Analytical and Biological Studies of Kanji and Extracts of its Ingredient, Daucus carota L.

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Summary: A fermented beverage, Kanji, prepared from roots of Daucus carota L. subsp. sativus (Hoffm.) Arcang. var. vavilovii Mazk. (Apiaceae), despite long usage history has not been investigated for analytical studies and biological activities. Therefore, the present study aimed to investigate different types of Kanji samples and various types of extracts/fractions of root of the plant for a number of analytical studies and in vitro antioxidant activities. The Kanji sample, Lab-made Kanji, having better analytical and biological profile was further investigated for preliminary clinical studies. The analytical studies indicated that Lab-made Kanji was having comparatively higher contents of phytochemicals than that of the commercial Kanji samples, different types of extracts and fractions (P < 0.05). All the Kanji samples and aqueous and ethanol extracts of fresh roots exhibited comparable antioxidant activities in DPPH assay (52.20 - 54.19%) that were higher than that of methanol extract (48.78%) of dried roots. The antiradical powers (1/EC50) of Lab-made Kanji samples showed higher activity than that of the methanol extract, but comparable to that of the vitamin-E and butylated hydroxyl anisole (BHA) (P < 0.05). A preliminary clinical evaluation indicated that Kanji has no harmful effect on blood components, liver function and serum lipid profile. The results of the present study indicate that Kanji is an effective antioxidant beverage.

Keywords: Kanji; Daucus carota L.; Apiaceae; Antioxidant activity; phytochemical constituents, Clinical evaluation

Introduction

Beside nutritional significance, vegetables due to their antioxidant properties can reduce the incidence of mortality rate of ailments involving oxidative stress such as cancer and cardio- and cerebro-vascular diseases [1]. Due to nutritional status, vegetables have varying popularity among people of different age groups throughout the world. Carrots are among those vegetables which are found and consumed in almost all parts of the world. On the basis of nutrition, these have been ranked as 10th among 39 fruits and vegetables as per the United States Agricultural Statistics [2]. The continuous research and proven health benefits of carrots are increasing their consumption and popularity day by day [3]. This vegetable has a number of cultivars – different colored carrots, subspecies and varieties, which are distributed in different parts of the world. Based on distribution, carrots are mainly grouped as Western carrots (yellow) and Eastern carrots (Black).

Daucus carota L. subsp. sativus (Hoffm.) Arcang (Black carrot) has six varieties; yellow (var. scharrovii Mazk.), violet (var. bissieerii Schweinf.), pink (var. rosseus Mazk.), orange (var. zhakovskii Setch.), white (var. albus Alef.) and black (var. vavilovii Mazk.). The roots of the variety vavilovii Mazk. are used to prepare a fermented beverage in some Asian countries particularly, Pakistan and India, whereby it is known as Kanji. It is a quite popular remedy for the treatment of indigestion, loss of appetite and liver disorders. It is prepared and used extensively as an appetizer in early summer season [4]. Since long, it is being prepared both in homes as a house-hold remedy and on a small industrial scale by road-side vendors. Despite such long usage history, it is neither characterized analytically nor evaluated biologically, which necessitates the investigation of this remedy to make it an evidence-based product.

The tuberous roots of black carrots, the main ingredient of Kanji, possess diuretic, digestive tract soothing, hepatoprotective and uterine stimulating properties [5, 6]. These are reported to have menstruation delaying and uterine contraction inducing effects [7]. The decoction of roots is efficacious in removing urinary tract stones [8]. The use of infusion of roots for treating edema, flatulence, indigestion and menstrual problems are other traditional claims [5].

The literature review indicated a number of pharmacological studies on roots of different cultivars of the plant. Two studies described the...
antioxidant activity of carrots using in vitro models [9, 10]. The seven different colored verities of carrots were biofortified to enhance their antioxidant potential, which was then evaluated using DPPH and (2, 2- azino-di [3-ethylbenzthiazoline-6- sulfonate] ABTS models [11]. Moreover, the juice of fresh roots had shown hepatoprotective activity in CCl₄ and lindane–induced intoxicated mice and rats [12, 13]. Surprisingly, all such reports are lacking in the correct identification of the subspecies or the variety used. Likewise, existing literature lacks reports on the chemical characterization and biological activity studies of Kanji.

Therefore, the present study aimed to investigate various types of Kanji samples, extracts and fractions of roots of Daucus carota L. subsp. sativus (Hoffm.) Arcang. var. vavilovii Mazk. for chemical characterization and antioxidant activity studies. It was further aimed to investigate well-characterized Lab-made Kanji in healthy individuals to find its effect on blood components, liver function and serum lipid profile. The findings of this study may provide analytical profiles prerequisite for standardization and scientific evidence to folklore claims of Kanji.

Results and Discussion

The results for the estimation of total contents of primary and secondary metabolites of different Kanji samples, extracts and fractions are given in Table-1. The linear regression equations used for the determining different groups of metabolites were as total protein (y = 1.2176x - 0.0031, R² = 0.9984), total polyphenols (y = 0.1520x - 0.0009, R² = 0.9996), total polysaccharides (y = 9.4258x - 0.011, R² = 0.9874) and total flavonoids (y =15.981x - 0.0182, R² = 0.991). These results indicate that the phytochemicals in Lab-made Kanji are significantly higher than that of its other types (P < 0.05). However, phytochemical constituents of all the commercial Kanji samples are alike. Total protein contents of Lab-made Kanji are significantly higher than other Kanji samples, extracts and fractions, except methanol extract (P < 0.05). Total polysaccharides, polyphenols, flavonoids and glycosaponins of all the Kanji samples are significantly higher than that of all the extracts and fractions (P < 0.05). Contrary to previous report, total phenol contents of ethanol extract of fresh root were found to be higher [14], which might be due to different cultivar, variety, growing conditions and age of the plant used in the present study.

The results of total solids (g/mL) and pH of Lab-made, homemade and six commercial samples of Kanji, without having the additional table salt or black salt, are presented in Fig. 1. All the Kanji samples were found to be having no significant difference in pH. On the other hand, the total solid contents of Lab-made, homemade and commercial samples (C5 and C6) of Kanji were similar, however higher than that of the other types of commercial Kanji samples (P < 0.05). Interestingly, all the samples of Kanji showed variation in weight/mL, which could be attributed to a number of probabilities such as non-availability of standard preparation protocol, addition of extra water to increase volume to maximize profit, ice cubes to keep it cool for the preservation of palatability, and use of different carrot variety. Moreover, the samples showing less total solid contents were light as well as hazy in color, whereas Lab-made and homemade Kanji samples were crystal clear having bright purple color. To rule-out the possibility of aforesaid factors, uv/visible profiles (scans from 800-200 nm) of all the Kanji samples were compared to each other. The overlay scan of different Kanji samples, extracts and fractions are given in Fig. 2. The similarity in metabolomics profiles of all the Kanji samples confirmed that the same carrots variety was used in the preparation of this beverage. However, the difference of peak intensities in metabolomics fingerprints was the clear evidence of using different quantities of carrots in the preparation of this drink or dilution made at the time of selling.

Fig. 1: Total solid contents (g/mL) and pH of different types of Kanji samples. C1- C6 (commercial samples).
Table-1: Total contents (mg/g) of phytochemicals of different types of Kanji and various types of extracts of root of *Daucus carota* L., and fractions of sequential methanol extract.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total proteins</th>
<th>Total polysaccharides</th>
<th>Total polyphenols</th>
<th>Total flavonoids</th>
<th>Total glycosaponins</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kanji</em> (Lab-made)</td>
<td>20.61</td>
<td>6.66</td>
<td>8.58</td>
<td>7.21</td>
<td>201.5</td>
</tr>
<tr>
<td><em>Kanji</em> (Home-made)</td>
<td>18.77</td>
<td>5.96</td>
<td>7.27</td>
<td>6.73</td>
<td>188.98</td>
</tr>
<tr>
<td><em>Kanji</em> (Com-1)</td>
<td>18.67</td>
<td>5.78</td>
<td>7.87</td>
<td>6.87</td>
<td>184.18</td>
</tr>
<tr>
<td><em>Kanji</em> (Com-2)</td>
<td>18.33</td>
<td>5.26</td>
<td>7.75</td>
<td>6.79</td>
<td>186.85</td>
</tr>
<tr>
<td><em>Kanji</em> (Com-3)</td>
<td>17.22</td>
<td>5.46</td>
<td>7.48</td>
<td>6.27</td>
<td>187.44</td>
</tr>
<tr>
<td><em>Kanji</em> (Com-4)</td>
<td>18.79</td>
<td>5.68</td>
<td>6.97</td>
<td>6.48</td>
<td>185.55</td>
</tr>
<tr>
<td><em>Kanji</em> (Com-5)</td>
<td>17.22</td>
<td>5.13</td>
<td>6.47</td>
<td>5.97</td>
<td>187.18</td>
</tr>
<tr>
<td><em>Kanji</em> (Com-6)</td>
<td>18.27</td>
<td>5.86</td>
<td>7.28</td>
<td>6.58</td>
<td>185.82</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>23.34</td>
<td>10.30</td>
<td>9.51</td>
<td>9.97</td>
<td>235.5</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>23.89</td>
<td>11.81</td>
<td>9.78</td>
<td>10.29</td>
<td>201.2</td>
</tr>
<tr>
<td>Hexane extract</td>
<td>0.69</td>
<td>1.43</td>
<td>2.19</td>
<td>0.64</td>
<td>0.00</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>1.97</td>
<td>3.29</td>
<td>2.86</td>
<td>1.70</td>
<td>0.40</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>20.71</td>
<td>0.2</td>
<td>9.31</td>
<td>8.17</td>
<td>19.20</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>1.05</td>
<td>0.82</td>
<td>0.39</td>
<td>5.94</td>
<td>0.00</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>0.94</td>
<td>0.88</td>
<td>1.32</td>
<td>0.95</td>
<td>0.35</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>4.04</td>
<td>0.97</td>
<td>4.39</td>
<td>2.02</td>
<td>0.42</td>
</tr>
<tr>
<td>n-butanol fraction</td>
<td>7.07</td>
<td>5.85</td>
<td>4.92</td>
<td>3.39</td>
<td>108</td>
</tr>
<tr>
<td>Water fraction</td>
<td>11.39</td>
<td>9.37</td>
<td>5.05</td>
<td>6.37</td>
<td>112</td>
</tr>
</tbody>
</table>

Fig. 2: UV/Visible scans of different types of Kanji samples, extracts and fraction of root of *Daucus carota* L. in a range of 800-200 nm. A [scans of Kanji samples, C1-C6 (commercial samples)]; B (Kanji and aqueous and ethanol extracts); C (sequential extracts); D (fractions of methanol extract).
The HPTLC profiles of all the Kanji samples and aqueous extract of fresh roots did not show any band under UV exposure, both at short wavelength (254 nm) and long wavelength (366 nm). Contrary to this ethanol extract of fresh roots and different types of extracts and fractions of dried roots showed bands at both the wavelengths. The absence of bands in Kanji and aqueous extract may be due to lower concentration of compounds, active at both the wavelengths.

The results of in vitro antioxidant activity of different Kanji samples, extracts and fractions of sequentially obtained methanol extract, determined by DPPH assay, are shown in Fig. 3. The antioxidant activities of all Kanji samples, ethanol extract and aqueous extract were not statistically different (P > 0.05). Likewise, all the extracts of dried material obtained by sequential extraction were not having any significant difference of antioxidant activity. The antioxidant profile of hexane, chloroform, ethyl acetate, n-butanol and water fractions of methanol extract was also found to be similar. However, activity of all the Kanji samples, extracts and fractions was lesser than that of the standards viz BHA, QTN and vitamin E (P < 0.05).
extracts and fractions have inhibited the peroxidation of linoleic acid due to the presence components having oxygen radical absorbing capacity, as reported previously for carrots [10]. It was also reported that antioxidant activity- using β-carotene assay of carrot juice kept at 2°C was increased until 30 min [9].

Fig. 5: Antioxidant activity of standards and different types of Kanji samples, extracts and fraction of root of Daucus carota L. using β-carotene assay (n=3). Vit E (vitamin E); BHA (butylated hydroxy anisole); C1-C6 (commercial samples); HF (hexane fraction); CF (chloroform fraction); EF (ethyl acetate fraction); BF (butanol fraction); WF (water fraction).

Fig. 6: Degradation kinetics of β-carotene in control and samples containing standards, extracts and fractions of fraction of root of Daucus carota L. using β-carotene linoleate model (n=3). BHA (butylated hydroxy anisole); HF (hexane fraction); CF (chloroform fraction); EF (ethyl acetate fraction); BF (butanol fraction); WF (water fraction).

The Pearson correlation analysis indicated that antioxidant activities of all the samples were correlated to their polysaccharide contents (P < 0.05) and total polyphenol and flavonoid contents (P < 0.01). Interestingly, the phytochemical constituents responsible for antioxidant activity were found to be present in all types of extracts, used in this experiment. The comparable profiles of aqueous and ethanol extracts indicate that water is a good solvent for the preparation of Kanji. The distribution of antioxidant components in hydrophobic and hydrophilic solvent extracts is supported by a previous study, wherein antioxidant activities determined using DPPH and ABTS assays are found in both types of solvent extracts [11]. The slightly higher activity of the ethanol extract was also in accordance to the reported previously [14].

The results of preliminary clinical study of chemically and biologically characterized Lab-made Kanji are shown in Fig. 8. These results show that except neutrophil count there is not any significant difference in blood components, liver function markers and serum lipid profile before and after the treatment. The presence of all the determined clinical parametrs within the normal limits indicate the usefulness of Kanji. However, all the individuals under study revealed certain additional benefits such as increase in appetite and urination after drinking Kanji. The increased urine volume was further found to be relieving burning sensation of urethra during mictuartion, very common problem in summer season. There was no change in body weight of all the volunteers during two weeks of the study. This data lend evidence that the use of Kanji is beneficail
and without harm. This is the first report of preliminary clinical evaluation of *Kanji*. However, as stated earlier, the extracts of black carrots had hepatoprotective effects in rodents [12, 13]. The findings of the present study may provide evidence to conduct a full scale clinical investigations on *Kanji* in patients suffering from liver disorders.

Fig. 8: Comparison of hematology, liver function test and lipid profile of male adult healthy volunteers (n=6) consuming 240 mL *Kanji*/day for two weeks.

**Experimental**

**Chemicals and Solvents**

The chemicals procured from Sigma Aldrich included 1, 1-diphenyl-2-picryl hydrazyl (DPPH), β-carotene, linoeleic acid, butylated hydroxy anisole (BHA), tocopheron (vitamin E), naringenin, gallic acid, bovine serum albumin, quercetin (QTN) and, folin ciocalteau’s reagent. The solvents/chemicals of E-Merck, purchased from the local market included ethanol, methanol, chloroform, hexane, ethyl acetate, n-butanol, sodium carbonate, copper sulphate, sodium hydroxide, potassium sodium tartrate, aluminum nitrate, enthrone reagent and glucose.

**Plant Material, Extraction and Fractionation**

The black carrots, purchased in the month of March from a local vegetable market were authenticated by Professor Dr. Zaheer ud Din Khan, Department of Botany, Government College University, Lahore, Pakistan. A voucher specimen was deposited in herbarium of Department of Botany, Government College University, Lahore, Pakistan, vide reference No. G. C. Bot. Herb. 958.

The roots were washed with water to remove extraneous matter and the residual water was dried in air. The material was divided into three portions; one to be used after drying whereas two to be used as fresh.

The fresh sliced material (250 g) was macerated overnight separately in 500 mL of ethanol and water. The extracts were collected and the residues were extracted again using the same procedure. Ethanol extracts were pooled and solvent was removed *in vacuo* at 40°C, whereas the pooled aqueous extracts were dried using freeze dryer (Christ, Germany).

The second portion of the material was crushed and dried under shade. The dried material (800 g) was extracted sequentially using solvents such as petroleum ether, chloroform and methanol in soxhlet apparatus. All the extracts were dried *in vacuo* at 40°C. Based on the yield, methanol extract was fractionated by partitioning using solvents such as hexane, chloroform, ethyl acetate, butanol and water. Except water fraction (dried in freeze dryer), all the fractions were dried *in vacuo* at 40°C.

**Preparation of Kanji and Collection of Samples from Market**

The 3rd portion of the fresh roots was used to prepare *Kanji* which was termed as Lab-made *Kanji*. It was prepared using the most commonly used traditional recipe as 113 g vertically sliced thin-long pieces of roots and 5 g each of red chilies, mustard seeds and table salt (sodium chloride) were added in a glass jar containing 1500 ml water. The container was covered and the contents were allowed to ferment spontaneously at room temperature for 4 days [15, 16]. Afterwards, it was stored in refrigerator (10 - 15°C) to be used whenever needed. The other ingredients, red chilies, mustard seeds and salt are added to impart good taste.

Six *Kanji* samples, each 250 mL, were randomly purchased from roadside vendors asking them not to add any other ingredient. These samples were designated as C1, C2, C3, C4, C5 and C6. Another 250 mL *Kanji* sample was collected from home of a resident of Lahore, Pakistan, which was labeled as homemade *Kanji*.

**Analytical Studies**

**Estimation of Total Proteins (TP):** Total protein was estimated by method of Lowry et al. [17], briefly 50 mg each of the samples was dissolved in 10 mL distilled water in a centrifuge tube using vortex. The tubes were centrifuged for 10 min at
2700 rpm and 0.1 mL aliquots of supernatant were transferred in test tubes and made the volume 1 mL with distilled water. The contents were mixed after adding 3 mL of reagent C – made by mixing 50 mL of reagent A (2% sodium carbonate in 0.1N sodium hydroxide) and 1 mL of reagent B (0.5% copper sulfate in 1% potassium sodium tartrate) – and 0.2 mL of folin-ciocalteau reagent. The tubes were incubated at room temperature for 30 min and absorbance was measured at 600 nm (UV-2550, Shimadzu Corporation Japan) against a blank having all the reagents except the sample(s). Bovine serum albumin (Fraction V) solutions in a concentration range 12.50-100 µg/mL, treated like the samples, were used to plot a standard curve for the determination of TP.

Estimation of Total Polysaccharides: Each of the samples (0.2 g) was dissolved in 7 mL of 80% hot ethanol in a centrifuge tube to remove soluble sugars. After vortex for 2 min, the tubes were centrifuged at 2700 rpm for 10 min and supernatant was collected. The procedure was repeated with the residue until washing did not give color with enetrone reagent. Then the final residue was dried on water bath, mixed with 10 mL mixture of distilled water and 25% HCl (1: 1 v/v) at 0 °C for 20 min, and then centrifuged at 2700 rpm for 10 min to collect supernatant. The extraction was performed twice and the supernatants were pooled, and the volume was made 100 mL with distilled water. Aliquots (0.1 mL) were taken in test tubes and made the volume 1 mL with distilled water, then 4 mL enetrone reagent was added. The contents were mixed, heated in boiling water bath for 8 min, cooled rapidly and the intensity of green color produced was measured at 630 nm against a blank having all the reagents except the sample. Glucose solutions having concentration 20, 40, 60, 100 and 200 µg/mL, treated like samples were used to plot the standard curve. The glucose concentration calculated from the standard curve was multiplied by factor 0.9 to determine total polysaccharides [18, 19].

Estimation of Total Glycosaponins: Total glycosaponins were determined as described by Hussain et al. [20], briefly, 1 g of each sample was refluxed in 50 mL methanol for 30 min and extract was collected. The process was repeated again and the pooled extract was concentrated to 10 mL using rotary evaporator, which was then added drop wise in a tarred beaker containing 50 mL acetone. The precipitate was dried in an oven at 100 °C, cooled to room temperature and weighed to determine total glycosaponins using the following equation.

\[
\text{Glycosaponins} = \left( \frac{\text{Weight of precipitate}}{\text{Weight of sample}} \right) \times 100
\]
Five hundred microliters of solution of each of the extracts and fractions having concentration 1 mg/mL were taken in test tube and volume was made 3 mL with methanol. Then each of the samples was scanned as mentioned above to get overlay of spectra.

**HPTLC of Kanji, Extracts and Fractions:**
The samples of Kanji and aqueous extracts (0.1 mg/mL) were analyzed using HPTLC system of CAMAG, Berlin, Germany, equipped with TLC plate scanner (Model-3), semi-automatic sampler (Linomat-5), 254/366 nm image recorder (PROSTER 3) and winCATS 4 software. The densitograms obtained were compared to each other.

**In Vitro Biological Studies**

**Free Radical Scavenging Activity by DPPH Model:** Each of the freeze-dried Kanji sample, extracts, fractions and the standards such as BHA, QTN and vitamin E was dissolved in methanol to get final solution of concentration 0.1 mg/mL.

The DPPH assay was performed as described by Brand-Williams et al. [23] and Robards et al. [24], briefly 2 mL of 0.1 mM methanolic solution of DPPH was mixed with 200 µL of each of the test samples/standards in a test tube. Then the volume was made 3 mL with methanol, the contents were mixed and all the tube was kept at room temperature for 60 min. A mixture of 2 mL the DPPH solution and 1 mL methanol, treated like sample/standard served as a control. The absorbance of all the samples, standards and the control was measured at 515 nm against the control. A blank was prepared by mixing 200 µL of ethanol and 3 mL of the emulsion. The absorbance of all the samples, standards and the control was measured at 515 nm against methanol as a blank. Free radical scavenging activity (FRSA) was determined using the following equation.

\[ \text{FRSA} = \left[ \frac{(\text{Ac} – \text{As})}{\text{Ac}} \right] \times 100 \]

Where “Ac” is absorbance of the control and “As” is the absorbance of the tested sample after 60 min.

**Antiradical Power by DPPH Assay:** The Lab-made Kanji, aqueous and ethanol extracts of fresh roots and methanol extract of dried material were further investigated for dose-response relationship and determination of anti-radical power. All the samples in a concentration range 10-100 mg/mL were treated as mentioned in DPPH assay, and the percentage remaining of DPPH was plotted against the concentration to calculate median effective concentration (EC_{50}) and antiradical power (1/EC_{50}) as described by Brand-Williams et al. [23].

**Antioxidant Activity by β-carotene Linoleate Model:** All the sample and the standard solutions were prepared as mentioned in DPPH model using ethanol as a solvent.

The assay was performed as described by Taga et al. [25], briefly, 1 mL of β-carotene (0.2 mg/mL in chloroform), 20 mg linoleic acid and 200 mg tween 40 (polyoxyethylene sorbitane monololeate) were mixed in a round bottom flask and chloroform was removed by rotary evaporator at 40 °C. The residue was mixed with 10 mL distilled water and volume was made 50 mL with oxygenated water. Aliquots (3 mL) of this emulsion were transferred into test tubes containing 200 µL of the sample/standard solutions. A control was prepared by mixing 200 µL of ethanol and 3 mL of the emulsion. A blank was prepared by mixing 200 µL of ethanol and 3 mL of the emulsion, without having β-carotene. All the test tubes were incubated in water bath at 50°C and the absorbance was measured at 470 nm against the blank at 0 min and then after every 15 min until color of β-carotene in the control disappeared (120 min). The degradation rate (R) of β-carotene was calculated using the following equation.

\[ R = \ln (\frac{a}{b}) \times \frac{1}{t} \]

The terms of the equation are as “ln” (natural log), “a” (absorbance at 0 min), “b” (absorbance at 120 min) and “t” (time in min).

Percentage antioxidant activity (%AA) was calculated using the following equation.

\[ \% \text{AA} = 100 \times \left( \frac{(R_{\text{control}} – R_{\text{sample}})}{R_{\text{control}}} \right) \times \frac{1}{120} \]

**In Vivo Biological Studies of Lab-Made Kanji**

The study was conducted as per approved protocol of the Human Research Review Board of University College of Pharmacy, University of the Punjab, Lahore, Pakistan. Written consent was acquired from all the participants. Six, consented, adult healthy male volunteers, aged (21.6 ± 1.14 years) and weight (78.40 ± 8.68 Kg) were selected for the study. Prior to treatment, the blood of each of the individuals was analyzed at Clinical Laboratory, Health Center, University of the Punjab, Lahore, Pakistan for blood cellular components, liver function markers and serum lipid profile, which served as a baseline. One glass of Kanji (240 mL) was given daily at noon to all the individuals for two weeks. No restriction of diet and daily activity was imposed on the volunteers throughout the study period. After two weeks treatment, blood samples of all the subjects
were analyzed in the same laboratory for the above stated parameters.

Statistical Analysis

All the samples were analyzed in triplicate and results were presented as mean ± Standard deviation. The data were analyzed using one way ANOVA with Post Hoc multiple comparison Bonferroni. A P value less than 0.05 were taken as significant.

Conclusion

*Kanji* is a beneficial and safe antioxidant remedy for the protection of liver from oxidative stress and hot weather effects. However, there is a need to devise and adopt a standard protocol for its preparation.

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References