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Comparison of almond and buckwheat oils on blood biochemical and liver function in rats with non-alcoholic fatty liver

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ABSTRACT

Introduction: almonds and buckwheat are known as useful oil seeds. So our objective was to determine the effects of their oil on the blood biochemical and liver function in rats with non-alcoholic fatty liver disease. *Materials and Methods*: Forty adult rats were divided into five groups. The experimental groups for 28 days were as follows; 1) control standard (normal rats), NAFL rats groups orally received 2) 1ml normal saline (control sham), 3) 1.5 ml/kg almond oil, 4) 5 ml/kg buckwheat oil, and 5) 1.5 ml/kg almond+5 ml/kg buckwheat oils. The weight of the body and liver were recorded. Liver weight was significantly higher in the high fat diet group comparing to the control groups ($P<0.05$). *Results*: Results showed that serum levels of blood urea nitrogen (BUN), creatinine,

IL-6 and cholesterol were significantly lower than those in the sham groups, whereas HDL concentration was higher. Compared with control-sham groups, the administration of almond oils significantly Decrease levels of creatinine, cholesterol, and LDL. Liver enzymes, inflammatory markers and BUN levels were lower in almond and buckwheat oil supplemented rats compared to control-sham, Also, the serum concentration of HDL was markedly increased in rats fed with dietary almond and buckwheat oils treatments, although, oils supplementation were significantly reduced serum concentration of LDL and cholesterol in NAFL rats. Following almond+buckwheat oils administration, slightly decreased ($P<0.05$) serum levels of BUN, LDL, and IL-6 comparing to almond and buckwheat alone. Also, almond+buckwheat oils supplementation showed higher values for serum Na and K levels comparing to almond and buckwheat alone.

Keywords: Almonds, Liver enzyme, Buckwheat, Liver, Non-alcoholic fatty liver

1. INTRODUCTION

The health science has faced with major challenges for management of liver disorder. Nowadays, lifestyle modifications consisted of diets rich in seed plant and low in saturated fat foods, orderly participation in physical activity and avoids the pollutants could affect the liver function, and common therapeutic methods often results in unsatisfactory results. Also, there is an increasing interest in this topic (natural functional food), due to the overall liver damage increase in industrialized countries, but there is still much to know concerning the effect of natural oils consumption on the liver system.

Approximately 30 million people in the USA and 29 million people in the European Union suffer from chronic liver disease (Miltonprabu et al., 2016). Non-alcoholic fatty liver (NAFL) disease is one of the common forms of liver diseases, and is closely related to metabolic syndrome and its related conditions, diabetes mellitus and dyslipidemia (Bagherniya et al., 2017). Among the variety of tree plant foods (almonds, hazelnuts, and walnuts) that may improve liver health and function. Almonds are rich in mono-unsaturated fat, α-tocopherol and phytochemicals including phytosterols and polyphenols (Chen et al., 2015), and also contain non-lipid components such as antioxidant vitamins (vitamin E), plant protein rich in arginine, magnesium, copper and fiber (ROS, 2008). Buckwheat known as powerful functional properties for use in a novel class of food preparations known as Food for Specified Health Use (Brunori et al., 2009) and could possibly be considered as a prebiotic product (Prestamo et al., 2003). Also, buckwheat has been regarded as a natural ingredient which has beneficial health properties such as anti-cancer, anti-inflammatory, anti-hypercholesterolemic (Brensel et al., 2013; Gimenez-Bastida & Zielin, 2015; Misan et al., 2017; Seval et al., 2014), and preventing the development of obesity (Nishimura et al., 2016) and diabetes (Qiu et al., 2016).

However, no studies are available that examine the effect of almonds and buckwheat oils (especially combination form) on blood biochemical, liver enzymes, inflammatory markers and histology of liver in rats with non-alcoholic fatty liver (NAFL) disease. Therefore, we hypothesized that administration of almond and buckwheat oils would improve liver function in rats with NAFL disease via an increase in blood biochemical availability, liver enzymes, anti-inflammatory situation, and/or reduction in dyslipidemia.

2. MATERIALS AND METHODS

Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany). The kits (Cosmo Bio Co. Tokyo, Japan) were prepared to evaluate blood biochemical blood urea nitrogen (BUN), creatinine, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), sodium (Na) and potassium (K)), liver enzymes (aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP)) and inflammatory markers (interleukin 6 (IL-6) and C-reactive protein (CRP)).

Animals

Healthy adult male Wistar rats (180±20 g; 9-week-old) were purchased from the Pasteur Research Center (Karaj, Iran). Rats were kept in an air-conditioned animal room under 12 h light: dark cycle under standard environmental conditions (22±2 °C, 52±5% humidity) with free access to tap water and commercial dry pellet diet. Rats were housed in polypropylene cages lined with pine wood husk, changed every day. Experimental protocols were approved by the Ethics Review Committee of Medicine, Shiraz University of Medical Sciences (Permit Number: A: 20-1732).

Experimental design

Forty rats were divided into five groups in a factorial arrangement of almond and buckwheat oils treatments. High fat emulsion diet for inducing NAFL disease was prepared based on Zou et al. (2007), protocol. The experimental groups for 28 days were as follows;

1) control standard (normal rats), NAFL rats groups orally received 2) 1 ml normal saline (control sham), 3) 1.5 ml/kg almond oil (Zhao et al, 2007), 4) 5 ml/kg buckwheat oil (Seval Develi et al., 2014), and 5) 1.5 ml/kg almond+5 ml/kg buckwheat oils. The weight of the body and liver were recorded.

Hormonal assays

Blood was obtained via the tail vein (weekly) for the assay of blood biochemical, liver enzymes and inflammatory markers concentration. The serum was separated by centrifugation (4000 rpm for 10 min), kept at –20 °C and assessed for blood biochemical by radioimmunoassay (kit of Monobind Inc), as recommended by the manufacturer. The concentrations of AST, ALT and ALK in the serum were measured by an automatic analyzer. Measurements obtained in the fasting state.

Statistical analysis

Our data were analyzed by SPSS 17.0 software. In order to compare the treatments, MANOVA, t-test and Duncan test were used for multiple comparisons between groups ($P<0.05$). The results were expressed as the mean ± standard deviation (SD).

3. RESULTS

The results showed, there was a significant difference between the study groups in the inflammatory markers (pg/ml; ng/ml) ($p<0.01$). So that, the results of Tukey's post-hoc test showed that using black seed oil and almond oil and their combination significantly decreased the inflammatory markers of Interleukin-6 (IL-6) and C-reactive protein (CRP) in comparing to placebo group (Table 1 and Diagram 1). The results of this study showed, blood biochemical markers (mg/dl) were significantly different between study groups ($p<0.01$). So that, the results of Tukey's post-hoc test showed, using black seed oil and almond oil and their combination significantly decreased the blood urea nitrogen (BUN), creatinine and sodium (Na); and significant increase of potassium (K) in comparing of placebo group (Table 1 and Diagram 2).

Table 1 Effects of dietary black Seed oil and almond oil on inflammatory markers (pg/ml ; ng/ml), blood biochemical markers (mg/dl), lipid-related markers (mg/dl) and liver enzymes (U/L)

	Variable	Group	N	Mean	Std. Deviation	Df	F	Sig
Inflammatory Markers (pg/ml ; ng/ml)	Interleukin 6 (IL-6)	Black Seed Oil	8	71.682	8.788	4	20.676	0.000
		Almond Oil	8	86.057	9.205			
		Black Seed Oil / Almond Oil	8	62.1	6.255			
		Placebo Group	8	99.791	4.623			
		Control	8	74.2	13.627			
	C-reactive protein	Black Seed Oil	8	6.338	0.306	4	17.773	0.000
		Almond Oil	8	7.859	1.474			
		Black Seed Oil / Almond Oil	8	7.71	0.926			
		Placebo Group	8	9.388	0.511			
		Control	8	5.913	0.94			
Blood Biochemical Markers (mg/dl)	Blood urea nitrogen (BUN)	Black Seed Oil	8	22.38	2.504	4	36.496	0.000
		Almond Oil	8	22.63	1.302			
		Black Seed Oil / Almond Oil	8	15.63	2.446			
		Placebo Group	8	29	3.117			
		Control	8	16.38	2.973			
	Creatinine	Black Seed Oil	8	0.736	0.076	4	11.178	0.000
		Almond Oil	8	0.77	0.036			
		Black Seed Oil / Almond Oil	8	0.843	0.044			
		Placebo Group	8	1.207	0.382			
		Control	8	0.682	0.037			

Lipid-related Markers (mg/dl)	Sodium (Na)	Black Seed Oil	8	142.5	3.742	4	44.514	0.000
		Almond Oil	8	140.75	2.053			
		Black Seed Oil / Almond Oil	8	146.25	2.493			
		Placebo Group	8	179.88	15.77			
		Control	8	136.5	1.604			
	Potassium (K)	Black Seed Oil	8	4.75	0.922	4	27.575	0.000
		Almond Oil	8	5.262	0.792			
		Black Seed Oil / Almond Oil	8	5.512	0.705			
		Placebo Group	8	1.837	0.388			
		Control	8	4.462	1.007			
	Cholesterol	Black Seed Oil	8	215.875	6.998	4	103.801	0.000
		Almond Oil	8	229.25	9.192			
		Black Seed Oil / Almond Oil	8	228.625	5.262			
		Placebo Group	8	269.5	14.909			
		Control	8	154.5	16.818			
	High-density lipoproteins (HDL)	Black Seed Oil	8	46.5	3.78	4	18.844	0.000
		Almond Oil	8	41.38	7.596			
		Black Seed Oil / Almond Oil	8	44.5	8.418			
		Placebo Group	8	26.75	4.287			
		Control	8	58.63	10.806			
	Low-density lipoprotein (LDL)	Black Seed Oil	8	109.88	7.53	4	122.325	0.000
		Almond Oil	8	126.63	5.927			
		Black Seed Oil / Almond Oil	8	113.13	1.959			
		Placebo Group	8	169	10.596			
		Control	8	104.88	3.72			
Liver Enzymes (U/L)	Aspartate aminotransferase (AST)	Black Seed Oil	8	101	9.577	4	94.399	0.000
		Almond Oil	8	105	8.552			
		Black Seed Oil / Almond Oil	8	101.88	4.016			
		Placebo Group	8	145.63	22.841			
		Control	8	29.13	6.833			
	Alanine aminotransferase (ALT)	Black Seed Oil	8	68.63	13.763	4	49.418	0.000
		Almond Oil	8	73.38	13.049			
		Black Seed Oil / Almond Oil	8	74.25	9.513			
		Placebo Group	8	109.5	7.483			
		Control	8	34.38	8.28			
	Alkaline phosphatase (ALP)	Black Seed Oil	8	723.5	103.761	4	13.811	0.000
		Almond Oil	8	811.25	162.925			
		Black Seed Oil / Almond Oil	8	853.75	73.673			
		Placebo Group	8	1280	562.213			
		Control	8	291.13	41.049			

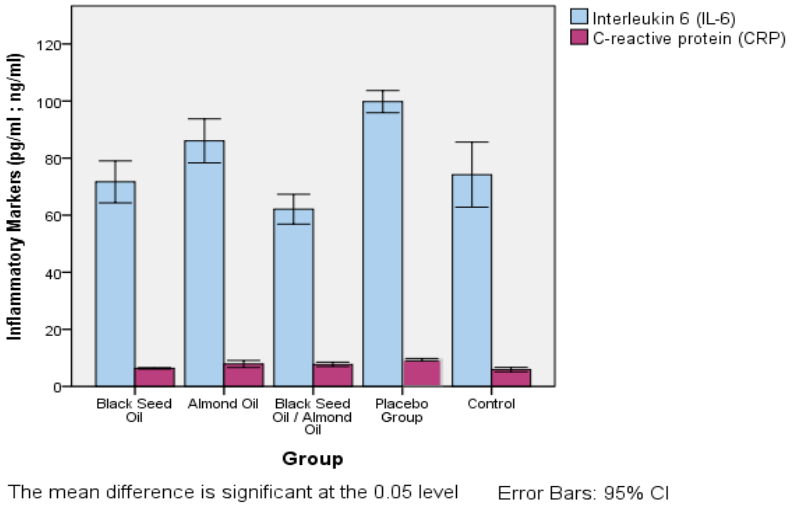

Diagram 1 Comparison of changes in inflammatory markers (pg/ml; ng/ml) of IL-6 and CRP in study groups

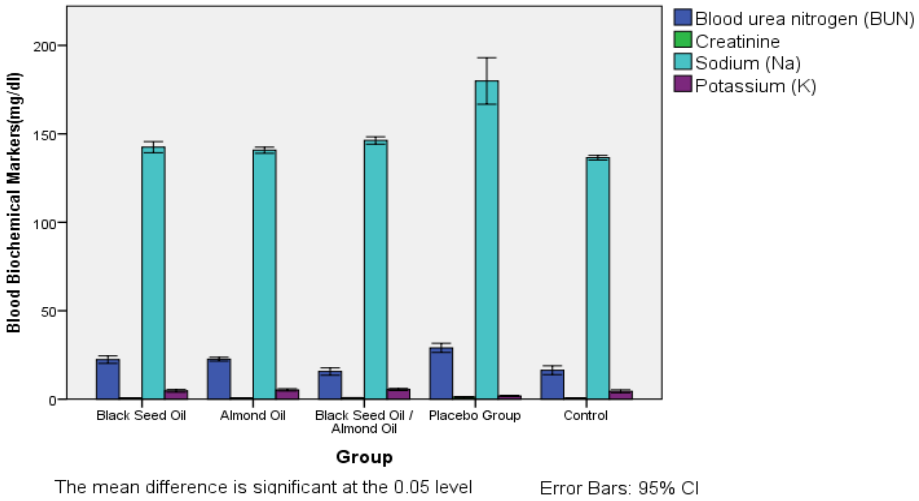

Diagram 2 Comparison of changes in blood biochemical markers (mg/dl) of blood urea nitrogen (BUN), creatinine, sodium (Na) and potassium (K) in study groups

Also, in lipid-related Markers (mg/dl) there was a significant difference between study groups ($p<0.01$). So the results, Tukey's post-hoc test showed that using black seed oil and almond oil and their combination significantly decreased the cholesterol and low-density lipoprotein (LDL) in comparing of the placebo group; and increased the serum level of high-density lipoproteins (HDL) (Table 1 and Diagram 3).

According to the results of this study, between groups studied in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) liver enzymes significant difference was observed ($P< 0.01$). So that, the results of Tukey's post-hoc test showed that black seed oil and almond oil using and their composition significantly decreased AST, ALT and ALP in comparing of placebo group (Table 1 and Diagram 4).

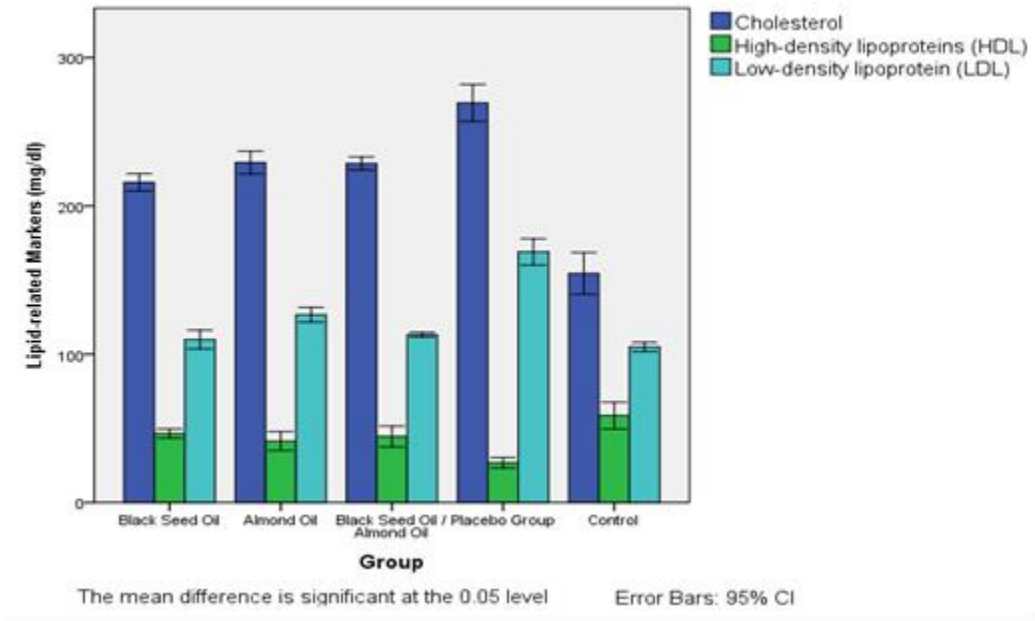

Diagram 3 Comparison of changes in lipid-related Markers (mg/dl) of cholesterol, high-density lipoproteins (HDL) and low-density lipoprotein (LDL) in study groups

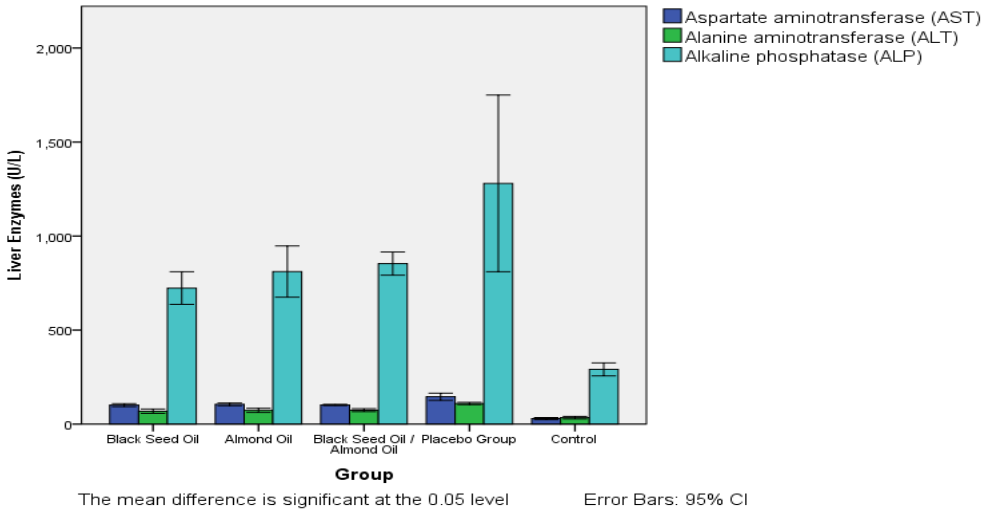

Diagram 4 Comparison of changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) liver enzymes in study groups

4. DISCUSSION

In the recent, years, new technologies and techniques initiate innovation. Further understanding of natural oils and their effects on the liver system could help improving liver disorder in patients. Animal models of NAFL disease are valuable for studying the physiological changes and treatment of liver disorder as well as its relationship to other metabolic syndrome. To the best of our knowledge, this is the first comprehensive experiment exploring the mechanistic basis for the beneficial liver effects of almond and buckwheat (single and combined) by using NAFL disease rat models. Traditionally, natural oils nut and plant seed could have commonly been used to treat free radicals, and tissue injured after inflammation. The difference in blood biochemical, liver enzymes, inflammatory markers and histology of liver between control and high-fat fed rats is likely attributed to differences in daily diet intake. Following continuous feeding with high-fat emulsion diet (10 ml/kg) for 8 weeks, HDL was lower than (37.62 mg/dl) those in the normal control group (53.87 mg/dl). This finding, in line with the results Zou et al. (2007) suggests that high-fat emulsion administrations could be cause for decreasing of HDL concentration after NAFL disease, which likely associated with the

antiatherogenic properties of HDL. Also, this observation supports existing evidence that HDL facilitates the uptake of cholesterol from peripheral tissues and its transport to the liver for catabolism and excretion (Miller, 1975). It is proposed that a reduction of serum HDL concentration may accelerate the development of NAFL disease.

Research on almonds suggests they may have beneficial actions on serum cholesterol, body weight, glucose homeostasis, inflammation, and oxidative stress (Griel & Kris-Etherton, 2006; Kamil & Chen, 2012). Further, Chen et al. (2015) reported that the addition of almonds to diet did not significantly modify the serum lipid profile and CRP. Whereas, our results showed that during the 4 weeks of almond oils (1.5 ml/kg bw) treatment, the serum lipid profile (cholesterol and HDL) was significantly improved, and the general condition of the rats remained satisfactory. Some of the components of almonds such as arginine, magnesium, fiber and vitamin E have demonstrated anti-inflammatory properties (Blomhoff et al., 2006; Kris-Etherton et al., 2016). Similarly, a Mediterranean diet with nuts was effective in lowering IL-6 levels compared with a control diet without nuts (Estruch et al., 2006).

This result indicated that regular consumption of almond oils leads to modify the serum lipid profile in the NAFL disease rats. Also, results showed that orally administrated of almond oil, reduced the serum concentration of LDL in rats submitted to NAFL disease. Whereas, studies have shown that the use of antioxidant plants in diet can prevent the oxidative changes created by free radicals and other reactive species (Soler et al., 2000). Antioxidants may also prevent oxidation of LDL in lambs (Aoudi et al., 2014). In confirmation of these findings, clinical studies in healthy and hypercholesterolaemic adults (Mukuddem-Petersen et al., 2005; Griel & Kris-Etherton, 2006), have shown that nuts lower LDL-cholesterol while improving the overall blood lipid profile.

The results presented in this study demonstrated that supplementation of almond oil at NAFL disease rats, improved the serum concentration of liver enzymes (such as ALT, ATS and ALK were measured to specify the antioxidant activity *in vivo*). Results are in agreement with those reported by Jamshed and Gilani (Jamshed & Gilani, 2014). Finally, it seems that the consumption of almond oil, as an antioxidant, alleviates negative effects of NAFL disease on liver system through blocking or preventing *reactive oxygen species (ROS*) production and partially restored liver. The idea is confirmed by other researchers who showed that essential oils, or antioxidants, prevent ROS production through interaction with peroxide radicals (Carrasco-Pozo et al., 2012; Gimenez-Bastida & Zielin, 2015). With regards to dietary inclusion of buckwheat, Prestamo et al. (2003) have reported that dietary inclusion of buckwheat could as well be considered as a healthy food due to the diminution on total cholesterol. Compared with normal rats, treatment of the high-fat group of rats with buckwheat oil significantly decrease blood lipids profile (Table 1, $P<0.05$). Whereas, a previous study has reported that no significant differences in lipid metabolism parameters such as TC, HDL, and LDL existed between the tartary buckwheat and the control group. However, the ingestion of the tartary buckwheat significantly decreased body weight (Nishimura et al., 2016).

During recent years, many herbal supplements are used in traditional therapy for their protective and therapeutic properties against liver disorders. Among their bioactive components, flavonoids have been found to be active against liver dysfunction and damage caused by liver diseases (Miltonprabu et al., 2017) Improved antioxidant statue in almond and buckwheat oils (single and combined) can be due to antioxidant properties and phenolic components of mentioned oils that reduces effects of NAFL disease on liver system and could help to absorb the amino acids. Serum CRP and IL-6 levels were significantly lower on the almond+buckwheat diet compared with the control high fat diet (sham). These findings were confirmed by other researchers who showed frequent nut and seed consumption is associated with lower levels of inflammatory markers such as CRP (a peripheral marker of inflammation), IL-6 and fibrinogen, even after adjusting for confounding factors (Jiang et al., 2006). High-sensitivity C-reactive protein has been developed and uses as a marker to predicting coronary vascular diseases in metabolic syndrome, and it was recently used as a predictor for NAFL disease in correlation with serum markers that indicated lipid and glucose metabolism (Kogiso et al., 2009).

5. CONCLUSION

With due attention to the positive effect of almond and black seed oil on the improvement of oxidative stress related indicator and liver function and since using the chemical drugs usually have side effects in treatment of fatty liver disease, therefore daily use of vegetable oils as a harmless method to prevent and improve this disease is effective.

List of Abbreviations

NAFLD: Non-alcoholic fatty liver
AST: Aspartat Aminotansferase
ALT: Alanin Aminotansferase
ALP: alkaline phosphatase
BUN: blood urea nitrogen

HDL: high density lipoprotein
LDL: low density lipoprotein
IL-6: interleukin 6
CRP: C-reactive protein

Conflict of interest statement

The authors report that they have no other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Author contributions

All authors have an equal contribution to writing, concept design and data collection.

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