Indian J. Anim. Nutr. 2021. 38 (3): 293-298
doi: 10.5958/2231-6744.2021.00043.8

Indian Journal of
Animal Nutrition

Effect of Edible oil on Lipid metabolism and Expressions of Scavenger Receptor Class-B Type 1 in Rabbit Liver

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ABSTRACT

This experiment was conducted to investigate the effect of edible oil on HDL and scavenger receptor class-B type 1 in rabbit liver. SR-B1 is extremely expressed in various tissues, including the liver, where its pronouncement is modulated by different mechanisms, which includes the transcriptional activity of nuclear receptors. Eighteen albino rabbits were fed with rabbit food pallets for 21 days, of which 06 were fed normal diet (Group-I), and the other 06 were fed with edible oil at 2 ml/kg + normal feed (Group-II), third group of 06 rabbits were fed edible oil at 5 ml/kg + normal feed (Group-III). Estimation of HDL levels in serum and the expression levels of scavenger receptor class-B type 1 genes in rabbit liver were determined by using real-time fluorescent quantitative PCR, ELISA and Western blot. The expressions of SR-B1 mRNAs, protein levels and SR-B1activity in Group-II and group - III were significantly over expressed as compared with those in Group-I. Low HDL levels were observed in serum of Group -III compared with Group-II and Group-I. It was concluded that edible oil showed regulative effect on liver by a potential mechanism of overexpression SR-B1 genes, protein abundance, activity of SR-B1 and low concentrations of high density lipoprotein (HDL) in serum, accordingly holding down the cholesterol synthesis in rabbit liver.

Key words: Cholesterol, Edible oil, High density lipoprotein, Scavenger receptor class-B type 1

INTRODUCTION

Lipids are vital component and major sources of energy second to carbohydrates (Acton *et al.,* 1996). They play specific roles in membrane signaling events. Some of the lipids are indicators and also take vital role in cell development. Concentration of lipids could represent physiological conditions of cells (Rigotti *et al.,* 1997). Cholesterol takes an essential role for the cellular functions, in addition to maintenance of plasma membrane fluidity and integrity. It is acquired from plasma lipoproteins by selective cholesterol uptake pathway (Glass *et al.,* 1983). The High-density lipoprotein (HDL) is very important cholesterol carrying lipoprotein. It has two essential roles; first it improves reverse cholesterol transport (RCT) and it regulates inflammation. The scavenger receptor, class B type I (SRB1) is a crucial cholesterol receptor for HDL that could mediate particular HDL lipid uptake into cells. It was first characterized by Acton *et al.* (1996). SRB1 was recognized as the first HDL receptor and it was focused to perform in the selective transport and adjustment of cholesterol and lipids (Acton *et al.,* 1996, Lee *et al.,* 2017). This is significant, as HDL cholesterol levels are shown to be inversely related with a risk of stroke (Glass *et al.,* 1985). SRB1 plays major role in production of CE for steroid hormone synthesis, as well as regulating plasma metabolism of cholesterol. SRB1 can bind HDL through interconnection with its apolipoproteins (Rigotti *et al.,* 1997). Animals that do not express SRB1 have enhanced levels of plasma concentrations of cholesterol associated with enlarged HDL elements with increased levels of unesterified cholesterol content and rehabilitated apolipoprotein composition (Rigotti *et al.,* 1997; Miettinen *et al.,* 2001; Mardones *et al.,* 2001; Braun *et al.,* 2003; Van *et al.,* 2004). Unesterified and esterified cholesterols are unchanged in hepatocyte but reduced biliary cholesterol level (Varban *et al.,* 1998; Miettinen *et al.,* 2001). Hepatic clearance of HDL cholesterol from blood is controlled by hepatic SR-BI expression in mice (Out *et al.,* 2004; Brundert *et al.,* 2005; Kozarsky *et al.,* 1997). In contrast, up-regulation

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of SR-B1 expression in liver of mice results in decreased levels of HDL cholesterol from blood and enhanced biliary secretion compared to down regulation levels of SRB1 (Wang *et al.,* 1998; Ueda *et al.,* 1999; Zhang *et al.,* 2005). Modern studies modeling RCT from macrophages have established that hepatic SRB1 plays major role in driving this process (Temel *et al.,* 2002). Down regulation of SRB1 gene expression results in reduced cholesterol storage in steroidogenic cells and ovaries (Rigotti *et al.,* 1997; Miettinen *et al.,* 2001), due to the main function of SRB1 in HDL cholesterol consumption by steroidogenic cells (Mardones *et al.,* 2020). Numerous studies indicated the major physiological role played by SRB1 in cellular consumption of HDL cholesterol including cholesterol ester, free cholesterol and bioactive lipids, such as α-tocopherol (vitamin E) (Luo *et al.,* 2010). In this study, we have investigated the effect of palm oil on rabbit liver and mRNA expression in up- regulation of SRB1 confirmed by quantitative RT-PCR analysis, Western blot and ELISA as well as measured HDL levels in blood of experimental rabbits.

MATERIALS AND METHODS

Eighteen Male albino rabbits weighing ~1750 g aged 3-4 months, obtained from a breeding farm of Bangalore were individually housed in 80 cm × 60 cm × 60 cm rabbit cages under standardized temperature between (15-21°C), humidity (45-60%) and light (12 h light-dark) and dark period for 21 days with a mixed diet of pellets, green leafy vegetables, and water *ad libitum.* Animals were divided into 3 groups with 6 rabbits in each group. The group- I was kept as control, with a normal diet and water supplied regularly. Group- II was supplemented with 2 ml/kg of body weight dose of palm oil and group- III animals were given 5 ml/kg BW of palm oil for 21 days. After experimental period, they were sacrificed and serum used for HDL assessment, extracted liver tissues used for ELISA, Western blot and qRT-PCR.

After 21 days experimental period incisions were made into their thoracic cavities. Blood withdrawal was performed in the morning at around 8:00–9:00 am, following a 12-h overnight fast. The blood was collected by heart puncture and allowed to clot in sample vials. Blood was centrifuged (10 min, 1500×g, 4 °C), the supernatant was harvested by simple aspiration subsequently used until analysis of high density lipoprotein. The automated analyzer Hitachi – 7170 was used and measured concentration of HDL in serum sample.

SRB1 activity of liver tissue was measured with the ELISA kit (R&D Systems, Minneapolis) according to the manufacturer's instructions. Briefly, samples and a diluted series of standards were incubated with a mouse monoclonal antibody to SR-B1 and SR-B1 conjugate in a 96-well plate at room temperature for 3 h. After washing systematically, substrate solution and stop solution were added, and the optical density of each well at 450 nm was was determined. For expression levels of SRB1 in rabbit, proteins were collected from liver of each experimental group. For quantitative analysis, the same amount of protein (30 μg) was loaded for each sample. Proteins were separated on 4–15% SDS-PAGE and relocated to a nitrocellulose membrane. Membranes were incubated overnight with 5% milk TBS-Tween solution at 4°C, washed and incubated for 2 h with rabbit primary polyclonal antibody SRB1. After second incubation with a peroxidase conjugated IgG secondary antibody was accomplished. The bound antibody bands were detected using Uptight US HRB Blot chemiluminescence as well as β-actin was used as control. Densitometries analyses of protein bands in the Western blots were done using G: Box Imager software. Relative band intensities were derived by using the software to calculate integrated optical density for each band, and then each band was normalized with the integrated optical density of the corresponding β -actin band. The bands were read in the air dried membrane.

RNA extraction and cDNA synthesis: (Trizol method): Liver tissue samples of all three groups were frozen in liquid nitrogen, followed by RNA isolation using Trizol Reagent (Thermo fisher Scientifics) under aseptic conditions. Concentrations of RNA were quantified by the optical density at 260 nm wavelengths

Table 1. Sense and antisense primers of SRB1 & β-actin.

Gene	Primers	Temp
SR-B1	Sense: 5'-AGGGTGTTCGAAGGCATCC-3'	58 °C
(128-bp product)	Antisense: 5'-GACCCGTTGGCAAACAAAGT-3'	
β-actin	Sense: 5'-CATGCCATCCTGCGTCTGGACC-3'	62 °C
(169-bp product)	Antisense: 5'-TACTCCTGCTTGCTGATCCACATCTGC-3'	

using the Nanodrop ND-1000. Purity was checked for RNA quantity in sample and cDNA was synthesized using applied biosystem kit following manufacturer's instructions.

Real-Time qRT-PCR: Each sample was evaluated in triplicate alongside with negative control. qRT-PCR was used to amplify the target genes using the cDNA according to the manufacturer's instructions. Briefly, the RT Mix was prepared as per the manual by preparing10 µl of cDNA sample, a final PCR volume of 20 µl containing 10 µl of ready master mix having, 10X RT random primers, 25X dNTP Mix, 10X RT buffer, MultiScribe™ Reverse Transcriptase, RNAse inhibitor, and nuclease-free water. On a Real-Time PCR System 7500 (Applied Biosystems), the reaction was performed. The sense and anti-sense primers used are tabulated (Table 1).The PCR Setup included 5.0 µl of template, 1.0 µl of forwarding Primer, 1.0 µL of Reverse Primer, 12.5 µl of master mix (PowrerUp SYBR Green), and 5.5 µL of PCR water making up to a total volume of 25 µl.

All the experiments were performed on three different occasions and data are presented as mean ± SD. The data was analyzed by one-way ANOVA. Data were statistically evaluated with SPSS/10 software. A value of $^{*}P<0.05$ was considered to indicate a significant variation among groups.

RESULTS AND DISCUSSION

The findings show that SR-B1 protein abundance in Group-III and group-II animals are significantly ($P<0.05$) higher than that found in group-I (Fig. 1). β - Actin was used as positive control by using the technique Western blot in the Rabbit liver with definite antibodies. In Lane III and Lane II no significant changes of Beta Actin have seen in comparison to Lane I

Fig. 1. Expression of SR-B1 in Lane I SR-B1 (catalog No. AB137829) examined as untreated (control) group; Lane II SR-B1 with 2 ml/kg dose; Lane III of 5 ml/kg dose

(Fig. 2). Outcomes of the present work showed the relationship between palm oil supplementation with hepatic over-expression of SRB1 in As compared to control, Group-III manifested reduced plasma levels of HDL. The effect of edible oil on HDL values is presented in Fig 6. HDL levels were significantly lower

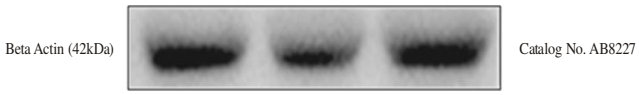

Fig. 2. Expression of Beta Actin (catalog no AB8227) in Lane I in untreated (control) Group; Lane II (2 ml/kg) and Lane III (5ml/kg) treated group

in Group-III rabbits as compared to control animals. In Group-II rabbits, HDL level decreased when compared to control group, but compared to Group-III the HDL levels increased which is due to moderate effect ofedible oil at 2 ml/kg. HDL mediated cholesterol

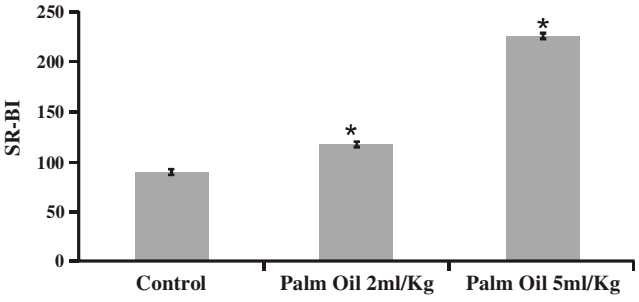

Fig. 3: Effect of palm oil on rabbit liver SRB1 level was quantified using ELISA.

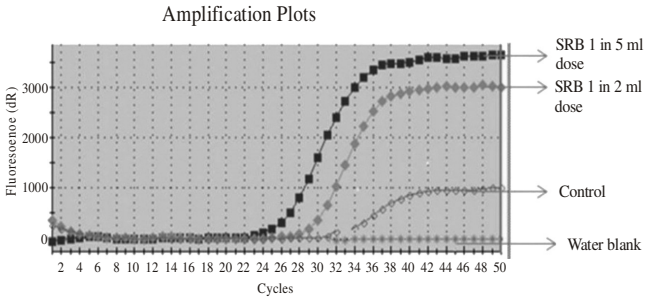

Fig. 4. Quantification of SRB1 found in rabbit liver using the RT-PCR method shows an increase in level of SRB1 in liver tissue of group-III

elimination is the standard restrictive step of RCT. In experimental animals, supplemented with high fat and high cholesterol diets overcome SR-B1 expression and enhanced the levels of HDL (Ji *et al.,* 1997). The effect of oil supplementation on serum SRB1 levels was assessed by ELISA. Enhancement in liver SRB1 levels was observed when oil was supplemented for the long term. Precisely, medium and high-dose of oil produced 1.8, 2.7 fold ($P<0.05$) augmentation in serum SRB1 levels, respectively, when compared with control (Fig 3). The gene expression of SRB1 in liver was analyzed by qRT-PCR. Palm oil supplementation may increase the gene expression of the SRB1 in Group-II and Group-III compared to group-I. The results of this study are similar to our previous study in which we observed an increase in the expression of PPARγ and PPAR α after 21 days of experimental feeding of polyphenol rich palm oil (Ahamed *et al.,* 2021). PPAR genes also

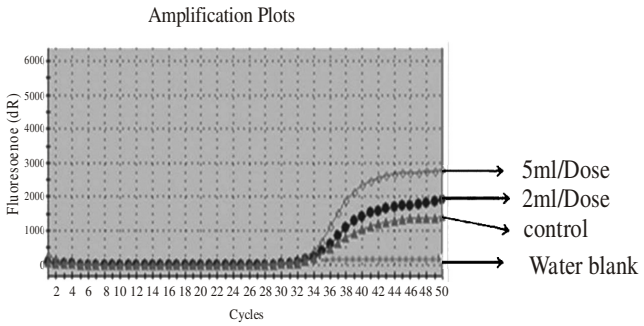

Fig. 5. Quantification of β-Actin found in rabbit liver using the RT-PCR method. Level of β-Actin was found more in 5 ml/kg oil supplemented rabbits (Group-III)

was reported to enhance the expression of SR-B1 (Babiker *et al.,* 1997). Expressions of mRNA for SR-B1 and β-actin are presented in Fig 5. Concentrations of RT-PCR products for SR-B1 were significantly increased in the tissues of oil supplemented group (Fig 4). The SRB1 plays key role in the metabolism of HDL cholesterol and CE in the liver. This receptor also aids in enabling the preliminary step of HDL arbitrate cholesterol efflux (Zhang *et al.,* 2005). In our study, overexpression of SR-B1 in liver might have enhanced the macrophage (Arai *et al.,* 1999). We report here that SR-B1 mRNA expression was increased in Group-III (5 ml/kg palm oil treated animals) and SRB1 protein abundance compared to remaining two groups. Slight increased amount of SRB1 protein was observed in group-II compared to control groups, but compared to group-III lesser protein level was observed. In agreement with our study, high expressions of SRB1 in the liver, while decreasing levels of HDL in plasma was reported earlier (Wang *et al.,* 1998; Kozarsky *et al.,* 2000). Whereas dietary edible oil in hypercholesterolemic

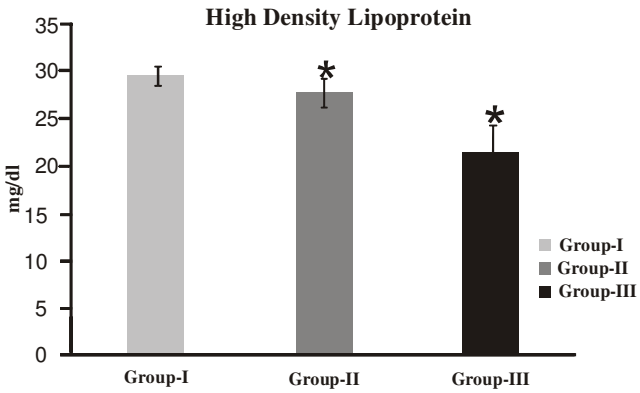

Fig. 6. All values are expressed in Ct Mean ± SD (n=6), the values were analyzed with ANOVA *= P<0.05; Group-I Control animals, Group-II 2 ml/kg palm oil + feed, Group-III 5ml/kg palm oil + feed

rabbits (Group-II andGroup-III) increases SRB1 protein levels, previous studies demonstrated that it correlates with reduced plasma HDL compared to control group rabbits. In a comparable study reduced HDL levels in plasma and over expression of SRB1 was found in transgenic mice controlled by the apolipo

protein A-I promoter (Rigotti *et al.,* 2003). This demonstrated that over expression of liver SRB1 may promote RCT. It is well identified that overexpression of SRB1 enhance the rate of consumption of HDL by the liver (Ueda *et al.,* 1999; Ji *et al.,* 1999; Alam *et al.,* 2001). These studies described SRB1 may directly arbitrate essential role in liver HDL metabolism, in resolving HDL concentrations and in regulating cholesterol concentrations in plasma and thus may influence the development of atherosclerosis and gallstone disease.

CONCLUSION

In conclusion, our study showed that supplementation of high concentration of edible oil in feed leads to imbalance of cholesterol levels in experimental rabbits and also resulted in over-expression of SRB1 gene in liver of experimental rabbits.

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Received on 09.10.2021 and accepted on 03.12.2021