Assessment of impact of feeding fermented karonda (Carissa carandas) whey beverage on biochemical parameters of albino rats

Pushkraj Jaywantrao Sawant*, Dinesh Chandra Rai and Satya Prakash Yadav

Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India.

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ABSTRACT
The present study was conducted to evaluate the impact of feeding fermented karonda whey beverage (FKWB) on biochemical parameters of albino rats. A total of 24 albino rats (male) of age 28±3 days and weighing 80 to 95 g were randomly divided into four groups (n=6) and assigned to experimental dietary treatment. Experimental groups consisted of control group fed with basal ration only and groups fed with basal ration containing 10%, 20% and 30% FKWB. Results showed that levels of BUN, creatinine, SGOT, SGPT were significantly (p<0.05) lower in FKWB fed groups, signifying positive impact of FKWB on functioning of kidney and liver. FKWB consumption had no significant (p>0.05) effect on cholesterol and protein levels, while its consumption reduced triglyceride level.

Key words: Albino rats, Biochemical parameters, Fermented whey beverage, Karonda (Carissa carandas).

INTRODUCTION
In recent times there is growing awareness and interest for medicinal agent present in the nature, commonly known as herbal medicines for treatment of various ailments, whose use is practiced from ancient times. Fruits forms integral part of various herbal medicines formulation, which are rich in concentration of bioactive compounds including antioxidants, beneficial for the health. Karonda (Carissa carandas) is common herb of Apocynaceae family found throughout the India, mainly in semi arid regions. Whole plant and its parts are traditionally used for treatment of various ailments. Fruits of this plant are berry sized, commonly used for preparation of chutney, pickles, jelly, jams, squash, tarts, etc. They are very rich source of iron and vitamin C, therefore, used ethno-medically for curing anemia and scurvy, as an astringent and forms remedy for biliousness. It is even consumed to treat liver dysfunction, to break fever and to prevent putrefaction of blood (Malik et al., 2010).

Whey is a highly nutritious by product of milk, obtained during manufacturing of casein, cheese, paneer, shrikhand and channa. Whey possesses half of the milk solids and is a rich source of lactose, water soluble vitamins, minerals and immunologically active proteins (Durham et al., 1997). Whey protein is mixture of globular proteins and includes β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulin and thermostable fractions of protease peptones (Nagar and Nagal, 2013). They are one of the nutritionally most valuable proteins as they possess high content of essential amino acids (EAAs), notably lysine, cysteine, methionine and cystine (Jelicic et al., 2008) and branched-chain amino acids (BCAAs) viz., leucine, isoleucine, valine which are important for tissue growth and repair. Leucine is a key BCAA in protein synthesis and plays a critical role in insulin and glucose metabolism. Whey composition is very variable and significantly depends on the technology of whey production. Whey possesses preventive and curative elements and is especially used to treat widespread of ailments such as arthritis, anemia and liver complaints (Jelen, 1992).

Several studies has been carried out all over the world reporting different health benefits of consuming probiotic dairy products, especially yoghurt and fermented milk products (Wang et al., 2012). Yoghurt culture is typically composed of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. The probiotic potential of yoghurt culture is confirmed by various studies proving it ability to survive in stomach acids and then in the intestinal ecosystem along with imparting health benefits for gastrointestinal conditions such as lactose intolerance, constipation, diarrheal diseases, colon cancer, inflammatory bowel disease, Helicobacter pylori infection, and allergies (Mokoena et al., 2016 and Balamurugan et al., 2014).

Thus the aim of present study was to determine the effect of supplementation of fermented karonda whey beverage on biochemical parameters of albino rats to further consolidate the above mentioned facts and to access potential therapeutic effect of their supplementation in combination.

*Corresponding author’s e-mail: sawantpushkraj@gmail.com
MATERIALS AND METHODS

The entire experiment was conducted in the laboratory of Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P., India. The study was approved by Central Ethical Committee of University (No. Dean/2016/CAEC/46).

Preparation of fermented karonda whey beverage: The fermented karonda whey beverage (FKWB) was prepared using standardized and established processing parameter. The ingredients used for preparation of FKWB were procured from local market of Varanasi. The FKWB mixture composed of paneer whey and karonda juice in ratio of 79.68:20.32, respectively, to which 9.77% sugar and 0.4% carboxymethyl cellulose (CMC) stabilizer was added. The mixture was pasteurized at 85°C for 5 minutes and allowed to cool to 37°C, which was then inoculated with 2% yoghurt culture (Code no. NCDC-144 procured from NDIRI, Karnal, Haryana, India) composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The mixture was incubated for 15 hours at 37±1°C and then stored at 4±1°C till further use.

Preparation of basal ration: The ingredients of basal ration were procured from University’s Dairy Farm and from local market of Varanasi. The basal ration was formulated as per NRC (1995) to meet the nutritional requirement of rats, which composed of 54% maize, 18% bengal gram, 20% soyabean meal, 2% soyabean oil, 4% milk and 2% mineral mixture. The fresh homogenous mixture of basal ration was prepared twice a day prior feeding.

Experimental animals: Out of 100 albino rats, 24 albino rats of similar sex (male) and body conformation were selected at 28±3 days of age for the study from Animal House of University. The rats having body weight ranging from 80 to 95 gm were randomly divided in four groups (n=6). The rats were caged in polycarbonate cages, with free access to water.

Study design: The study to access the impact of dietary treatment was carried out for 28 days (4 weeks). The dietary treatment combinations of for four groups in the present study was as follows,

Group 1 (G1) - Control group with basal ration only
Group 2 (G2) - Basal ration + 10% FKWB
Group 3 (G3) - Basal ration + 20% FKWB
Group 4 (G4) - Basal ration + 30% FKWB

The rats were fed twice daily at 8 am and 4 pm. At the end of experiment, three rats (off feed for 24 hours) of each group were transferred to the box possessing cotton pads treated with anesthetic ether and were allowed to lose their conscious. Anesthetized rats were pinned up on the dissection tray and blood was sucked from the heart by disposable syringe. Blood from each rat was collected separately. Blood was collected in well cleaned, dry, sterilized test tubes and allowed for clotting. After clotting, the tubes were centrifuged at 3000 rpm for 10 minutes to collect the sera for biochemical parameters estimation.

Biochemical parameter measurement: Biochemical analysis parameter such as blood urea nitrogen (BUN), creatinine, cholesterol, triglyceride, serum glutamate oxaloacetate transaminase / aspartate aminotransferase (SGOT/AST), Serum glutamate pyruvate transaminase/alanine aminotransferase (SGPT/ALT), total protein, serum albumin, serum globulin were analyzed using commercially available kits in an auto biochemical analyzer (Robonik Prietest Touch).

Statistical analysis: The data were statistically evaluated by one way analysis of variance (ANOVA) using SPSS software (Version: IBM SPSS Statistic 23). Duncan multiple range tests (Montgomery, 1977) were used to detect differences among mean values at level of 0.05. Data are presented as mean ± standard error of the mean (Mean±SE).

RESULTS AND DISCUSSION

The mean and standard error for biochemical parameters of albino rats fed on FKWB has been presented in Table 1.

Biochemical parameters

Blood urea nitrogen (BUN) and creatinine: The BUN and creatinine test is done to access functioning of kidney. Their higher levels in blood are indicator of kidney impairment or dysfunction. The levels of BUN and creatinine are significantly (p<0.05) lower in the groups fed with FKWB as compared to control group. The BUN level was significantly lower in group fed with 30% FKWB (G4), while creatinine levels were at par each other in groups fed with FKWB (G2, G3, and G4). The similar result was reported in nephrotoxicity induced rats fed on methanolic extract of Carissa carandas fruit extract (Dhodi et al., 2015). The results even corroborated with finding reported by Ranganathan et al. (2005) and Ranganathan et al. (2009) when rats and patients suffering from chronic kidney disease were fed on probiotic formulation, respectively. Patra et al. (2014) reported anti uremia (a major feature of chronic kidney diseases) activity of *Streptococcus thermophilus* in uremia induced experimental rats.

Cholesterol and triglyceride: Feeding FKWB had non-significant (p>0.05) effect on cholesterol levels of rats, while triglyceride levels were lower in FKWB fed groups as compared to control. Triglyceride levels were significantly (p<0.05) lower and at par with each other in groups fed with 20% and 30% FKWB (G2 and G4). The probable reason for higher cholesterol level in FKWB groups may be due to presence of sugar in beverage and retention of HDL-cholesterol. The similar result for retention of higher level...
of HDL-cholesterol and decrease in triglyceride was reported by Mallick and Khan (2016) in hyperlipidemic rats fed on sweet orange and by Jonsson and Ellegard (2006) in healthy adults fed on apple juice. Beena and Prasad (1997) observed no hypercholesterolemic or hypocholesterolemic effect of fermented milk product and ordinary yoghurt on 68 healthy volunteers and rats, respectively. Baroutkoub et al. (2010) and Hunter (2008) reported increase in HDL-cholesterol on feeding yoghurt to hypercholesterolemic and healthy volunteers, respectively. On contrary to present findings, Yadav et al. (2006) reported decrease in serum cholesterol level on feeding yoghurt to rats.

The present finding for serum triglyceride is in agreement with findings of AL-Kehayaz (2015) and Halaby et al. (2015) who reported decrease in serum triglyceride level on feeding yoghurt and fermented milk (Miao et al., 2016) to rats. Kawase et al. (2000) reported a decrease of triglyceride levels in humans when fed with combined supplementation of Lactobacillus casei and Streptococcus thermophilus. Nwaka et al. (2014) and Mustafa and Abdelrahman (2015) reported decrease in serum triglyceride level on feeding Shaddock citrus fruit and pomegranate juice to albino rats. However elaborate work is required to support the present interpretation for increase in total cholesterol on consumption of FKWB.

Serum glutamate oxaloacetate transaminase/aspartate aminotransferase (SGOT/AST) and Serum glutamate pyruvate transaminase/alanine aminotransferase (SGPT/ALT): The SGOT and SGPT enzymes are considered to be two most important tests to detect liver injury as these enzymes are associated to liver parenchyma cells. The SGOT and SGPT levels are significantly (p<0.05) lower and at par with each other in groups fed on FKWB as compared to control group. The lower level of SGOT and SGPT enzymes in rats fed on FKWB may be due to hepatoprotective activity of karonda juice and yoghurt bacteria. The similar result was reported in hepatotoxicity induced rats fed on ethanolic extract of karonda fruit (Bodakhe et al., 2014). The strong antioxidant activity of fermented karonda juice also might be reason for hepatoprotective activity of beverage. Antioxidant activity is responsible for greater hepatoprotective activity (Hegde and Joshi, 2009). The percent DPPH radical scavenging activity of karonda (Carissa carandas), a measurement for antioxidant potential, was 52.79±3.91% (Chaudary et al., 2014), showing strong antioxidant potential of karonda fruit. Almost similar result were reported by authors who fed fruits such as mango (Pourahmad et al., 2010), grapes (Jassim et al., 2010), bitter orange (Arbo et al., 2009) and lactic acid bacteria formulation (Han et al., 2005 and Yun et al., 2007) and pomegranate yoghurt (El Din et al., 2014).

**Total protein, albumin, globulin and A/G ratio:** The total protein content was significantly (p<0.05) higher in group

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<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
<th>HDL/LDL ratio</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>Total Protein (gm/dl)</th>
<th>Albumin (gm/dl)</th>
<th>Globulin (gm/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>27.8 ± 0.176</td>
<td>1.70 ± 0.173</td>
<td>134.3 ± 2.906</td>
<td>139.0 ± 2.082</td>
<td>89.47 ± 10.717</td>
<td>3.23 ± 0.067</td>
<td>89.47 ± 10.717</td>
<td>4.13 ± 0.033</td>
<td>98.37 ± 4.070</td>
<td>7.33 ± 0.033</td>
<td>4.13 ± 0.033</td>
<td>3.23 ± 0.067</td>
</tr>
<tr>
<td>G2</td>
<td>17.1 ± 0.536</td>
<td>1.07 ± 0.033</td>
<td>143.0 ± 8.963</td>
<td>136.7 ± 1.453</td>
<td>51.80 ± 10.335</td>
<td>3.07 ± 0.088</td>
<td>51.80 ± 10.335</td>
<td>4.10 ± 0.033</td>
<td>63.02 ± 7.361</td>
<td>7.17 ± 0.145</td>
<td>4.10 ± 0.033</td>
<td>3.07 ± 0.088</td>
</tr>
<tr>
<td>G3</td>
<td>15.2 ± 1.790</td>
<td>1.23 ± 0.033</td>
<td>144.7 ± 0.882</td>
<td>131.3 ± 0.333</td>
<td>49.63 ± 8.320</td>
<td>3.00 ± 0.035</td>
<td>49.63 ± 8.320</td>
<td>4.03 ± 0.033</td>
<td>56.63 ± 7.055</td>
<td>7.03 ± 0.145</td>
<td>4.03 ± 0.033</td>
<td>3.00 ± 0.035</td>
</tr>
<tr>
<td>G4</td>
<td>12.1 ± 1.059</td>
<td>1.20 ± 0.115</td>
<td>142.7 ± 4.910</td>
<td>129.3 ± 2.028</td>
<td>43.17 ± 10.277</td>
<td>2.83 ± 0.096</td>
<td>43.17 ± 10.277</td>
<td>3.40 ± 0.058</td>
<td>54.20 ± 3.470</td>
<td>7.63 ± 0.067</td>
<td>3.40 ± 0.058</td>
<td>2.83 ± 0.096</td>
</tr>
</tbody>
</table>

Values are mean± standard error of three replicates per treatment. Means in the same column with different letters are significantly different at 5% level (Duncan test, p<0.05).
of rats fed on 30% FKWB (G₄), while it was lowest in group fed with 20% FKWB (G₃). The dietary treatments in all groups had non-significant (p>0.05) effect on albumin levels and A/G ratios of the groups, while globulin level was significantly highest in rats fed on 30% FKWB (G₄). It can be interpreted from the present investigation that there was not that significant effect of different levels of FKWB on serum total protein, albumin, globulin and A/G ratio of rats fed on it. The present findings are in agreement with findings of Olukanni et al. (2013) and Hadijah et al. (2015), who reported non-significant (p>0.05) effect of lemon and mixed fruit juice on serum total protein of rats fed on it, respectively. The total protein content of calves was not affected by experimental dietary treatment of probiotic yoghurt (Noori et al., 2016) and lactic acid bacteria formulations (Frizzo et al., 2010). Ranasinghe et al. (2013) observed no significant differences in total protein level between control and Streptococcus thermophilus and Lactobacillus bulgaricus formulation treated groups of guinea pigs. The values in present study are near about similar to normal range of serum total protein, albumin and globulin, which is 5.6-7.6 g/dl, 3.8-4.8 g/dl (Anonymous, 2014) and 2.8 to 3.2 g/dl (Burns and De Lannoy, 1996), respectively.

**CONCLUSION**

From the present study it can be concluded that feeding fermented karonda whey beverage (FKWB) to albino had positive impact on functioning of their kidney and liver as the levels of marker tests i.e. BUN, creatinine, SGOT and SGPT for accessing its functioning reduced in blood. FKWB consumption had no significant effect on cholesterol and protein levels, while its consumption reduced triglyceride level. It was even confirmed from the study that consuming FKWB was safe as it did not had any adverse effect on biochemical parameters of albino rats.

**REFERENCES**

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