Effect of sub-acute and sub-chronic administration of Vicia faba's cooking medium containing EDTA on hepato-renal functions in albino mice

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Abstract

To decrease the *Vicia faba* beans cooking time, some chefs added ethylene diamine tetra-acetic acid (EDTA) during the cooking process. This study addressed the effect of sub-acute and chronic administrations of faba bean's cooking media containing EDTA (C.M./EDTA) on hepato-renal functions in mice. Ninety male mice were divided into three groups (n = 30). The group 1 (Gp1) divided into Gp1a and Gp1b, both were served as control for sub-acute and sub-chronic treatments. Gp2 divided into Gp2a and Gp2b then administered with 200 µl of C.M. for 15 and 90 days, respectively. Gp3 divided into Gp3a and Gp3b then administered 200 µl of C.M./EDTA (10 mg/kg) for 15 and 90 days, respectively. Hematological, biochemical, and histopathological alterations were investigated. The results showed that sub-acute administration of C.M./EDTA did not show any signs of toxicity, however, sub-chronic administration showed substantial toxicity evidenced by alterations in some biochemical and histological investigations.

Introduction

Faba bean (*Vicia faba*, L.) is an important legume crop used for human and animal nutrition (Hefni and Witthoft 2014). It belongs to family Fabaceae (Leguminosae). *V. faba* beans are rich with macro-nutrients such as carbohydrates (58%), lipids (2%), proteins (26%) and micronutrients such as vitamins, minerals. Secondary metabolites including phenolics, flavonoid and other constitutes were reported (Baginsky et al. 2013). Due to their nutritional importance, people have shifted their consumption to *V. faba* as a protein source instead of animal proteins. The seed of *V. faba* beans is very hard, therefore, the time of cooking process of these beans are relatively long (Farajvand et al. 2017; El-Naggar et al. 2020). For this reason, different additives were used to decrease the time of cooking process. Among of them, some chemicals were routinely added to increase the cooking process such as sodium bicarbonate, citric acid (Laniga et al. 2002; Lamas et al. 2014).

Ethylene diamine tetra-acetic acid (EDTA) is a chelator agent, potentially used for vascular disease treatment, keratopathy and increased the efficacy of chemotherapy (Kobayashi et al. 2015; Ferrero 2016; El-Naggar et al. 2019a; El-Naggar et al. 2019b). EDTA may inhibit bleomycin nuclease activity, which in turn induces cancer cell apoptosis (Feril et al. 2017). Recently, unfortunately some chefs added EDTA to decrease faba beans cooking time from 9 hours to only 90 minutes (El-Naggar et al. 2019c). Previous study showed that EDTA addition to black beans (*P. vulgaris* L.) decreased starch gelatinization degree (Mubaiwa et al. 2017). Very few reports demonstrated the effect of EDTA addition during the cooking process on the nutritional and toxicological levels. In our previous study, we have reported that EDTA addition to faba beans during the cooking process decreased the nutritional values of macronutrient (El-Naggar et al. 2019c). In addition, it has been reported that cooking of faba beans in presence of EDTA decreased the levels of minerals, but not affect the levels of vitamins. Furthermore, it was reported that addition of EDTA during the cooking process of faba beans decreased their phytochemical contents of phenolic, flavonoid, anthocyanin and saponin contents. EDTA addition to faba beans during cooking process also decreased, increased, and diminished some phytochemicals constitute (El-Nashrty 2021).

Acute administration of EDTA may cause gastrointestinal upset, kidney injury, transient bone marrow depression and muscle cramps (Bingham et al. 2001). EDTA administration also decreased coagulation factor activity and some biochemical parameters in the serum (Khalil et al. 2008). Administration of 750 mg/kg/day of EDTA was reported as the lowest dose that caused a toxic effect and induce DNA damage or chromosomal breaking in human and mice with no carcinogenic effects (Lanigan and Yamarik 2002). Recently, *in vivo* study showed that the administration of C.M./EDTA (20 or 40 mg/Kg) for 90 days caused pathophysiological alterations evidenced by increase the adverse of liver and kidney function biomarkers in mice (El-Naggar et al. 2019a). Therefore, this study was extended to address the adverse effect of sub-acute and chronic administrations of C.M./EDTA (10 mg/Kg) on hepato-renal functions in experimental mice.

Materials And Methods

Chemicals

EDTA was purchased from Sigma-Aldrich. Biochemical kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, cholesterol, triglycerides, HDL, LDL-cholesterol, malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) kits were purchased from Biodiagnostic (El-Dokky, Egypt).

Preparation of cooking media containing EDTA

Fifty grams of *Vicia faba* beans (faba seeds) was completed to 500 ml distilled water in presence of 1 g of EDTA/L. Faba seeds were cooked for 90 minutes and then the C.M. was filtered and stored at -20 °C until use. 50 g of faba seeds were completed to 500 ml of distilled water and boiled under the same condition and the C.M. was used as a negative control.

Mice and experimental design

Male albino mice with average weights 20 ± 2 g were obtained from National Research Center (NRC, Cairo, Egypt). Mice were kept at the Animal Facility, Zoology Department, Faculty of Science, Tanta University. Mice were housed under standard laboratory condition of temperature and humidity according to the suggested National ethical guidelines for the care of laboratory animals as the Animal Ethics Committee, Faculty of Science, Tanta University, Egypt. The institutional animal care committee at the Zoology Department, Faculty of Science, Tanta University-Egypt, approved the experimental design (IACUC-SCI-TU-0098).

Post a week of adaptation, mice were divided into three groups (n=30). The group 1 (Gp1) further divided into two subgroups (Gp1a and Gp1b), both of them were served as control for 15 days (subacute) and 90 days (chronic) of administrations. Gp2 was divided into two subgroups (Gp2a and Gp2b) that administered orally with 200 µl of C.M. for 15 and 90 days, respectively. Gp3 was divided into two subgroups (Gp3a and Gp3b) that administered orally with 200 µl of C.M./EDTA (10 mg/kg) for 15 and 90

days, respectively. Blood samples were collected via orbital plexus bleeding for hematological and biochemical analysis and then mice were sacrificed to harvest liver and kidney tissues for histopathological examinations.

Determination of hematological and biochemical parameters

The blood was withdrawn from retro-orbital plexus to estimate the total red blood cells (R.B.Cs), hemoglobin content (Hb), hematocrit (Hct%), the total white blood cells (W.B.Cs) and differential count using auto hematology analyzer (BC-3200, Mindray, China). Biochemically, serum ALT, AST, urea, and creatinine were assayed by the colorimetric method using kit (Diamond-Diagnostics, Egypt). Furthermore, serum cholesterol, triglycerides, and HDL-cholesterol were determined using quantitative kit based on the previously described methods (Burstein *et al.* 1970; Allain *et al.* 1974; Fossati and Prencipe, 1982). LDL was calculated according to Friedewald *et al.* (1972) as follows: LDL = total cholesterol – HDL – VLDL. MDA was assessed based on the method that have been previously described by Li and Chow, 1994. According to Paglia and Valentine, (1967), the levels of GSH was determined. Catalase activities were measured following the methodology of Aebi, (1984). SOD activity was determined as described by Paoletti and Mocali, (1990).

Histopathology investigations

Liver and kidney tissues of the experimental groups under the study were collected. Small pieces were immediately fixed in 10% neutral buffered formalin for 24 hours. The tissue samples were dehydrated in ascending grades of ethyl alcohol, cleared by xylene, and embedded in paraffin. Sections of 5µ thickness were mounted and stained with haematoxylin and eosin (HandE) (Bancroft and Gamble, 2002). All slide preparations were microscopically examined for the histopathological investigation.

Statistical analysis

Dunnet test was used to compare all groups against the control group to show the significant effect of treatment. The criterion for statistical significance was set at $p < 0.05$. All data are presented as mean $\pm$ SD.

Results

Subacute and sub-chronic administrations of C.M./EDTA did not influence body weight changes

Administration of C.M. alone for 15 days (Gp2a) or 90 days (Gp2b) orally did not induce changes in body weight when compared to their control group. Also, administration of C.M./EDTA for 15 days (Gp3a) or 90 days (Gp3b) did not decrease the body weight of mice post 15 and 90 days of the administrations (Fig. 1A). The percentages of body weight changes (% b.wt) in Gp1a, Gp2a, and Gp3a post 15-days of

administration were 28.1, 30.3, and 28.2%, respectively. While the % b.wt in Gp1b, Gp2b, and Gp3b post 90-days of administration were 43.6, 41.1, and 40.2%, respectively (Fig. 1B).

Sub-chronic but not sub-acute administration did alter some hematological parameters

The results showed that there were no significant changes in the hematological parameters post 15 days of administration (subacute) either with C.M. alone (Gp2a) or with C.M./EDTA (10 mg/kg) (Gp3a) when compared to their control group. Post 90 days of administration (sub-chronic) with C.M. alone, the differential leucocytes count did not alter when compared to control group. Administration with C.M./EDTA for the same time did change these percentages when compared to Gp1b and Gp2b (Table 1).

Table 1
Total leucocyte counts and their differential percentages post 90 days of administration.

Groups	W.B.Cs ($\times 10^3/\text{ul}$)	Differential leucocyte count		
		Lymph. (%)	Neut. (%)	Mono. (%)
Gp1b	9.2 ± 1.7	81.7 ± 2.5	14.5 ± 2.0	4.7 ± 2.0
Gp2b	9.5 ± 2.8	79.5 ± 4.5	11.5 ± 1.5	6.7 ± 2.2
Gp3b	8.5 ± 1.9	$55.4 \pm 5.5^*$	$28.7 \pm 2.9^*$	$8.5 \pm 1.9^*$
Gp1b: control administered with H ₂ O, Gp2b: mice administered with cooking media (C.M.) alone for 90 days, Gp3b: mice administered with C.M./EDTA (10 mg/Kg) for 90 days. * Means significant change ($p \geq 0.05$).				

Sub-chronic administration of C.M./EDTA altered liver transaminases, urea, and creatinine levels

Administration of C.M. alone (Gp2a) or C.M./EDTA (10 mg/kg) (Gp3a) for 15 days (subacute) led to no significant changes in the ALT, AST, urea, and creatinine levels when compared to their control group (Gp1a). Post 90 days of administration with C.M. alone (Gp2b), this parameter did not alter when compared to control group (Gp1b). Sub-chronic administration of C.M./EDTA (10 mg/kg) (Gp3b) increased these parameters in sera when compared to control group (Gp1b) (Table 2).

Table 2
ALT, AST activities, urea, and creatinine levels post 15 and 90 days of administration.

Groups	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Post 15-days (Sub-acute)				
Gp1a	52.3 ± 1.7	210.8 ± 6.5	42.5 ± 0.6	0.42 ± 0.06
Gp2a	52.3 ± 1.0	233.5 ± 5.3	46.3 ± 0.9	0.45 ± 0.08
Gp3a	42.3 ± 1.3	242.5 ± 6.3	48.7 ± 0.7	0.41 ± 0.05
Post 90-days (Sub-chronic)				
Gp1b	49 ± 0.9	179.6 ± 2.1	38.89 ± 0.9	0.35 ± 0.06
Gp2b	62 ± 1.1	185.5 ± 3.2	41.24 ± 0.8	0.49 ± 0.02
Gp3b	88 ± 0.5*	197.5 ± 2.8	48.35 ± 1*	0.87 ± 0.03*
Gp1a and Gp1b: control administered with H ₂ O, Gp2a and Gp2b: mice administered with cooking media (C.M.) alone, Gp3a and Gp3b: mice administered with C.M./EDTA (10 mg/Kg) Gp1a, Gp2a, and Gp3a were administrated for 15 days, Gp1b, Gp2b, and Gp3b were administrated for 90 days. ALT: Alanine transaminase, AST: Aspartate transaminase. * Means significant change (p ≥ 0.05).				

Cooking media/EDTA did not affect the lipids profile post 90 days of administration

Administration with C.M. alone (Gp2a) or with C.M./EDTA (Gp3a) for 15 days did not change the levels of cholesterol, triglycerides, HDL-C, and LDL-C in sera when compared to control (Gp1a). Similarly, lipids profile was detected when mice were administered for 90 days either with C.M. alone (Gp2b) or with C.M./EDTA (Gp3b) when compared to their levels in control (Gp1b) (Table 3).

Table 3
Lipids profile post 15 and 90 days of administration.

Groups	Cholesterol (mg/L)	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Post 15-days (Sub-acute)				
Gp1a	113.5 ± 1.4	115.5 ± 11.6	78.5 ± 1.5	13.5 ± 1.7
Gp2a	115.8 ± 1.4	120.5 ± 11.7	70.3 ± 1.5	12.5 ± 1.3
Gp3a	110.2 ± 1.2	103.7 ± 11.5	82.5 ± 2.16	14.2 ± 1.4
Post 90-days (Sub-chronic)				
Gp1a	116 ± 1.8	119.5 ± 13.7	83.7 ± 1.5	15.5 ± 1.2
Gp2a	125 ± 1.9	135.5 ± 12.6	76.3 ± 1.5	14.5 ± 1.8
Gp3a	130.25 ± 1.5	101.5 ± 12.8	72 ± 2. 6	19.25 ± 1.9
Gp1a and Gp1b: control administered with H ₂ O, Gp2a and Gp2b: mice administered with cooking media (C.M.) alone, Gp3a and Gp3b: mice administered with C.M./EDTA (10 mg/Kg) Gp1a, Gp2a, and Gp3a were administrated for 15 days, Gp1b, Gp2b, and Gp3b were administrated for 90 days. HDL-C: High density lipoprotein-Cholesterol, LDL-C: Low density lipoprotein-Cholesterol. * Means significant change (p ≥ 0.05).				

Sub-acute administration did not oxidants/antioxidants biomarkers however, sub-chronic administration did

Oxidant/antioxidant biomarkers (MDA, GSH, CAT, and SOD) were measured 15- and 90-days post administration of different groups. The results showed that sub-acute and sub-chronic administrations of C.M. alone did not alter these biomarkers. Sub-acute administration of C.M./EDTA (Gp3a) did not alter these biomarkers (Fig. 2), however, chronic administration of C.M./EDTA (10 mg/kg) (Gp3b) did increase MDA, and decrease GSH levels, SOD, and CAT activities when compared to control group (Gp1b) (Fig. 3).

Administration of C.M./EDTA for 90 days altered the architectures of liver and kidney tissues

Liver section of mice administered with C.M alone for 15 days (Gp2a) showed normal central veins (Cv), normal hepatic strands (H), some hepatocytes are degenerated and vacuolated (V), others are binucleated (arrows), regular blood sinusoids with distinct phagocytic Kupffer cells (double arrows). Liver section of mice administered with C.M./ EDTA (10 mg/Kg) for 15 days (Gp3a) displaying organized hepatic architecture and central vein (Cv), few cellular infiltrations (arrow) and Kupffer cells (K) were noticed. Some histopathological alterations were seen in liver sections obtained from mice administered for 90 days with C.M./EDTA (Gp3b), including cellular disorganization, degeneration, necrosis of many hepatocytes especially in the centrilobular zone and cytoplasmic vacuolation, pronounced nuclear

changes, karyolitic nuclei and pleomorphic ones as well as dilated and congested central vein with cellular infiltration and accumulation of large dark brown pigments in its lumen (Fig. 2C).

Kidney sections of mice administered either with C.M. alone (Gp1a) or with C.M./EDTA (Gp3a) for 15 days exhibiting normal like structure of the renal tissue, normal glomeruli with regular Bowman's space (*), mostly renal tubules are normal (R), few numbers are dilated and damaged (arrows). However, kidney sections of mice administered with C.M./EDTA (10 mg/Kg) for 90 days (chronic) displaying disorganized glomeruli (G) with narrow Bowman's space (*), mostly renal tubules are damaged, destructed and atrophy of its lining epithelia (R), others are intermixed with each other (thin arrow) and intratubular hemorrhage was damaged (thick arrow) (Fig. 2C).

Discussion

Faba bean (*V. faba*, L.) is a popular nutritional legume crop in most countries around the world (Hefni and Witthoft 2014). EDTA and its salt derivatives have been widely used in the field of food industry. Prolonged oral administration of EDTA could be toxic depending on the administration route and the doses (Ramadan et al. 2016). Previous studies reported that cooking could alter phytochemicals constitute and hence influence the bioactive compounds and antioxidant capacity in food (Podsede et al. 2008; Chan et al. 2009; Rawson et al. 2013; Gunathilake et al. 2018). Using of chemicals in cooking negatively affect the nutritional value of faba seeds and could be harmful upon eating on human health (El-Naggar et al. 2019a; El-Naggar et al. 2019b).

This study was conducted to evaluate the effect of subacute and chronic administration of C.M./EDTA (10 mg/Kg) on the hepatorenal functions in the experimental mice. Previous study showed that administration of 20 mg/Kg or 200 mg/Kg led to toxic effects on the liver and kidney tissues in experimental animals that evidenced by the increase in ALT, AST, urea, and creatinine biomarkers and altered histological architectures of liver and kidney tissues (El-Naggar et al. 2019a). In the present study, administration of C.M alone for 15 days (sub-acute), or 90 days (sub-chronic) did not significantly alter the body weight changes when compared to their control. Administration of C.M./EDTA (10 mg/Kg) for 15 days did not alter the hematological parameters, however, administration of C.M./EDTA (10 mg/Kg) for 90 days alter the differential leucocytes. This finding could be due to the toxic effect of EDTA byproducts on the bone marrow or spleen. Previous study showed that EDTA could alter hematological parameters post chronic administration (El-Naggar et al. 2019b; El-Naggar and El-Said 2020). The ALT, AST, urea, and creatinine biomarkers did not alter post administration with C.M. alone or with C.M./EDTA for 15 days, however, these parameters increased post 90 days of C.M./EDTA (10 mg/Kg) administration. These findings postulated that the administration of C.M./EDTA altered the biochemical and physiological features of liver and kidney tissues that might be due to the formation of some toxic compounds upon the boiling of EDTA with beans. These findings were agreed with El-Naggar et al. (2019a) who reported that administration of C.M./EDTA increased liver and kidney biomarkers. Furthermore, previous studies reported the toxic effects of EDTA administration in experimental animals that promoted biochemical alterations (Wang et al. 2004; Khalil et al. 2008).

Previous study demonstrated that multiple sessions of intravenous EDTA chelation therapy with or without vitamin C decrease the oxidative stress and lipid peroxidation (Roussel et al. 2009). In the present study, the levels of oxidants/antioxidants biomarkers did not change post 15 days of administration of C.M. alone or with C.M./EDTA. These results postulated that subacute administration of C.M./EDTA was not toxic at the subacute phase, however, post 90 days (sub-chronic) was toxic due to the alteration in oxidants/antioxidants homeostasis. This disturbance in antioxidant status upon administration of C.M./EDTA to mice might be due to the negative influence of EDTA on enzyme activities. González-Cuevas et al. (2011) reported that EDTA treatment induces the activity of antioxidant enzymes, decreases lipid peroxidation, hepatic inflammation, and liver fibrosis in experimental animals. Furthermore, it has been reported that EDTA showed neuroprotective potential against lead toxicity due to their ability to react faster with peroxy radicals there by reducing the severity of biochemical variable indicative of oxidative damage (Basha et al. 2013).

In experimental animals, EDTA administration promoted histological alterations (Wang et al. 2004; Khalil et al. 2008; Ramadan et al. 2016). Consistent with the hematological and biochemical findings, the present study indicated that the effect of C.M./EDTA administration for 15 days did not alter both liver and kidney architecture, however, post 90 days of administration, C.M./EDTA (10 mg/Kg) induced histopathological alterations in the liver and kidney tissues. Similar findings were found on the histopathological alterations of liver and kidneys upon administering the experimental animals with C.M./EDTA for long time (El-Naggar et al. 2019a). Wang et al. (2004) reported that there was marked hepatocyte damage in rats administered with EDTA. Abdel-Wahhab et al. (2002) reported that EDTA administration showed kidney dysfunction. Furthermore, Ramadan et al. (2016) reported that having food contaminated with EDTA caused degeneration in the interstitial cells in mice and decreased the fertility.

Conclusion

The present study reported that sub-acute administration of C.M./EDTA at 10 mg/Kg did not show significant alterations on the hematological, biochemical, and histopathological investigations. However, sub-chronic administration did alter the functionality of liver and kidney organs.

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request and included in this article.

Author contribution

Sabry Ali El-Naggar: conceptualization, funding acquisition, writing original draft, and supervision. Mona Elwan and Doaa Ibrahim Kabil: conceptualization, data analyses, visualization, writing original draft preparation. Abdelaziz Zidan: investigation, writing review and editing. Karim Samy El-Said: conceptualization, funding acquisition, writing original draft, supervision, investigation, writing review and editing.

Ethics approval

The institutional animal care committee at the Zoology Department, Faculty of Science, Tanta University-Egypt, approved the experimental design (IACUC-SCI-TU-0098).

Consent for publication

Not applicable.

Competing interests

All authors declared that there is no competing interest.

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Figures

Figure 1

(A) Kinetics of body weight changes (B) Percentages of body weight changes in all groups under the study. Gp1a and Gp1b: control administered with H₂O, Gp2a and Gp2b: mice administered with cooking media (C.M.) alone, Gp3a and Gp3b: mice administered with C.M./EDTA (10 mg/Kg) Gp1a, Gp2a, and Gp3a were administrated for 15 days, Gp1b, Gp2b, and Gp3b were administrated for 90 days.

Figure 2

Oxidative stress biomarkers post 15 days. (A) Malondialdehyde (MDA), (B) Reduced glutathione (GSH), (C) Catalase (CAT), and (D) Superoxide dismutase (SOD) in all groups under the study. Gp1a: control administered with H₂O, Gp2a: mice administered with cooking media (C.M.) alone, Gp3a: mice administered with C.M./EDTA (10 mg/Kg) Gp1a, Gp2a, and Gp3a were administrated for 15 days.

Figure 3

Oxidative stress biomarkers post 90 days. (A) Malondialdehyde (MDA), (B) Reduced glutathione (GSH), (C) Catalase (CAT), and (D) Superoxide dismutase (SOD) in all groups under the study. Gp1b: control administered with H₂O, Gp2b: mice administered with cooking media (C.M.) alone, Gp3b: mice administered with C.M./EDTA (10 mg/Kg). Gp1b, Gp2b, and Gp3b were administered for 90 days.

Figure 4

Liver and kidney sections stained with H & E of different groups under the study post 15 days of administration. (A, B, and C) Liver sections of control, C.M., and C.M./EDTA administered mice (10 mg/Kg). (D, E, and F) kidney sections of control, C.M., and C.M./EDTA administered mice (10 mg/Kg).

Figure 5

Liver and kidney sections stained with H & E of different groups under the study post 90 days of administration. (A, B, and C) Liver sections of control, C.M., and C.M./EDTA administered mice (10 mg/Kg). (D, E, and F) kidney sections of control, C.M., and C.M./EDTA administered mice (10 mg/Kg).