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**ARTICLE**

**Evaluation of effects of various rapeseed oils on serum lipids and cardio-hepatic enzymes in experimental rats**

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Md. Hafizur Rahman, Shaikh Shahinur Rahman, Dipa Mandal  
and Md. Nazibur Rahman

*Full Length Research Paper*

## Evaluation of effects of various rapeseed oils on serum lipids and cardio-hepatic enzymes in experimental rats

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Rapeseed oils are commonly used as edible oil in rural and urban areas in Bangladesh. This study used various rape seeds like mustard, rai and canola oils to investigate the effect on lipids and cardio-hepatic enzymes in experimental rats. Body weight, food efficiency ratio (FER), serum lipids and cardio hepatic enzymes were measured in this study. Body weights were reduced in fried rai (37.67%) and mustard oils (33.18%) in comparison with the control and canola oils (19.78%). While both wild and hybrid mustard oils (20.97 and 24.72%, respectively) and rai oils (23.08 and 24.76%, respectively) also decreased the body weight. FER of rats was high in control group (0.11%) and lower in both fried mustard and rai oils (0.05%). Total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL) were significantly high in fried rai (56.11, 144.1 and 16.25 mg/dl, respectively) and fried mustard oils (55.03, 142.01 and 14.68 mg/dl, respectively) while the lowest were in control (39.04, 90.02 and 7.07 mg/dl, respectively) and canola oils (40.46, 95.06 and 7.80 mg/dl, respectively). On the contrary, high density lipoprotein (HDL) was raised in control group (28.06 mg/dl) and decreased in fried rai and mustard oils (11.12 and 12.07 mg/dl, respectively). This result also showed that cardio hepatic enzymes like creatine kinase-MB (CK-MB), aspartate amino transferase (AST) and alanine amino transferase (ALT) were increased in fried rape seeds than control and canola oils. Fried mustard oils were more detrimental than wild mustard oils and canola oils due to the highly concentrated erucic acids.

**Key words:** Mustard oils, canola, lipid profiles, aspartate amino transferase (AST), alanine amino transferase (ALT), creatine kinase-MB (CK-MB).

### INTRODUCTION

Rapeseed oils are one of the leading edible oils in Bangladesh and India (Pradeepkumar, 2008). In most cooking practices in Bangladesh, these oils are used for frying. Due to the presence of high amount of mono and polyunsaturated fatty acids and less saturated fatty acids

and no trans fats, it is good for health (Alternative Medicine, 2006). While De Wildt and Speijers (1984) showed that rapeseed oils have significantly higher erucic acid (20 to 55%) which have detrimental effect on health. Roine et al. (1960) also demonstrated the toxic effects of

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rapeseed oil. Hence, United States, Canada and most of the European countries banned culinary rapeseed oils (Malaysian Palm Oil Fortune, 2011; Economic Research Service, 2011). Although rapeseed oil reduced body weight by enzymatic disturbance of metabolic system, histopathological study showed changes in tissues of liver, heart and kidney (Badawy, 1994). Several varieties of rapeseed oil in Bangladesh contain high level of erucic acid (51.56 to 67.98%) which is raised upto 69.75 to 72.77% in fried form (Hafizur et al., 2017). Naturally, erucic acid is not present in body fat, but when the diet contains high erucic acid, it is found in depot fat, organ fat and milk fat (Borg, 1975). Severe myocardial lipidosis was evident in rats fed with high erucic acid rapeseed (HEAR) oil. Cardiac triglycerides and free fatty acids were also increased in HEAR oil fed rats (Kramer et al., 1992). Repeated or reused cooking oil notably increased erucic acid content during frying (Chacko and Rajamohan, 2011) and chronic intake of this oil could be harmful to health and increase risk of many diseases including hypertension and cancer (Srivastava et al., 2010; Soriguer et al., 2003). On the other hand, canola oil is the third most widely consumed vegetable oil in the world and contains low levels of erucic acid (Canola Wikipedia the Free Encyclopedia, 2017). Although different varieties of rapeseed oils are randomly used for cooking, frying and other purposes, the effect of these oils on health were not yet documented systematically. Thus, the aim of this study was to assess the effect of rapeseeds and canola oils on serum lipids and cardio-hepatic enzymes on rats.

## MATERIALS AND METHODS

### Experimental oils

Rapeseed oils like mustard and rai (wild, hybrid and fried formed) and canola oil (origin Canada) were collected from the local areas of Bangladesh.

### Frying procedure

Mustard and rai oils were heated according to the method of Owu et al. (1998). About 500 ml of oil was fried in a stainless steel pan for 10 min at 180°C and heated oil was obtained. The process was repeated two times to obtain three times heated oil with a cooling interval of at least 3 h. Then the heated samples were analyzed after cooling for the chemical analysis to evaluate the changes caused by frying.

### Chemicals

Analytically grade biochemical kits for the estimation of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), creatine kinase-MB (CK-MB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were procured from Atlas medical, Cambridge, CB4 OWX, UK. Analytical process was done according to manufacturer's protocol.

### Experimental animals and diet

Adult healthy Wistar male rats of age 5 weeks and average body weight of 70 to 100 g were purchased from the Department of Jahangirnagar University, Savar Dhaka, Bangladesh. They were housed in poly carbonate cages with steel wire tops and wood-cube bedding (5 rats per cage) at constant room temperature with relative humidity (55+5%) and 12h:12h dark-light cycle with available supply of distilled water and feed. The normal diet of the rats was the mixture of wheat flour (which contributes 30% of diet), molasses (which is a rich source of sugar mainly sucrose, glucose and fructose), and fish-meal that is an excellent source of protein.

### Experimental design

After one week of acclimatization with normal diet, Wistar rats were divided into eight groups (n=5) named as A, B, C, D, E, F, G and H. Group-A is considered as control (normal diet) and Group-B to H groups were given 0.3 g mustard (wild), mustard (hybrid), mustard (fried), rai (wild), rai (hybrid), rai (fried) and canola oil respectively per day with normal diet. According to Chakraborty (2003), 15% of erucic acid rich rapeseed oil is responsible for the development of heart lesion and 400 to 1500 mg/kg erucic acid significantly increased myocardial lipidosis in rats (Kramer, 1992). This study was carried out over a six week period and body weight, supplied foods and water *ad libitum* were recorded daily.

### Determination of average body weight gain and food efficiency ratio (FER)

After 6 weeks, the average body weight gain and FER of each rat was calculated by the following equation:

Cardiac lipidosis muscle and Body weight gain = (Final weight of average rat) – (Initial body weight) and Food efficiency ratio = Body weight gain / Food consumed.

### Statistical analysis

Statistical analysis was performed with SPSS for windows, version 11.5. Data were expressed as mean  $\pm$  standard deviation (SD) or mean  $\pm$  standard error (SE). Differences between the body weight of all groups and differences between serum indices of different groups of rats were analyzed using T-test. Significance was accepted at the  $p<0.05$  and highly significance was accepted at the  $p<0.01$  levels.

## RESULTS

Body weights of the experimental rats during the initial day were 80 to 92 g and after 6th weeks were 136 to 158 g. After 6th weeks, the body weight of the control group was increased 87.73% while the experimental oils reduced body weight gradually. Among these oils, fried form of rai (37.67%) and mustard oils (33.18%) significantly reduced body weight whereas mustard and rai of both wild and hybrid oils were 20.97, 24.72, 23.08 and 24.76%, respectively. Canola oil decreased only 19.78% body weight (Table 1). FER of the experimental groups was lower than control group (0.11) and the lowest one was 0.05 ( $p<0.01$ ) for both fried mustard and

**Table 1.** Effects of rapeseed oils on body weight changes.

Group	Diet provided	Effect of oils on body weight			
		Initial body weight (g) ±SD	Final body weight (g) ±SD	Change (%)	Reduction of body weight (%)
Group A (Control)	Normal diet	80.76 ±2.00	151.63±17.43	87.73	-
Group B	Mustard (wild)	90.50±3.98	153.25±2.86	69.33	18.4
Group C	Mustard (hybrid)	91.20±1.99	151.43±6.25	66.04	21.4
Group D	Fried mustard	85.90±12.22	136.26±10.25	58.62	19.11
Group E	Rai (wild)	90.10±5.31	150.90±4.50	67.48	20.25
Group F	Rai (hybrid)	91.00±6.20	151.06±8.58	66.00	21.73
Group G	Fried Rai	89.90±5.10	139.06±1.44	54.68	33.05
Group H	Canola	92.93±13.81	158.33±21.12	70.37	17.36

Values are mean ±SD; n=5.

**Table 2.** Effects of rapeseed oils on Food Efficiency Ratio of rats (FER).

Group	Diet provided	Initial body Weight (g)±SD	Final body Weight (g)±SD	Weight gain (g)±SD	Food intake (g)±SD	FER±SD
Group A (Control)	Normal diet	80.77±2.00	151.63±17.43	70.86± 15.43	644.18± 1.83	0.11±0.02
Group B	Mustard (wild)	74.36±7.88	135.00±7.77	62.73±0.11	704.0± 2.74	0.086±0.01
Group C	Mustard (hybrid)	91.20±1.99	151.43±6.25	60.23±4.26	752.88±2.00	0.08±0.02
Group D	Fried Mustard	85.97±24.24	136.33±18.77	50.36±5.47	970.23±1.95	0.05** <sup>a,b</sup> ±0.01
Group E	Rai (wild)	77.47±5.31	138.27±4.51	60.8±0.8	715.29±2.12	0.085±0.01
Group F	Rai (hybrid)	91.00± 6.21	151.06±13.08	60.06±6.87	816.71±2.16	0.07 <sup>a</sup> ±0.01
Group G	Fried Rai	9.9±5.10	139.06±1.44	4.16±1.27	915.4±1.1	0.05** <sup>a,b</sup> ±0.01
Group H	Canola	92.93±13.82	155.93±19.76	63.0±5.94	654.0±1.83	0.09±0.02

RSP: Rapeseed powder; FER: Food Efficiency Ratio; Values are mean ±SD; n=5; \*significant values p<0.05; <sup>a</sup>Values significant differ from control; \*\*High significant values p<0.01; <sup>b</sup>Values significant differ from canola oil.

rai oils (Table 2), while FER of wild mustard, rai and hybrid rai oils were almost same (0.08%) and hybrid rai oil was 0.07% (p<0.05). Table 2 also shows that FER of canola oil was 0.09%.

Table 3 shows that fried oils significantly raised TC, TG and LDL. While serum HDL was lowered when compared with the control group. Although serum TG level of canola oil (95.06 mg/dl) was almost similar to control rats (90.02 mg/dl), TG level of different rapeseed oils were drastically increased (126.07 to 144.10 mg/dl). In case of HDL, both wild and hybrid rapeseed oils had non-significant level of HDL as the control group (28.06 mg/dl) except fried mustard (12.07 mg/dl) and fried rai (11.12 mg/dl) which were significantly decreased. Serum HDL of canola oil (30.81 mg/dl) was slightly increased than control (Table 3).

Table 4 also shows that cardiovascular and liver enzymes such as CK-MB, SGOT and SGPT for control groups were 21.03, 110.04 and 43.77 U/L, respectively. However, these enzymes were significantly raised (p<0.05) in different forms of experimental rapeseed oils, but the rising rate of these enzymes were highly

significant (p<0.01) in both fried mustard (31.0, 173.0 and 58.0 U/L, respectively) and fried rai oils (33.0, 175.0 and 59.06 U/L, respectively). Hybrid rapeseed oils were superior to wild and canola oils. Although cardio-hepatic enzymes were slightly increased in canola oils (22.05, 113.34 and 45.0 U/L, respectively) than normal diet feeding rats, this oil was better than rapeseeds oil.

## DISCUSSION

Erucic acid containing rapeseed oils causes growth rate retardation and disturbances in metabolism (Ray et al., 1979; Hornstra, 1972; Borg, 1975). During the process of frying, erucic acid content is increased upto 72.77% (Hafizur et al., 2017) and proceeds to the formation of free radicals and other harmful agents. Oxidation reaction, hydrolysis of triglycerides and polymerization of triglycerides give rise to peroxides which are starting point for the formation of several free radicals (Boatella, 2000). This study evaluates the effect of high and low erucic acid containing rapeseed oils on body weight,

**Table 3.** Effects of rapeseed oils on serum Lipid profile (TC, TG, HDL-C and LDL-C).

Group	Diet provided	Blood serum indices			
		TC (mg/dl)±SD	TG (mg/dl)±SD	HDL (mg/dl)±SD	LDL (mg/dl)±SD
Group A (Control)	Normal diet	39.04± 5.32	90.02±2.56	28.06±2.85	7.07±1.06
Group B	Mustard(wild)	45.12± 3.52	126.07***a **b ± 4.27	28.10±2.761	8.20±0.60
Group C	Mustard (hybrid)	46.13±5.566	137.08***a **b ± 3.94	27.06±2.67	9.11±1.13
Group D	Fried Mustard	55.03*a *b ±0.23	142.01***a **b ±3.00	12.07***a **b ±1.70	14.68***a *b ±0.70
Group E	Rai(wild)	46.08±3.75	131.06***a **b ±6.55	28.11±3.44	8.60±1.63
Group F	Rai (hybrid)	47.00± 3.35	138.07***a **b ±4.83	26.88±2.88	9.80±1.65
Group G	Fried Rai	56.11*a *b ±3.79	144.10***a **b ±4.10	11.12***a **b ±1.461	16.25***a *b ±1.52
Group H	Canola	40.46±4.54	95.06±2.30	30.81±3.10	7.80±1.32

TC: Total cholesterol; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; RSP: rapeseed powder; Values are mean±SD; n = 5; \*Significant values p<0.05; <sup>a</sup>Values significant differ from control; \*\*High significant values p<0.01; <sup>b</sup>Values significant differ from canola oil.

**Table 4.** Effects of rapeseed oils on serum CK-MB, AST and ALT.

Group	Diet provided	Blood serum enzymes (U/L)		
		CK-MB ±SD	AST (SGOT)±SD	ALT (SGPT)±SD
Group A (Control)	Normal diet	21.03 ±2.26	110.04 ±7.8	43.77 ±3.20
Group B	Mustard (wild)	27*a ±2.64	150.66 ±17.92	45.41 ±4.59
Group C	Mustard(hybrid)	29.03**a*b ±3.00	167***a**b ±8.88	47.00± 4.35
Group D	Fried mustard	31*a*b ±3.46	173***a**b ±9.64	58.00**a*b ±3.60
Group E	Rai (wild)	28*a ±2.64	158***a**b ±10.53	46.00± 6.55
Group F	Rai (hybrid)	30.14**a*b ±2.96	170.06*a ±24.37	48.03 ±3.82
Group G	Fried Rai	33*a*b ±3.60	175.0***a**b ±10.44	59.06**a*b ±4.40
Group H	Canola	22.05 ±2.70	113.34 ±7.63	45.00± 4.35

CK-MB: Creatine kinase-MB; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Values are mean ±SD; n=5; \*\*Indicate high significant values p<0.01; <sup>a</sup>Value significant differs from control; <sup>\*</sup>Indicate significant values p<0.0; <sup>b</sup> Values significant differ from canola.

FER, lipid profile (TC, TG, HDL-C and LDL-C) and cardio-hepatic enzymes (CK-MB, AST and ALT). After six weeks intervention, body weight change of all experimental rats was lower than control (Table 1) similar to the earlier study of Christoffersen (1972) and Hornstra (1972). Dasgupta and Bhattacharyya (2007) showed that erucic acid reduced FER. This study found that high erucic acid containing fried rapeseed oils (both mustard and rai) had the lowest FER ( $p<0.01$ ), while low erucic acid containing canola oil increased FER than control rats, this might be due to the improvement in digestion. Dasgupta (2007) also reported that 10% erucic acid rich rapeseed oil did not elevate serum triglyceride (TG) level, while Mohamed (1989) and Badawy et al. (1994) demonstrated high erucic acid (20 to 55%) rapeseed oil increased TG level. Chakraborty (2003) also showed that heart lesion occurs with 15% erucic acid containing rapeseed oil. This study indicated that both mustard and rai oils with various forms like wild, hybrid and fried oils significantly increased serum TC, TG and LDL level ( $p<0.05$ ) where fried rai and fried mustard oils

were highly significant ( $p<0.01$ ). On the other hand, serum HDL of both fried oils was significantly lower (Table 3). However, no significant changes occurred in canola oil in compare with control rats. This result concurs with Przybylski et al. (2005) who found that serum triacylglyceride and very low-density lipoprotein (VLDL) decreased with canola oil regimen.

This study also noticed that cardio-hepatic enzymes like CK-MB, AST and ALT were increased with rapeseed oils (both mustard and rai), where fried oils were more significant ( $p<0.01$ ). Serum CK-MB is mainly found in heart muscle and helps the cells to perform their normal functions, but elevated level of CK-MB indicates injury of the cells (Panteghini, 1998). High erucic acid is poorly oxidized by the mitochondrial  $\beta$ -oxidation system, especially by the myocardial cells, which results in an accumulation of erucic acid, producing myocardial lipidosis and reduces the contractile force of the heart (Mori et al., 1998). In case of canola oil, less or no significant changes were found (Table 4). AST is an inflammatory marker of the heart; this might be indicated

development of atherosclerosis. Many researchers have established that increased plasma concentration of several inflammatory markers indicates atherosclerosis (Osterud, 2003; Libby and Ridker, 2004). The elevation of ALT activities might be due to the presence of high erucic acid rapeseed oil. ALT, AST and ALP normally found in the cytosol and released into blood stream. Estimation of these enzymes level in the serum has been considered as a useful quantitative marker to describe the extent and type of hepatocellular and cardiac cell damage (Kumar et al., 2004). This study found that serum SGPT and SGOT were highly significant with fried rai and fried mustard oils ( $p<0.01$ ) while hybrid rapeseed oils were also considerable ( $p<0.05$ ) than wild (both mustard and rai) and canola oils. Canola oil reduced low-density lipoprotein and TC levels, and as a significant source of the essential omega-3 fatty acid, it is associated with reduced cardiovascular mortality (Ansari, 1991). Thus, canola oil is better than fried, wild and hybrid rapeseed oils.

## Conclusion

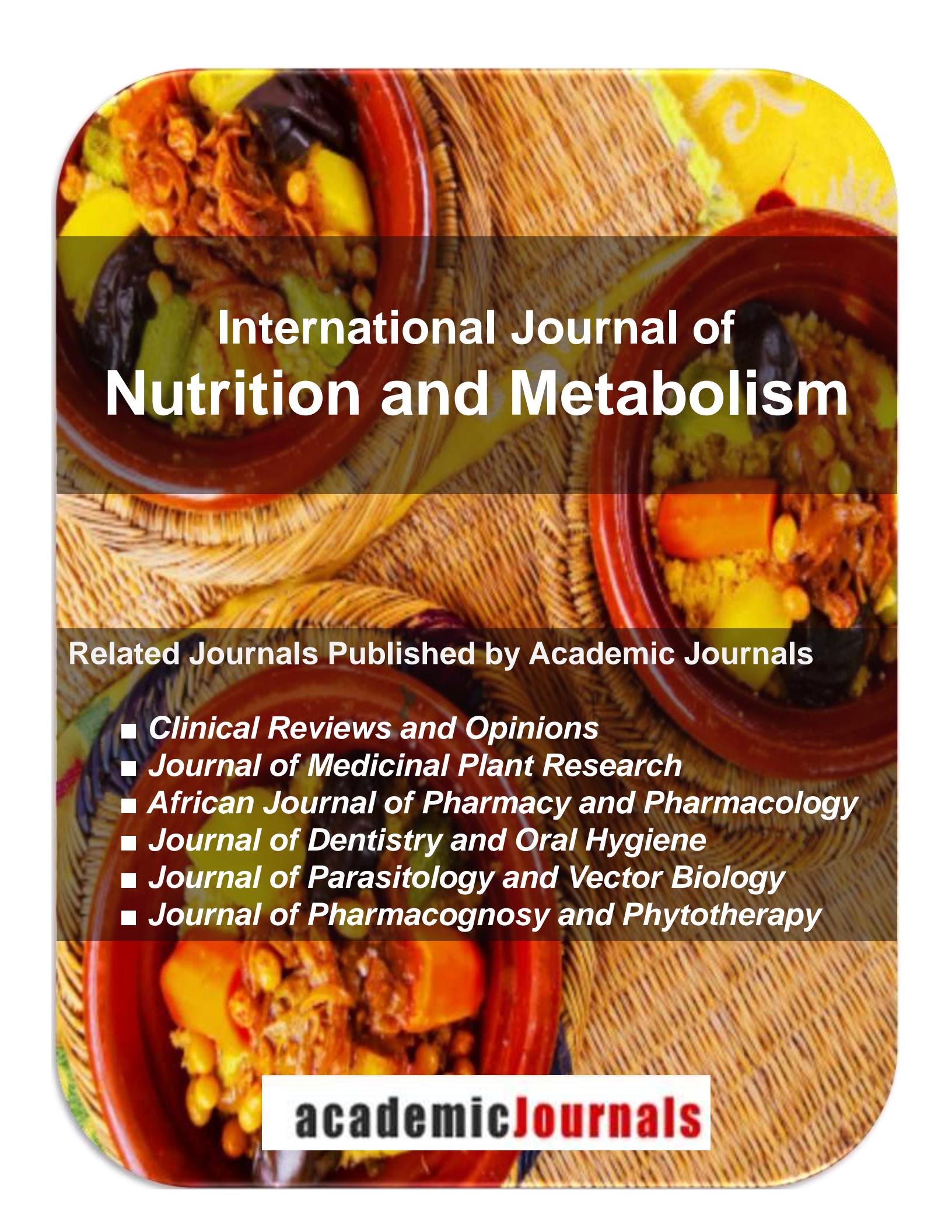
This study found that fried rapeseed oils (both mustard and rai) were more toxic for the rat physiology due to significantly high level of total cholesterol, triglycerides and low density lipoprotein with raised cardio-hepatic changes. However, low erucic acid containing canola oil was better than wild and hybrid rapeseed oils.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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