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Biochemical evaluation of liver and kidney function after subchronic administration of *Chaenomeles maulei* fruit juice to rats

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Chaenomeles japonica var. maulei (Mast.) Lavall,e, belonging to the *Chaenomeles* genus, contains high concentrations of polyphenols with antioxidant and anti-inflammatory activities. The aim of the current research was to evaluate the effects of *Chaenomeles maulei* fruit juice (CMFJ) on liver and kidney functions after subchronic juice administration to rats using biochemical parameters.

Male healthy Wistar rats (n=64) were used, 32 of them treated for 14 days and the rest 32 animals treated for 30 days. For each treatment period, the animals were divided in 4 groups of 8 rats: Control, $CMFJ_{2.5}$, $CMFJ_5$ and $CMFJ_{10}$. The serum enzymes used for the assessment of liver function were: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) determined on the 14^{th} and 30^{th} day. The indices measured for kidney function were serum concentrations of creatinine and urea on the 14^{th} and 30^{th} day.

The serum levels of AST were significantly reduced after 30 days of CMFJ (10 ml/kg) administration ($p<0.05$) in comparison with the Control. The rest of the liver enzyme tests did not show a significant difference between the control animals and the CMFJ-treated animals. There was also no significant change in the indices of kidney function after 14 and 30 days of CMFJ administration.

In conclusion, the results from the current investigation showed the safety of the subchronic CMFJ administration. The highest dose of CMFJ produced a reduction in the levels of AST, probably because of its polyphenolic content known to be antioxidant and organo-protective.

Key words: *Chaenomeles maulei* fruit juice, polyphenols, liver, kidney, subchronic, rats

INTRODUCTION

Many of the current understandings of modern clinical practice find their roots in the traditional medicine. *Chaenomeles japonica var. maulei (Mast.) Lavall,e* (*Chaenomeles maulei*) belongs to the *Chaenomeles* genus, *Rosaceae* family. *Chaenomeles maulei* is a subtype of *Chaenomeles japonica* and comes from China and Japan, where it has been widely used in traditional medicine. In the traditional Chinese medicine, the fruits from *Chaenomeles* plants were beneficial as anti-inflammatory and antioxidant, and were used in conditions like rheumatism, cholera, migraine, liver- and stomach ache [1,2]. Currently *Chaenomeles maulei* fruit juice (CMFJ) is prepared for the production of bio juice, available on the market.

The chemical constituents of the fruit juice include mainly polyphenols and vitamin C at high concentrations. Polyphenolic compounds are believed and proven to be suppressive against inflammation, bacteria, cancer cell activation and protective against free radicals [3-5]. The high concentration of vitamin C also contributes to the antioxidant effect of the juice. The antioxidant potential of phenolic compounds is attributed to their redox capacities, meaning they act as reducing agents. This is in support of the idea that plant constituents with antioxidant properties might be protective against oxidative stress and the conditions related to it [6].

Since there is no data on the safety and effects of CMFJ on liver and kidney functions, the focus of the current research was to evaluate these effects after subchronic juice administration to rats using biochemical parameters.

EXPERIMENTAL

Chaenomeles maulei fruit juice

Chaenomeles maulei fruit juice was obtained from the region of Troyan, Bulgaria, where the plants were grown. The fresh fruit juice was used, so that after handpicking, the fruits were grinded, crushed and squeezed. The juice was filtered, preserved with potassium sorbate (1.0 g/l) and stored at 0 °C till the experiments. The spectrophotometric Folin-Ciocalteu assay [7] was used to determine the total phenolic content. Absorbance was read at 760 nm. As a standard in this assay, gallic acid was used. The spectrophotometric assay showed a very high content of phenolic compounds – 8900.00 mg gallic

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acid equivalents per liter of juice. The HPLC analysis showed that vanillic acid, caffeic acid and chlorogenic acid were the phenolic acids found at the highest concentrations. From the flavonoids, the most abundant were epicatechin, catechin and quercetin [8].

Animals

The animals used in this study were healthy male Wistar rats with a mean weight of 250 ± 30 g, bred in the Animal Centre of Medical University of Varna. The animals were housed in plastic cages in a well ventilated room maintained at 22±1 °C and on a 12/12 light/dark cycle. They received standart rodent pelleted diet and water *ad libitum.*

All procedures concerning animal treatment and experimentation were conducted in conformity with the national and international laws and policies (EU Directive 2010/63/EU for animal experiments) and were approved by Bulgarian Food Safety Agency (Document 141/23.06.2016).

Experimental design

For this experiment, 64 healthy male Wistar rats were used, for 2 treatment periods: 14 days (32 animals) and 30 days (32 animals). For each treatment period, the animals were divided in 4 groups of 8 rats: Control, $CMFJ_{2.5}$, $CMFJ_5$ and $CMFJ_{10}$. The Control groups received orally distilled water at a volume of 10 ml/kg, while $CMFJ_{2.5}$, $CMFJ_5$ and $CMFJ_{10}$ groups received CMFJ at doses of 2.5, 5 and 10 ml/kg, respectively. The doses of 5 and 10 ml/kg were diluted with distilled water to a volume of 10 ml/kg. On the 14th and 30th day, the animals from the respective groups were anesthetized with diethyl ether and blood from the sublingual veins was obtained. It was centrifuged and serum was obtained for biochemical analyses.

Biochemical measurements

Biomarkers for the assessment of liver function were serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) on the 14th and 30th day. Serum activities of liver enzymes AST, ALT and ALP were determined spectrophotometrically (Aurius 2021 UV-VIS, Cecil Instruments Ltd, UK) using standard test kids (BioSystems, Spain).

An elevated AST activity is frequently associated with liver damage, but also can be linked to skeletal muscle and heart injury [9]. ALT is an intracellular cytoplasmic enzyme. In the serum, it is derived from the normal turnover of cells. Increased serum levels of this enzyme indicates tissue damage arising from liver cell inflammation or necrosis. Elevatated levels of serum ALP indicates injury to the canalicular membrane or biliary epithelial cells. ALP is a biomarker enzyme, used to assess membrane integrity, indicating its possible injury [10].

The indices measured for kidney function were: serum concentrations of creatinine and urea on the 14th and 30th day. They were determined spectrophotometrically (Aurius 2021 UV-VIS, Cecil Instruments Ltd, UK) using standard test kids (BioSystems, Spain). Creatinine and urea are both used for the assessment of glomerular filtration and kidney function [11]. Creatinine is a product from muscle metabolism and its serum concentration is maintained by its generation and excretion rate. The serum creatinine level reveals the efficiency of kidney clearance. Urea is a waste product from dietary protein and plays a role in metabolism of nitrogen-containing compounds. The serum urea level rises when kidney failure occurs [12].

Statistical analysis

The results obtained were expressed as mean ± SEM. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post hoc test. A level of $p<0.05$ was considered significant. All analyses were performed using GraphPad Prism statistical software.

RESULTS

Aspartate aminotransferase (AST) levels

The serum levels of AST after 14 days of CMFJ administration did not change significantly when compared to the Control. There was a slight reduction in AST in $CMFJ_5$ group (176.9±12.5 U/L) compared to the control AST values of 215.5±19.9 U/L. The groups $CMFJ_{2.5}$ and $CMFJ_{10}$ had AST levels of 229.5±18.1 U/L and 230.4±22.8 U/L, respectively (Fig. 1A). The assessment of AST levels after 30 days of CMFJ administration showed a significant AST reduction in $CMFJ_{10}$ group (157.8±22.8 U/L, $p<0.05$) when compared to the Control (250.3±32.2 U/L). The results for groups $CMFJ_{2.5}$ (210.3±19.9 U/L) and $CMFJ_5$ (236.2±54.8 U/L) were similar to the control values (Fig. 1B).

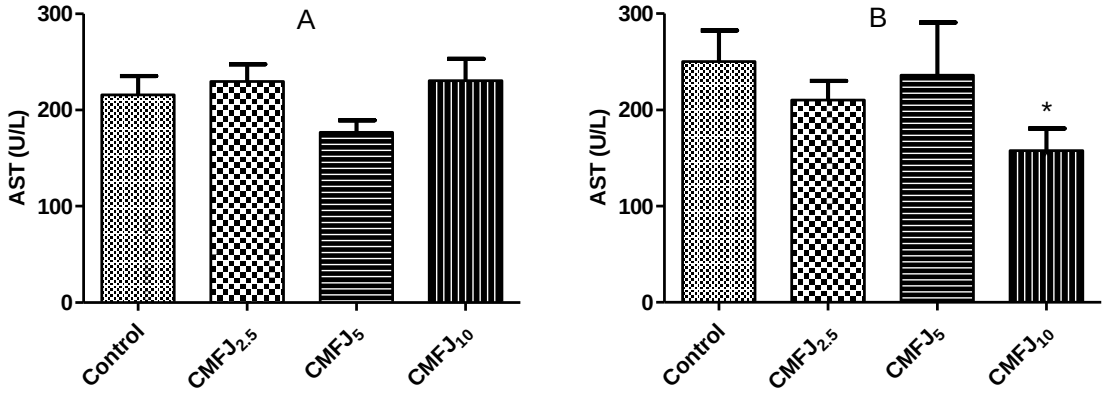

Fig. 1. Effect of *Chaenomeles maulei* fruit juice (CMFJ) at doses 2.5, 5 and 10 ml/kg on serum AST levels in rats after 14 days (plot A) and 30 days (plot B) of administration. The results are expressed as mean ± SEM; *p<0.05 vs. Control

Alanine aminotransferase (ALT) levels

The assessment of serum ALT levels after 14 days of CMFJ administration did not show significant statistical changes. The levels were: for the Control group – 45.6±4.1 U/L, for $CMFJ_{2.5}$ group – 48.9±5.2 U/L, for $CMFJ_5$ group – 58.9±4.4 U/L and for $CMFJ_{10}$ group – 59.2±4.5 U/L (Fig. 2A). The 30-days treatment also did not produce significant changes with only a slight reduction in the $CMFJ_{10}$ group levels (29.6±8.9 U/L) when compared to the Control (34.3±5.1 U/L). For $CMFJ_{2.5}$ and $CMFJ_5$ the results were 45.4±7.6 U/L and 46.3±12.0 U/L, respectively (Fig. 2B).

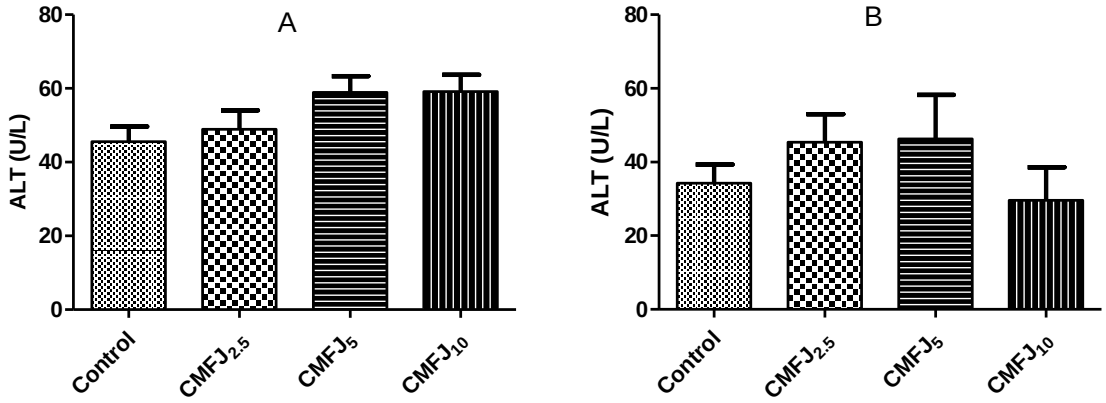

Fig. 1. Effect of *Chaenomeles maulei* fruit juice (CMFJ) at doses 2.5, 5 and 10 ml/kg on serum ALT levels in rats after 14 days (plot A) and 30 days (plot B) of administration. The results are expressed as mean ± SEM

Alkaline phosphatase (ALP) levels

The ALP levels did not differ significantly after 14 days of CMFJ administration when compared to the Control (284.2±14.1 U/L). ALP levels were 323.3±23.4 U/L for $CMFJ_{2.5}$ group, 340.1±17.6 U/L for $CMFJ_5$ group and 340.1±17.1 U/L for $CMFJ_{10}$ group (Fig. 3B). After the treatment period of 30 days, there was also no significant change in ALP concentration. The ALP levels of Control group were 534.4±74.4 U/L, of $CMFJ_{2.5}$ group – 519.9±16.3 U/L, of $CMFJ_5$ group – 688.8±45.1 U/L and of $CMFJ_{10}$ group – 597.1±26.7 U/L (Fig. 3B).

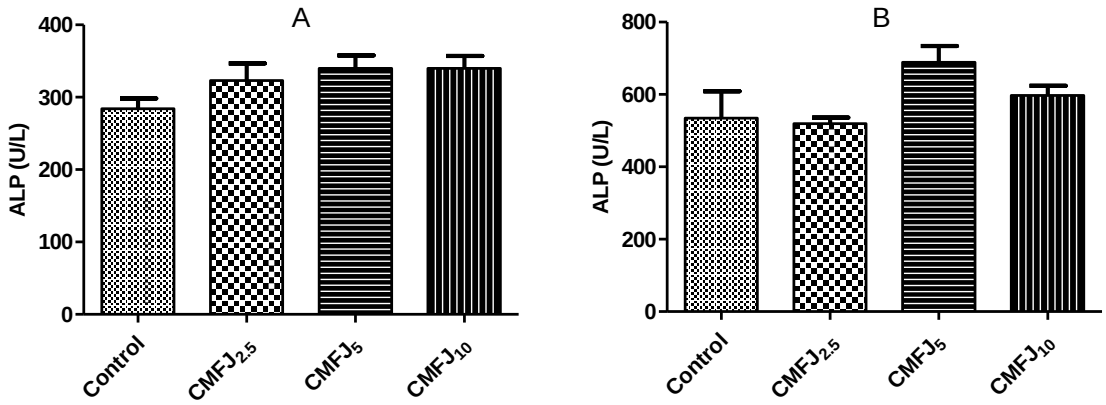

Fig. 2. Effect of *Chaenomeles maulei* fruit juice (CMFJ) at doses 2.5, 5 and 10 ml/kg on serum ALP levels in rats after 14 days (plot A) and 30 days (plot B) of administration. The results are expressed as mean ± SEM

Creatinine levels

There was no significant change in kidney function, measured by creatinine levels after 14 days of CMFJ administration when compared to the Control. The creatinine levels were 61.5±15.3 μmol/L for Control group, 58.3±14.4 μmol/L for $CMFJ_{2.5}$ group, 62.5±14.7 μmol/L for $CMFJ_5$ group and 47.9±9.7 μmol/L for $CMFJ_{10}$ group (Fig. 4A). The 30 days administration did not produce significant changes in the levels of creatinine when compared to the Control (50.4±10.3 μmol/L). Creatinine levels were as follows: for $CMFJ_{2.5}$ – 65.3±13.4 μmol/L, for $CMFJ_5$ group – 71.9±6.2 μmol/L and for $CMFJ_{10}$ – 69.5±6.5 μmol/L (Fig. 4B).

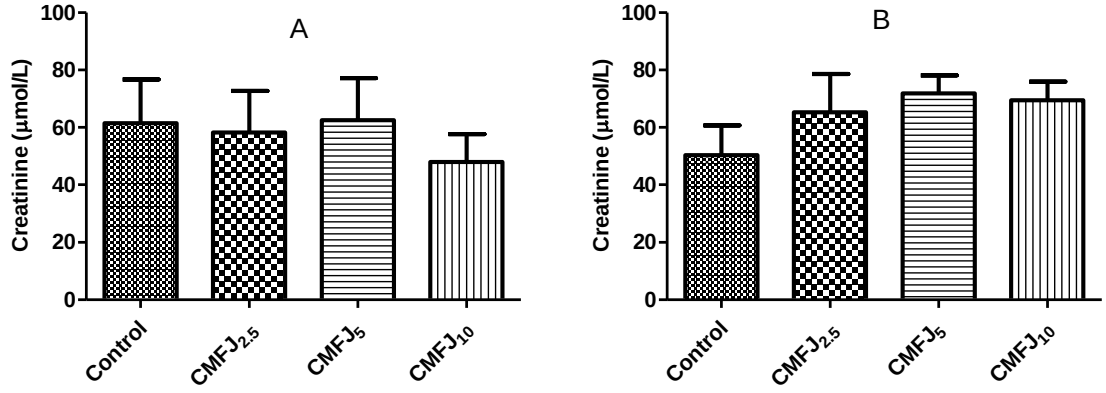

Fig. 3. Effect of *Chaenomeles maulei* fruit juice (CMFJ) at doses 2.5, 5 and 10 ml/kg on serum creatinine levels in rats after 14 days (plot A) and 30 days (plot B) of administration. The results are expressed as mean ± SEM

Urea levels

The urea levels in rats after 14 days of CMFJ administration did not show significant changes when compared to the Control (8.6±0.6 mmol/L). The urea levels measured for $CMFJ_{2.5}$ group were 7.2±0.6 mmol/L, for $CMFJ_5$ group were 6.8±0.6 mmol/L and for $CMFJ_{10}$ group were 7.0±0.7 mmol/L (Fig. 5A). The urea levels after 30 days of CMFJ administration were very similar to the Control level (7.6±0.8 mmol/L). The urea levels for $CMFJ_{2.5}$ group were 7.4±0.5 mmol/L, for $CMFJ_5$ group were 8.6±0.5 mmol/L and for $CMFJ_{10}$ group were 8.0±0.3 mmol/L (Fig. 5B).

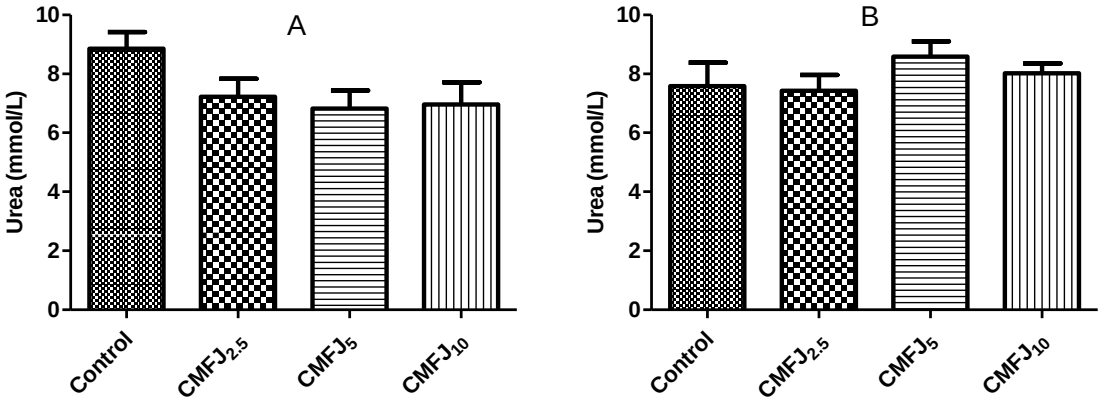

Fig. 4. Effect of *Chaenomeles maulei* fruit juice (CMFJ) at doses 2.5, 5 and 10 ml/kg on serum urea levels in rats after 14 days (plot A) and 30 days (plot B) of administration. The results are expressed as mean ± SEM

DISCUSSION

There are only a few toxicological assessments reported on plants or extracts from the *Chaenomeles* genus. The safety profile remains of utmost significance [13].

The current study showed the effects of CMFJ on liver and kidney functions after its subchronic administration to male rats. This is the first study to confirm the safety of CMFJ.

Widely accepted biomarkers for the function and integrity of the liver and heart are the levels of aminotransferases (ALT and AST) [14]. They are usually released from damaged liver cells. Elevation in their serum levels has been associated with tissue necrosis, cardiovascular damage and nonalcoholic fatty liver disease [15]. In the current study, there was no change in the levels of aminotransferases during the 14-days treatment period. The results from the 30-days treatment period showed a significant reduction in the levels of AST for $CMFJ_{10}$ group, when compared to the Control. These results suggest not simply the safety of the fruit juice, but also its possible hepatoprotective effects. This effect might be due to its polyphenolic ingredients.

ALP is a membrane bound enzyme and elevation in ALP levels is usually observed in cancer, heart infections and liver damage [16]. There was no significant change in the levels of ALP after 14 or 30 days of CMFJ administration, suggesting that CMFJ did not produce cell membrane disintegration.

Serum creatinine and urea are both widely accepted and used markers to evaluate the renal function. Urea is a waste product of protein metabolism and its serum levels rise in patients with kidney failure, leading to uremia [17]. Creatinine is produced by the breakdown of creatine phosphate in muscles [18]. Its values depend on muscle composition and function, activity, diet and health status [19]. Creatinine is a commonly used biomarker of kidney function, allowing to monitor the progression of renal failure. An accumulation of creatinine may cause itching and nerve damage [20]. The 14-days and the 30-days CMFJ administration to rats did not produce changes in the levels of creatinine or urea when compared to the Control. These results suggest that CMFJ at the used doses was completely safe and did not induce kidney damage. There are data that *Chaenomeles* plants exert even a nephroprotective effect in hyperuricemic mice [21].

In conclusion, the biochemical results from the current investigation suggest that the subchronic administration of CMFJ at different doses to rats did not induce any liver and kidney damage. The highest dose of CMFJ (10 ml/kg) caused a reduction in AST levels, suggesting a hepatoprotective effect.

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