



Hemogram results and renal function before and after administration of black cumin seed oil in healthy volunteers

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ABSTRACT

Black cumin seed oil (BCSO) is widely used to maintain health and strengthen the people of Indonesia's immune system and proved to be safe for kidneys. However, the effect of BCSO usage in a healthy kidney is remaining unknown. This study was aimed to determine the impact of the 20-day administration of BCSO on kidney function in healthy volunteers. We conducted a pre- and post-control design trial in 36 healthy volunteers. The volunteers had to hold a valid health certificate from the hospital, be willing to be involved in the research as evidenced by their willingness to complete and sign an informed consent, and be aged between 18 and 50 years. Pregnant women and participants allergic to BCSO were excluded from the study. BCSO was given for 20 days in three dose regimes: 3×1 , 3×2 , and 3×3 capsules/day. Blood pressure, pulse, weight, and blood chemistry of kidney function was observed, and hemograms and urinalyses were performed before and after treatment. One-way ANOVA and a repeated measures analysis were used to examine the mean difference of clinical parameters outcomes. BCSO administration with the three-dose levels for 20 days did not change blood pressure, hemogram, blood urea levels, blood creatinine levels, or specific gravity and urine pH ($P > 0.05$). Based on the result, it can be concluded that the administration of BCSO for 20 days in healthy volunteers has not been able to show its effect on kidney function. It is necessary to plan a study with a longer duration and a higher dose to determine the effect of BCSO on the kidney function.

Keywords: BCSO, healthy volunteers, kidney function, pre- and post-controlled design trial

INTRODUCTION

Black cumin (*Nigella sativa* L.) seed oil is widely used to maintain health and strengthen the people of Indonesia's immune system. The black cumin seed oil (BCSO) contains active compounds such as long-chain unsaturated fatty acids and thymoquinone (TQ).^[1-3] Unsaturated fatty acids have been shown in the laboratory to serve as powerful antioxidants and immunomodulators. TQ has been shown to have acted as an anti-inflammatory and antioxidant, so it is useful as a chemopreventive. The

anti-inflammatory mechanism of *N. sativa* crude fixed oil in rat peritoneal leukocytes is through cyclooxygenase and lipoxygenase pathways inhibition.^[4-9] The BCSO in capsules is widely available in Indonesia. Empirically, Indonesians consume BCSO capsules as a supplement to increase immunity or immune booster.^[1,9] Immunobooster agents are commonly used for 7–14 days,^[10,11] Huseini *et al.* (2013)^[12] reported that BCSO administration at a dose of 5 ml/day for 8 weeks in healthy volunteers could reduce both systolic and diastolic blood pressure. The data are in line with the results of *in vivo* research by Azzubaidi *et al.* (2017), where TQ has been shown

to reduce blood pressure in normotensive mice.^[13] A decrease in blood pressure can affect the kidney filtration rate and is thought to affect kidney function. Confirmation is needed about the effect of BCSO administration on kidney function.

The BCSO is a safe preparation with a wide dosage range, but it is still possible to cause unexpected side effects. LD₅₀ oral administration of BCSO was 28 ml/kg BW, whereas subacute administration for 12 weeks *in vivo* at a dose of 2 ml/kg BW showed changes in blood parameters but did not cause test death animals.^[14] Preclinical studies of doses of 0.01–0.1 ml/kg BW BCSO have to be shown to be safe and effective as an antioxidant and immunomodulatory agent in rats.^[15] Based on the preclinical data and the dose conversion formula, the equivalent effective dose for a human is between 0.082 and 0.82 ml/kg BW BCSO.^[16] The BCSO at a dose of 1 × 1.5 ml/day as an adjunct therapy for anti-hyperglycemic and anti-hypertensive drugs for 40 days has been shown not to affect blood pressure reduction but improved the decrease in blood sugar and HbA1c levels in patients at risk of metabolic syndrome.^[9,17] Nevertheless, other studies have shown that a 5 ml/day of BCSO for 8 weeks lowers healthy volunteers' blood pressure.^[12] In general, this is a preliminary sub-chronic study to determine the effect of the BCSO dosages of 3 × 1, 3 × 2, and 3 × 3 BCSO capsules/day for 20 days on kidney function in healthy volunteers.

MATERIALS AND METHODS

Research Design

This experiment was a pre- and post-control design. All research procedures were carried out by sound clinical practice principles as written in the research protocol. The protocol was reviewed and approved by the Ahmad Dahlan University Research Ethics Committee (UAD) with an ethical clearance number of 021903003. The research subjects were 36 healthy men and women, as evidenced by a health certificate from the authorized hospital, aged 18–50 years and willing to volunteer (by signing an informed consent). The number of subjects for phase I research recommended by the Food and Drug Supervisory Agency is between twenty to eighty volunteers; therefore, the study used 36 volunteers divided into three groups of doses.^[18]

Volunteer invitation was disseminated through brochures and leaflets on the UAD campus area, the University of Gadjah Mada (UGM) Campus, and the area around the UGM campus. Interested prospective subjects were asked to contact the telephone number included in the brochures and leaflets. They were then invited to attend a meeting, where they explained the study and were asked to complete and sign the informed consent. Subsequently, they underwent physical and laboratory examinations in a legitimate hospital by a competent health worker to ensure their health status before being randomly divided into three groups.^[19]

BCSO capsules were purchased from the traditional medicine pharmaceutical industry (CV Al Afiat) under the license of the Republic of Indonesia's Food and Drug Supervisory Agency with registration number 163 394 411 (TR 163 394 411). Each of which contained 500 mg of BCSO. The manufacturing process of BCSO is carried out in compliance

with the Indonesian Food and Drug Administration's good manufacturing process guidelines. Based on active ingredient analysis, the BCSO was found to contain 2.72% TQ, 75.54 % unsaturated fatty acid, and 24.44% saturated fatty acid. Each BCSO capsule contains 13 mg of TQ, the equivalent of 362 mg of unsaturated fatty acids, and 117 mg saturated fatty acids. BCSO capsules are recommended to be consumed at a dose of ×3 1–3 capsules/day.

The healthy volunteers were randomly divided into three groups of 12. Group 1 received 3 × 1 BCSO capsule; Group 2 received 3 × 2 BCSO capsules; and Group 3 received 3 × 3 BCSO capsules. Under the Ahmad Dahlan University research ethics committee's advice, based on practical use in society and previous research, the BCSO was administered in 20 days for 3 × 1, 3 × 2, and 3 × 3 capsules/day.^[17,19]

Data Analysis

Measuring pulse and blood pressure

Blood pressure was measured using a mercury sphygmomanometer by trained professionals using the procedure and protocols recommended by the American Heart Association.^[20] Measurement of blood pressure and heart rate was done 3 times, namely, before the treatment, on the 10th day of treatment, and on the 20th day of treatment.

Examining peripheral blood features

A total of 2 ml of peripheral blood were taken from the cubital vein by trained analysts using a protocol reviewed by the UAD research ethics committee. The blood underwent a hemogram profile and testing for urea and creatinine levels, blood glucose level, cholesterol, and triglyceride level using the spectrometer method with hemato-analyzer, as done in the previous studies.^[19] Examination of hemogram profiles, creatinine ureum levels, glucose levels, cholesterol levels, and triglyceride levels was carried out before and after treatment. Urine density and pH were examined using urinalysis before and after treatment.

Statistical Analysis

The significance of differences in average age, blood pressure, and pulse among groups were tested with one-way ANOVA. A repeated measures test was conducted to determine the difference in the mean of blood pressure, pulse, a renal function parameter, and hemogram among measurements in one group. The mean deviation of blood pressure, pulse, a renal function parameter, and hemogram among groups in one measurement was tested by one-way ANOVA using SPSS 17 free edition. Statistical tests were carried out at a 95% confidence level.

RESULTS AND DISCUSSION

Characteristics of Healthy Volunteers

The volunteers were 36 people consisting of 10 men (22.92%) and 26 women (77.08%). Table 1 shows no significant differences ($P > 0.05$) in healthy volunteers' demographic characteristics between groups. The volunteers were people aged 18–50 years old: 30 subjects (83.33%) were ≤25 years,

and 6 (16.67%) were >25 years. Based on body mass index (BMI), they were divided into two categories: 26 (72.22%) were BMI < 25 (non-obese), and 10 (27.78%) were BMI \geq 25 (obese). Two subjects (5.55%) had a history of hypertension, while the other 34 (94.45%) did not have such a history. Based on the level of education, most volunteers (75%) were university students or graduates, while the rest (25.00%) graduated from high school.

The Clinical Condition of Volunteers Before Treatment

Table 2 shows that the volunteers' age, weight, pulse, and blood pressure were within normal limits. Before treatment, volunteers' cell blood, blood sugar, cholesterol, and triglyceride levels were similar among groups. There were no significant differences in age, weight, heart rate, and blood pressure among groups ($P > 0.05$). Before the treatment, cell blood, blood sugar, cholesterol, and triglyceride levels were also within normal limits. Based on the literature, normal blood pressure for healthy males/females aged <65 is <140/90 mmHg. Normal blood sugar, triglyceride, and cholesterol levels according to the American Diabetic Association are <5.17 mmol/L, 1.7 mmol/L, and 5.17 mmol/L, respectively.^[22]

Blood Pressure and Pulse Before and After Treatment

Blood pressure and pulse rate were measured on day 0 (pre-treatment as controls), day 10, and day 20 during the research process. Table 3 presents the average blood pressure in the

control and treatment groups. Blood pressure and heart rate measurements were performed on day 0 (pre-treatment as controls), the 10th day, and the 20th day to monitor the volunteers' clinical condition during the research process. Table 3 shows that BCSO administration for 20 days did not affect blood pressure. The average of blood pressure in both systole and diastole as a whole in the measurement of day 0 compared to the 10th day did not differ significantly ($P > 0.05$).

Table 3 also shows that the BCSO treatment did not affect healthy volunteers' heart rate ($P > 0.05$). This study's results are in line with a preclinical *in vivo* study, wherein healthy test animals, the administration of black cumin seeds for 2 months did not influence cardiac hemodynamic.^[23] Black cumin seed administration for 20 days is not shown to change myocardium contractions' strength or frequency. This result is different from the previous study that the BCSO administration of 5 ml/day for 8 weeks in healthy volunteers could reduce both systolic and diastolic blood pressure.^[12] However, in this study, the BCSO dose given to healthy volunteers was smaller, and the duration of administration was relatively shorter than in the previous research.^[12] Thus, the treatment group's BCSO administration did not significantly affect blood pressure and pulse rate among the treatment groups compared to the control.

Hemograms Before and After Treatment

Table 4 shows that the administration of BCSO dosages of 3 \times 1, 3 \times 2, and 3 \times 3 capsules/day for 20 days in healthy volunteers did not affect the number of erythrocytes, lymphocytes, and platelets, and other blood components. Average Hb levels are >13 mg/dl in

Table 1: Demographic characteristics of healthy volunteers for the study of the effects of BCSO administration on renal function in Yogyakarta

Characteristics	Group			Total (%)	P
	Group 1(%)	Group 2(%)	Group 3(%)		
Sex (Male/female)	3/9 (33.33)	3/8 (37.5)	4/9 (44.44)	10/26 (38.46)	0.94
Age>25 year (yes/no)	2/10 (20)	2/10 (20)	2/10 (20)	6/30 (20)	1
BMI \geq 25 (Yes/No)	3/9 (33.33)	3/8 (37.5)	4/9 (44.44)	10/26 (38.46)	0.95
Hypertension history (yes/no)	1/12 (8.33)	0/11 (0)	1/11 (9.09)	2/34 (5.88)	0.63
Education level (Senior high school/University)	3/9 (33.33)	3/9 (33.33)	3/9 (33.33)	9/27 (33.33)	1
Job (Private/Student)	3/10 (30)	3/9 (33.33)	2/9 (22.22)	8/28 (28.57)	0.91
Marital status (Yes/No)	2/11 (18.18)	2/10 (20)	1/10 (10)	5/31 (16.13)	0.85

Table 2: Clinical state of the volunteers before the BCSO administration

Clinical characteristics	Groups			P-value
	Group 1	Group 2	Group 3	
Age (year)	25.83 \pm 6.58	24.08 \pm 4.48	23.83 \pm 4.45	0.6
Bodyweight (kg)	57.42 \pm 11.33	58.00 \pm 16.18	61.50 \pm 17.66	0.8
Heart rate (BPM*)	73.00 \pm 4.39	76/16 \pm 7.79	75.50 \pm 5.60	0.6
Systolic blood pressure (mmHg)	116.25 \pm 16.80	117.00 \pm 14.19	114.35 \pm 17.89	0.8
Diastolic blood pressure (mmHg)	73.75 \pm 10.02	73.33 \pm 13.70	73.75 \pm 12.45	0.7
Blood glucose level (mmol/L)	4.95 \pm 0.74	5.24 \pm 0.82	5.19 \pm 0.92	0.7
Cholesterol (mmol/L)	4.58 \pm 0.60	5.09 \pm 0.63	4.75 \pm 0.81	0.2
Triglyceride (mmol/L)	1.10 \pm 0.39	1.69 \pm 1.08	1.12 \pm 0.61	0.1

*BPM: Beats per minute; Group 1: 3 \times 0.5 ml/day; group 2:3 \times 1 ml/day; and group 3: 3 \times 1.5 ml/day

Table 3: Mean blood pressure on day 1, day 10, and day 20 after BCSO administration for 20 days in healthy volunteers

Groups	n	Parameters	Pre-treatment (control)	10 th day	20 th day	P-value
Group 1	12	Systolic BP (mmHg)	116.25±16.80	109.50±11.29	110.83±11.64	>0.05
		Diastolic BP (mmHg)	73.75±10.02	70.08±7.26	69.58±9.40	>0.05
		Heart rate (BPM*)	73.00±4.39	75.00±5.42	76.33±8.97	>0.05
Group 2	12	Systolic BP (mmHg)	117.00±14.19	116.67±14.03	112.50±12.88	>0.05
		Diastolic BP (mmHg)	73.33±13.70	70.00±9.04	65.83±11.83	>0.05
		Heart rate (BPM*)	76.16±7.79	78.33±8.97	74.16±7.00	>0.05
Group 3	12	Systolic BP (mmHg)	114.35±17.89	112.08±14.37	107.92±16.98	>0.05
		Diastolic BP (mmHg)	73.75±12.45	72.91±9.40	70.41±12.14	>0.05
		Heart rate (BPM*)	75.50±5.60	74.83±5.74	77.33±7.92	>0.05

*BPM: Beats per minute; Group 1: 3 × 0.5 ml/day; group 2: 3 × 1 ml/day; and group 3: 3 × 1.5 ml/day

Table 4: Hemogram before and after BCSO administration of 3 × 1, 3 × 2, and 3 × 3 capsules for 20 days in healthy volunteers

Characteristics	Group 1		Group 2		Group 3		P-value
	Pre	Post	Pre	Post	Pre	Post	
Hb (g/dl)	13.58±1.32	13.33±1.47 ^a	13.18±1.02	13.62±1.60 ^a	13.88±1.42	14.12±0.57 ^a	>0.05
Erythrocytes (T/L)	4.92±0.47	4.87±0.31 ^a	4.62±0.17	5.09±0.41 ^a	4.12±0.27	5.06±0.46 ^a	>0.05
Hematocrits (%)	40.76±1.23	39.53±3.79 ^a	41.36±2.21	40.0±3.87 ^a	41.76±2.34	41.69±1.3 ^a	>0.05
Leukocytes (G/L)	8.21±2.71	8.34±1.64 ^a	7.42±2.51	8.44±1.85 ^a	9.25±2.91	7.61±1.63 ^a	>0.05
MCV (fL)	81.94±6.47	81.30±7.3 ^a	82.10±5.47	80.98±8.0 ^a	84.10±4.47	82.84±5.57 ^a	>0.05
MCH (pg)	27.18±1.52	27.38±2.75 ^a	26.45±2.52	26.9±3.45 ^a	26.68±2.52	28.04±1.98 ^a	>0.05
MCHC (pg)	33.28±0.95	33.66±0.81 ^a	33.08±0.45	33.13±1.32 ^a	32.26±0.65	33.86±0.56 ^a	>0.05
RDW (fL)	13.47±1.10	13.97±2.29 ^a	14.17±1.20	13.75±1.80 ^a	13.27±1.30	13.14±0.80 ^a	>0.05
Platelet (G/L)	301±66.44	302±70.56 ^a	311±76.44	312±80.71 ^a	300±46.44	305±52.66 ^a	>0.05
Lymphocytes(%)	32.69±7.82	31.75±4.98 ^a	31.69±7.82	33.33±7.86 ^a	32.99±7.82	33.58±8.24 ^a	>0.05
Monocytes (%)	6.94±1.36	7.00±1.54 ^a	6.64±1.06	6.67±1.23 ^a	6.14±1.39	8.42±3.90 ^a	>0.05
Neutrophil (%)	57.29±8.70	57.83±6.28 ^a	56.99±8.78	57.67±8.82 ^a	57.59±8.60	55.42±10.76 ^a	>0.05
Eosinophil (%)	3.08±1.91	3.42±2.81 ^a	3.78±1.61	2.33±1.44 ^a	3.38±1.31	2.82±2.02 ^a	>0.05
ESR first hours (mm/hour)	16.42±12.51	14.58±11.0 ^a	16.12±12.51	14.00±11.0 ^a	15.32±12.51	16.08±11.07 ^a	>0.05
ESR second hours (mm/hour)	33.98±21.93	34.25±20.67 ^a	32.78±21.93	30.75±22.12 ^a	31.98±21.93	32.75±20.63 ^a	>0.05

^aP>0.05 from dependent t-test intergroup, Hb: Hemoglobin, ESR: Erythrocytes sedimentation rate during the 1st/2nd h, RDW: Red cell distribution width, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

men and >12 mg/dl in women, and in this study, the volunteers' Hb levels were >13 mg/dl in all groups hence within normal limits both before and after the treatment. There was no difference in the mean Hb levels among groups after the BCSO administration for 20 days ($P > 0.05$). The number of normal erythrocytes was 3–7 million cells/dl. In this study, the numbers of erythrocytes in all groups were within normal limits ($>4 \times 10^6$ cell/dl). There was no difference in the mean number of erythrocytes among groups after the BCSO treatment for 20 days ($P > 0.05$). Overall, the number of leukocytes, erythrocytes, and platelet were all within the normal limits, and there was no statistically significant difference in blood cell count among groups ($P > 0.05$).

Until now, there is no clinical evidence of the effect of BCSO on human blood cell counts. Research data showed that consumption of 3 × 1, 3 × 2, and 3 × 3 capsules/day of BCSO for 20 days did not affect the blood cell count. The duration and dose of BCSO administration and subject characteristics appear to be determinants of the hemogram appearance of the

study results. The results of this study are also in accordance with the previous studies.^[19,21]

In vivo studies show that TQ administration in DM-made mice showed in the laboratory that TQ was shown to increase lymphocyte.^[24] The administration of *N. sativa* preparations improved biochemical parameters, blood composition, and immune response in mammary cancer model animals.^[25] This study found that BCSO standardized TQ did not affect the number of blood cells, including lymphocytes. The administration for 20 days with variations in daily doses of 3 × 1, 3 × 2, and 3 × 3 capsules/day in the healthy volunteers was vital to allay the uncertainty surrounding the BCSO safety.

Kidney Function Before and After Treatment

The parameters of kidney function before and after administration of BCSO are presented in Table 5. Table 5

Table 5: Levels of blood urea, blood creatinine, and pH urine in healthy volunteers before and after 20-day administration BCSO dosages of 3 × 0.5 ml, 3 × 1 ml, and 3 × 1.5 ml

Characteristics	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	P
Urea (mmol/L): pre	2.85±0.36	3.00±0.42	2.45. ±0.65	>0.05
Post	2.97±0.12 ^a	3.27±0.27 ^a	2.76±0.21 ^a	>0.05
Creatinine (umol/L): pre	62.78±9.73	62.78±10.11	63.66±14.13	>0.05
Post	62.78±6.19 ^a	68.97±13.26 ^a	62.78±14.67 ^a	>0.05
Urine density (kg/L): pre	1.01±0.01	1.01±0.01	1.02±0.01	>0.05
Post	1.02±0.01 ^a	1.01±0.01 ^a	1.01±0.01 ^a	>0.05
Urine pH: pre	5.83±0.71	5.79±0.72	5.79±0.33	>0.05
Post	5.23±0.21 ^a	5.53±0.41 ^a	5.53±0.61 ^a	>0.05

^aP>0.05 from dependent t-test pre-post internal group

presents the results of the investigation of renal function and urinalysis. It shows that urea levels and creatinine levels were normal in all groups both before and after the BCSO administration of 20 days. There were no differences in mean blood urea levels, blood creatinine levels, and density and pH urine before and after the treatment ($P > 0.05$).

This finding is consistent with the results of the other studies. Akrom *et al.* (2021) showed that consumption of 3 × 1, 3 × 2, and 3 × 3 capsules/day for 30 days in healthy volunteers who smoked did not affect platelet counts, blood clotting time, and kidney function.^[26] Clinical trials of additional therapy of BCSO 2.5 ml/day orally for 12 weeks in patients with CKD have been shown to improve renal function and be safe.^[27] The safety test of the combination of BCSO 2.5 ml/day and amino acid analogs for 12 weeks in CKD patients is safe and increases the therapeutic effect of amino acid analogs.^[28-30] However, the previous studies have shown that consumption of BCSO 5 ml/day for 8 weeks by healthy volunteers has been shown to reduce blood pressure (Huseini *et al.*, 2013). Of course, the incidence of lowering blood pressure in healthy volunteers is an unexpected effect.

It has been reported that *in vivo* studies of TQ or BCSO were nephroprotective.^[31] The previous research has shown that TQ plays a role in protecting the kidneys from the threat of damage through anti-inflammatory, antioxidant, and anti-apoptotic activities. The 2 ml/kg *N. sativa* Oil or 50 mg/kg TQ by oral consumption for 10 weeks improves renal function in STZ-induced diabetic nephropathy. Other studies have shown that TQ has protective effects against I/R injury to the experimental models' kidneys. Other researchers have shown that TQ acts to inhibit the renin-angiotensin system. Oral consumption of TQ (10 mg/kg/day) 4 days before the I/RI for 6 days improved the I/RI effects on the tubular renal functional and hemodynamic parameters as well as the expression of some renal damage markers, profibrotic, and proinflammatory cytokines, which showed TQ has renoprotective effects on I/RI-induced renal disorders.^[32,33] In line with *in vivo* studies, other researchers also pointed out the possibility of TQ toxicity. TQ toxicity generally occurs in large doses, more than > ×20 the effective dose. The maximum tolerated dose (MTD) of TQ, which is defined as the highest dose that is safe to administer to animal models in the absence of intolerable adverse effects, was determined in male and female Wistar rats. The findings indicated that the MTD for intraperitoneal injection was 22.5 mg/kg in male rats and 15 mg/

kg in females, whereas for oral administration, it was 250 mg/kg in both male and female rats. Two rats died after administration of 500 mg/kg of TQ due to bowel obstruction.^[34,35]

The results of this study were not as expected. The effect of administering BCSO doses of 3 × 1, 3 × 2, and 3 × 3 capsules/day for 20 days on hemogram images of blood and kidney function has not been determined. The BCSO capsules tested contained 500 mg of BCSO/capsule. Each BCSO capsule contained 13 mg of TQ, the equivalent of 362 mg of unsaturated fatty acids, and 117 mg of saturated fatty acids, at a dose of 3 × 1, 3 × 2, and 3 × 3 capsules/day that it is far below the MTD value. Hence, kidney function's effect is difficult to determine, especially with the short duration of administration (20 days).^[36]

BCSO was able to improve cardiometabolic risk factors in patients with low-calorie diets.^[37] Other researchers have reported that BCSO can reduce blood sugar levels in healthy volunteers,^[38] in metabolic syndrome,^[39] as a chemopreventive *in vivo*,^[40] and as an antioxidant *in vivo*.^[41] The active ingredient content of BCSO, TQ, can inhibit anti-stress oxidative responses through the CHEK pathway.^[42]

Apart from being a preliminary test regarding the effect of BCSO consumption on kidney function in healthy volunteers, this study's results can be used to determine the duration and initial dose of BCSO of testing in future studies.

Based on the result, it can be concluded that the administration of BCSO for 20 days in healthy volunteers has not shown its effect on kidney function. It is necessary to plan a study with a longer duration and a higher dose to determine the effect of BCSO on kidney function.

CONCLUSION

Based on the result, it can be concluded that the administration of BCSO for 20 days in healthy volunteers has not shown its effect on kidney function. It is necessary to plan a study with a longer duration and a higher dose to determine the effect of BCSO on kidney function.

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CONFLICTS OF INTEREST

There is no conflicts of interest in this research.

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