.72 \pm 0.707$ µmol/L and $40.91 \pm 0.489$ µmol/L, respectively. This indicated a significant increase ($P < 0.05$) in serum creatinine from G2 and a highly significant decrease ($P < 0.01$) from G1. The increase in serum creatinine in G3 from G2 was 3.69% ($P > 0.05$) whilst in G4 it was 8.84% ($P > 0.01$). An increase in serum creatinine in G4 from G3 was significant ($P < 0.01$).

The level of urinary creatinine (Figure 6b) was the highest in G1 as $90.14 \pm 7.198$ mg/dL, followed by G3 ($P < 0.05$) at $80.20 \pm 7.662$ mg/dL. The level of urinary creatinine in G4 was $78.40 \pm 4.722$ mg/dL.

G2 had the lowest urinary creatinine levels as $75.00 \pm 5.568$ mg/dL. The decrease in urinary creatinine in G2 from G3 ($P < 0.05$) was 6.4% whilst from G4 ($P < 0.05$) it was 4.33%. The result indicated that camel milk improved levels of urinary and serum creatinine. The difference between G3 and G4 was 2.24% ($P < 0.05$).

Serum and urinary creatinine levels are indicators of muscle function in the absence of kidney disease. Creatinine is a by-product of muscle metabolism. In the absence of renal disease, creatinine levels in the serum and urine are normal when the muscle mass is normal. In sarcopenia, urinary and serum creatinine levels decrease owing to a reduction in muscle metabolism (Tosato et al., 2017). Our results showed that serum and urinary creatinine levels in groups given CMP and camel milk were significantly higher as compared to the control. Zhang et al. (2017) investigated the serum and urinary creatinine levels in diabetic rats. Serum and urinary creatinine levels were higher in normal rats than in those with low muscle mass.

### 3.10 Liver function test

The liver is responsible for metabolism and detoxification of dietary components. Any condition affecting the liver elevates...
the enzyme levels in the liver. It was observed that the liver was damaged by chemotherapy-induced toxicity in tumor-bearing rats. ALT level was the lowest in G1 as 46.29 ± 1.976 U/L. ALT was most elevated in G2 by 38% of G1. The level of ALT in G3 elevated by 36% and in G4 by 33.49%. An increase in ALT in G2, G3 and G4 were significant (P < 0.01) from G1. Similarly, the AST level was the lowest in G1 as 118.00 ± 2.708 U/L whereas elevated in G2, G3 and G4. In G2, the AST level was elevated by 19.54%, in G3 by 16.31% and G4 by 15.1%. An increase in AST in G2, G3 and G4 were significant (P < 0.01) from G1. In the same manner, the level of ALP was 84.29 ± 2.563 U/L in G1 that elevated by 117.36%, 15.87% and 17.03% in G2, G3 and G4. The increase was significant (P < 0.01) from G1. The increase liver enzymes between G3 and G4 was non-significant (P > 0.05).

A non-significant difference existed among enzyme levels of G2, G3 and G4 that indicated camel milk and CMP does not possess any effect against chemotherapy-induced liver toxicity. The liver is damaged by chemotherapy-induced toxicity. Any condition affecting the liver elevates the enzyme levels in the liver. Owing to ethical constraints, liver function tests were performed (Rocha et al., 2019). The present results are in agreement with the findings of Rocha et al. (2019) analyzing the effect of chemotherapy on liver enzymes. A highly significant increase in liver enzyme levels was observed in rats treated with chemotherapy.

4 Conclusion
Our study showed that CMP may help to manage sarcopenia in cancer along with chemotherapy. During disease advancement and chemotherapy, the progression and severity of sarcopenia was the lowest in the group that was provided with CMP. The group given CMP showed improved skeletal muscle and neuromotor functions compared to the other groups. Thus, camel milk and pistachio-based product possess positive effects on cancer induced sarcopenia in rat modelling. It is recommended to promote awareness programs about the importance of camel milk and pistachio in the prevention of muscle wasting.

Conflict of interest
The authors declared no conflict of interest.

Funding
The research was not funded.

References

