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Lipid lowering and antioxidant activity of flavonoid diosmin and hesperidin supplementation in donkeys

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Abstract
Hesperidin and diosmin are a naturally occurring flavonoid and reported for having hypolipidemic properties. In present study effects of oral supplementation of hesperidin and diosmin on serum lipid profile, serum biochemical and oxidative stress markers were investigated in adult Poitou female donkeys. Donkeys were divided in two groups having 6 donkeys in each group; first group was kept as control while second group was kept as treatment group. Oral supplementation of diosmin and hesperidin @ 10 mg/kg body weight was given to donkeys of treatment group. Serum total lipids, serum triglyceride and serum cholesterol levels were found significantly decreased in treatment group. Serum malondialdehyde levels were found significantly lower in the treatment group and serum glutathione was found significantly higher in the treatment group. Results of the study demonstrated that the oral supplementation of hesperidin and diosmin combination have lipid-lowering and antioxidant properties and have potential to be used therapeutically in donkeys.

Keywords: cholesterol, diosmin, donkey, hesperidin, hyperlipaemia, malondialdehyde, oxidative stress

Introduction
Hyperlipaemia is abnormally elevated levels of fat in the blood, such as cholesterol and triglycerides. These disorders may manifest with the elevation of serum total cholesterol, very low density lipoprotein (VLDL), triglyceride concentrations and a decrease in the high density lipoprotein (HDL) concentration (Durham and Thiemann, 2015) [12]. The donkey has a particularly high risk for hyperlipaemia due to a number of factors including their metabolic efficiency with ability to utilize poor forage and subsequent weight gain if fed inappropriately (Mendoza *et al*., 2019).

Flavonoids are plant secondary metabolites having a polyphenolic structure. Hesperidin (HS) is a flavanone glycoside ($C_{28}H_{34}O_{15}$) flavonoid. It is abundant and in-expensive by-product of citrus cultivation, such as sweet orange *(Citrus sinensis L.)*, bitter orange (*Citrus aurantiumL.*) and lemon (*Citrus limon*) (Li *et al*., 2018) [20]. HS help in treatment of inflammatory kidney damage and liver tissue damage (Fouad *et al*., 2019; Turk *et al*., 2019) [30]. HS has antioxidant properties (Selvaraj and Pugalendi, 2010) [26], hypoglycemic and hypolipidemic properties (Kim *et al*., 2003). HS is found to reduce cholesterol (Monforte *et al*., 1995) [22] and blood pressure in rats (Ohtsuki *et al*., 2003) [24]. Diosmin is derived by dehydrogenation of the corresponding flavanone glycoside hesperidin (Campanero *et al*., 2010) [8]. Diosmin has an antihyperglycemic effect because it enhances the secretion of β- endorphin and increases the glucose uptake of tissues (Hsu *et al*., 2017) [16]. Diosmin treatment to high fat diet-induced hyperlipidemic mice caused significant decrements in the levels of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C).Diosmin resulted in significant increase in the levels of HDL-C and improvements in total protein levels and decreases in SGOT, SGPT and ALP enzymatic activities in hyperlipidemic mice (Firdous *et al*., 2021) [14]. Combination of hesperidin and diosmin is being used as a nutritional supplement in the USA and European countries (Elhelaly *et al*., 2019) [13]. Moreover, it has anti-inflammatory (Tahir *et al*., 2013) [29] and anti-hyperglycemic effects (Ahmed *et al*., 2016) [2]. The present study was aimed to evaluate lipid lowering efficacy and antioxidant activity of flavonoid diosmin and hesperidin oral supplementation in donkeys.

Materials and Methods

Experimental Animals

The study was conducted on apparently healthy non-pregnant, adult poitou female donkeys at Equine Production Campus, National Research Centre on Equines (EPC, NRCE) Bikaner. The experimental trials were approved by the Institutional Animal Ethics Committee and conducted under its guidelines.

Experimental supplementation

Commercial product of hesperidin and diosmin (Daflon®1000mg, Serdia Pharma) used in this study was purchased from the local market of Bikaner, Rajasthan India.

Experimental Design

Donkeys were divided into two groups having six animals in each. First group was taken as control group and second group was taken as treatment group. In treatment group animals, diosmin+hesperidin combination (90:10) was given orally @ 10 mg/kg body weight for 30 days. Blood samples were collected from donkeys of each group on 0 (pre treatment), 1st, 10th, 20th and 30th day after start of supplementation. Blood samples were collected for estimation of serum biochemical parameters (serum glutamic pyruvic transaminase, serum glutamic-oxaloacetic transaminase, alkaline phosphatase, total protein, total lipid, serum gamma-glutamyltransferase, serum albumin, serum glucose, serum triglycerides, serum cholesterol, blood urea nitrogen and creatinine) and oxidative stress markers (malondialdehyde and reduced glutathione). The blood for serum separation was collected in centrifuge tubes of capacity 15 ml. Serum was separated within half hours of collection by centrifugation of clotted blood at 3000 rpm for 10 minutes. Serum samples were stored at -20 ˚C for further use.

Biochemical Estimation

Biochemical analysis of serum samples were done for estimation of serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total protein, total lipid, serum gamma-glutamyltransferase (GGT), serum albumin, serum glucose, serum triglycerides (TG), serum cholesterol, blood urea nitrogen and creatinine. All test performed by semi auto biochemical analyzer by RMS manufactures, blood serum sample were analyzed by using standard kits supplied by SPINREACT, S.A./S.A.U. Ctra. Santa Coloma, 7-E-17176 SANT ESTEVE DE BAS-(Girona) SPAIN and ERBA diagnostics Mannheim GmbH, Mallustr, 69-73, D- 68219, Mannheim/Germany. Estimation of malondialdehyde was conducted by method developed by Okhawa *et al*. (1979) [23]. Estimation of reduced glutathione was conducted by method developed by Beutler *et al*. (1971) [5].

Statistical Analysis

The data were statistically analysed and compared using standard formula given for mean, standard error and student's 't' test as per the procedures explained by Snedecor and Cochran, 1994. The data was considered statistically significant when $p<0.05$ and $p<0.01$.

Results and Discussion

Effect on Lipid Profile

The levels of serum total lipids, serum triglyceride and serum cholesterol were found significantly decreased in the diosmin and hesperidin supplementation group on day 10 (Table 1). Further supplementation show no significant effect on 20th day onwards in the treatment group. In previous studies by Bok *et al*. (1999) [6], Kim *et al* (2003) [18] in rats, Akiyama *et al*. (2009) [3] and Firdous *et al*. (2021) [14] in mice, reported significant decrease in the levels of total cholesterol, triglycerides and total lipid in high fat diet induced hyperlipidemia when treated with hesperidin and diosmin. Chung *et al*. (2020) [9] also reported that diosmin significantly ameliorated dyslipidemia, by reducing serum cholesterol levels in mice. While Mohamadi *et al* (2019) in a systemic review of many papers concluded that hesperidin supplementation might not affect the lipid profile. In present study that hesperidin and diosmin supplementation showed effects for a limited period and thereafter no effect of supplementation was observed on the serum lipid profile. So results of the present study are in agreement to both the contrary views to some extent and advocates supplementation of hesperidin and diosmin for 10 days to the donkeys.

Effect on biomarkers of liver and kidney functions

The effect of treatment with diosmin and hesperidin supplementation on liver function test like serum glutamic pyruvic transaminase, serum glutamic-oxaloacetic transaminase, serum gamma-glutamyltransferase, alkaline phosphatase, serum glucose, total protein and serum albumin in donkey is shown in Table 2 & 3. In the present study, initially there was no significant difference was observed between treatment and control groups upto day 10 of the experiment. On day 20 & 30 significantly lower SGOT levels were observed in treatment group. Elhelaly *et al*. (2019) [13] in acryl amide induced liver damaged rats reported a significant decrease on oral administration of diosmin and hesperidin on the value of serum glutamic pyruvic transaminase and Firdous *et al*. (2020) reported that diosmin caused remarkable decreases in SGOT and SGPT enzymatic activities in hyperlipidemic mice. In earlier studies supplementation showed decrease in already increased SGPT levels, while in present study, SGPT levels were already normal in both the groups so supplementation may have not showed any significant effect on SGPT levels.

Serum gamma-glutamyltransferase (GGT) was comparable between the groups up to day 10th. On day 20 serum gamma-glutamyltransferase (GGT) was significantly higher in treatment group compared to control; GGT levels were also higher in the supplemented group on day 30 of experiment, but not significantly. Higher GGT levels are found in liver damage in mammals including equines (Dedar *et al.* 2019) [30]. Dominici *et al*. (2005) reported that gamma-glutamyltransferase (GGT) plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione as well as drug and xenobiotic detoxification. Increase in serum GGT levels in donkeys on 20th and 30th day might be associated with liver damage. Favourable effects on lipid profiles were also observed till day 10th only and thereafter no significant difference was found on the serum lipid profile in both the groups it might be due to compromised liver of the donkeys of the supplemented group.

No significant effect of treatment was observed on alkaline phosphatase up to day 10 of the experiment. While on day 20 & 30 significantly higher alkaline phosphatase levels were observed in treatment group compared to the donkeys of control group. Hong and Zhang (2020) [15] reported that hesperidin promotes the ALP activity. Hesperidin has been observed to inhibit osteoclast differentiation and maturation,

reduce expressions of bone resorption markers and increase ALP content of osteoblast (Zhang *et al*. 2018) [32]. Increased serum levels of ALP are also found in the liver damage (Dedar *et al*. 2019) [10]. So increase in ALP activity might be due to combined effect of hesperidin on osteoblasts and compromised liver. Likewise GGT, increase in ALP might be associated with liver damage due to supplementation. However, simultaneously decrease in SGOT remained unexplained in the present study. Serum glucose was similar between the groups up to day 20. On 30th day of trial significantly lower serum glucose was observed in treatment group compared to control. These findings are in agreement with the studies of Akiyama *et al*. (2009) [3] in rats, Srinivasan and Pari (2012) [28] in diabetic rats, Jain *et al*. (2014) [7] in rats and Chung *et al*. (2020) [9] in mice. Hsu *et al*. (2017) [16] reported significant decrease in serum glucose level in streptozotocin-induced diabetic rats when treated with oral administration of diosmin. There was no significant effect of oral supplementation of diosmin and hesperidin on serum albumin and total protein levels in donkeys. Likewise, no significant effect in mean serum albumin and total protein values were observed in the diosmin alone-treated group by comparison to control group (Wojnar *et al*. 2017; Bozdag and Eraslan, 2020) [31, 7]. Results of kidney function test revealed that values of blood urea nitrogen and serum creatinine showed a non significant effect of oral supplementation of diosmin and hesperidin (Table 4). The findings of present study deviate from the findings of Turk *et al*. (2019) [30] in rats, Elhelaly *et al*. (2019) [13] in male Wister albino rats and Alkhalaf (2020) [4] in Wister male albino rats who reported a significant decrease in the value of blood urea nitrogen and creatinine on oral administration of diosmin and hesperidin.

Effect on Oxidative stress markers

It was observed that malondialdehyde was significantly lower in the treatment group compared to control on day 20 and serum glutathione was found significantly higher on day 20 in treatment group compared to control (Table 4). The findings of present study are in agreement with previous findings reported by Abdel *et al*. (2012) [1] in rats, Srinivasan and Pari (2012) [28] in male albino wister rats, Kobo *et al*. (2014) [19] in winster rats, Ahmed *et al*. (2016) [2] in wister rats, Turk *et al*. (2019) [30] in rats and Elhelaly *et al*. (2019) [13].

Table 1: Effect on Lipid profile following oral supplementation of diosmin and hesperidin in donkey.

Days	Serum total lipid (TL) level (Mean ± SE) in (mg/dl)		Serum triglyceride (TGL) level (Mean ± SE) in (mg/dl)		Serum cholesterol level (Mean ± SE) in (mg/dl)	
	Control	Treatment	Control	Treatment	Control	Treatment
0	552.45±46.23	498.85±35.91	93.65±10.87	100.46±12.20	88.00±3.88	83.01±3.04
1st	492.03±60.02	430.35±46.33	124.57±21.61	115.99±22.06	89.41±3.89	82.33±3.44
10th	570.27±53.56	313.23±43.86**	120.88±4.28	74.07±11.83**	108.14±4.88	87.69±4.14**
20th	535.59±42.80	388.86±61.92	66.22±6.57	71.40±9.68	99.92±3.18	88.91±4.46
30th	455.36±81.68	429.53±66.35	75.44±20.43	71.39±16.64	89.13±2.07	93.74±6.45
*= P value<0.05 ,**= P value<0.01						

Table 2: Effect on Liver function test following oral supplementation of diosmin and hesperidin in donkey

Days	Serum glutamic pyruvic transaminase (SGPT) level (Mean ± SE) in (IU/L)		Serum glutamic oxaloacetic transaminase (SGOT)level (Mean ± SE) in (IU/L)		Serum GGT level (Mean ± SE) in (IU/L)		Serum Alkaline phosphatase level (Mean ± SE) in (IU/L)	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
0	7.53±1.34	13.11±3.94	120.72±6.73	145.67±14.90	16.23±3.83	19.66±3.69	178.09±12.87	243.55±53.30
1st	7.91±1.22	13.52±2.21	116.04±19.67	127.18±15.99	14.66±2.17	19.50±3.08	199.02±24.57	247.86±53.45
10th	16.57±1.30	13.12±2.84	121.64±18.41	70.32±23.59	18.16±2.84	24.50±2.82	212.44±23.46	304.79±61.44
20th	17.21±1.06	14.30±1.72	131.02±18.29	83.42±8.09*	24.83±6.26	51.66±3.28**	225.64±29.83	374.95±44.41*
30th	19.34±2.00	13.87±3.24	138.23±19.97	84.42±10.96*	33.16±7.17	51±3.47	258.28±33.29	419.93±46.62*
*= P value<0.05 ,**= P value<0.01								

Table 3: Effect on liver function test following oral supplementation of diosmin and hesperidin in donkey

Days	Blood glucose level (Mean ± SE) in (mg/dl)		Albumin level (Mean ± SE) in (g/dl)		Serum total Protein (TP) level (Mean ± SE) in (g/dl)	
	Control	Treatment	Control	Treatment	Control	Treatment
0	99.73±10.41	92.92±17.61	3.36±0.13	3.56±0.25	6.21±0.32	7.88±1.83
1st	82.78±7.79	82.40±3.47	3.44±0.13	3.75±0.27	6.97±0.23	6.57±0.14
10th	95.29±5.97	83.09±4.05	3.98±0.14	3.83±0.26	6.91±0.34	7.16±0.16
20th	116.43±14.17	85.61±5.35	4.06±0.20	3.99±0.14	7.45±0.13	7.43±0.17
30th	118.33±13.57	80.55±2.70*	4.17±0.21	4.10±0.14	7.29±0.18	7.34±0.28
*= P value<0.05 ,**= P value<0.01						

Table 4: Effect on Kidney function test, serum malondialdehyde and reduced glutathione following oral supplementation of diosmin and hesperidin in donkey

Days	Blood urea nitrogen level (Mean ± SE) in (mg/dl)		Serum creatinine level (Mean ± SE) in (mg/dl)		Serum malondialdehyde level (Mean±SE) in (nanomole/ml)		Serum reduced glutathione level (Mean±SE) in (mg/dl)	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
0	32.15±3.80	35.58±4.15	1.83±0.25	1.89±0.26	2.02±0.28	3.92±1.79	2.00±0.12	2.03±0.06
1st	32.33±1.44	36.62±1.89	1.70±0.18	1.59±0.21	1.89±0.14	2.62±0.27*	2.87±0.48	4.06±0.26
10th	38.41±2.47	35.39±2.80	1.57±0.15	1.67±0.15	3.43±0.32	2.94±0.43	2.78±0.58	2.42±0.47

20th	39.48±2.71	35.46±1.75	1.53±0.11	1.58±0.52	3.80±0.67	1.94±0.23*	1.49±0.17	2.15±0.21*
30th	37.77±0.44	34.10±2.37	1.14±0.10	1.24±0.03	1.54±0.18	1.28±0.14	1.38±0.36	2.51±0.46
*= P value<0.05 ,**= P value<0.01								

Conclusion

Based on the results of the present study in donkeys it may be concluded that hesperidin and diosmin supplementation @ 10 mg/ Kg body weight for 10 days show beneficial effects on serum lipid profiles and oxidative stress markers and they have potential to be used as therapeutic agent in the treatment of hyperlipemia in donkeys. For supplementation longer than 10 days there is need of further trials with reduced dose rates.

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