

AMELIORATION OF HEAT STRESS IN RABBITS THROUGH SUPPLEMENTATION OF *EMBLICA OFFICINALIS*

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SUMMARY

The present study was undertaken to study the effects of supplementation of *Embllica officinalis* on biochemical and enzymatic profile of rabbits. Rabbits weighing approximately 0.8-1.0 kg were divided into four groups: Group I included animals kept in normal conditions at 21°C, Group II included animals kept at high temperature (40-45°C), Group III included animals kept in normal conditions and supplemented with *E. officinalis* and Group IV included animals kept at high temperature and supplemented with *E. officinalis*. Group III and IV animals were administered *E. officinalis* @ 3000 mg/rabbit/day for 6 weeks. Blood samples were collected at weekly intervals to study the biochemical and enzymatic milieu in animals. Biochemical profile revealed significant decrease in concentration of total cholesterol and significant rise in high density lipoproteins in rabbits of Group III and IV supplemented with *E. officinalis*. There were non significant variations in the liver function tests in animals supplemented with *E. officinalis*, which suggests nontoxic effect of supplementing *E. officinalis* powder in feed. Thus, it can be concluded that *E. officinalis* facilitates the animals in ameliorating heat stress without producing any adverse effect on the liver.

Key words: Biochemical profile, *Embllica officinalis*, Heat stress, Rabbits

Rabbits maintain their homeostasis by respiration and heat dissipation through ear lobes. They are very prone to heat stress, since the sweat glands present in rabbits are non-functional and they perspire very less due to fur (Marai *et al.*, 2002). Heat stress leads to increased free radical generation in the animal's body that can produce a negative effect on biological activities (Halliwell and Gutteridge, 1989). *Embllica officinalis* is widely used in traditional Indian medicine and has been regarded as divine fruit in Indian mythology. *E. officinalis* is a rich source of vitamin C, iron, essential amino acids, vitamins, minerals, antioxidants and several polyphenols (Pandey and Pandey, 2011). Its fruit extract has been reported to have hypolipidaemic (Anila and Vijayalakshmi, 2002), antidiabetic (Sabu and Kuttan, 2002), anti-inflammatory (Asmawi *et al.*, 1993) and antioxidant properties (Anila and Vijayalakshmi, 2003). It has potential for restoration of liver function, kidney function and lipid profile in poisoned rats (Maiti *et al.*, 2014). Thus, the present study was undertaken to study the effects of high ambient temperature and supplementation of *E. officinalis* on biochemical and enzymatic profile of rabbit.

A total of 24 White New Zealand rabbits weighing approximately 0.8-1.0 kg were housed in individual cages. They received standard rabbit food and water ad lib. and were maintained on a 12-h light cycle in a room thermostatically controlled at 21°C. The rabbits were divided into four groups (n=6 each): Group I- Rabbit kept in normal conditions at 21°C, Group II- Rabbit kept at high temperature (40-45°C), Group III- Rabbit kept in normal

conditions supplemented with *E. officinalis*, Group IV- Rabbit kept at high temperature supplemented with *E. officinalis*. Group III and IV animals were fed *E. officinalis* @ 3000 mg/rabbit/day for 6 weeks. Blood sample was aspirated from the marginal ear vein from each rabbit for biochemical and enzymatic analysis at weekly interval for 6 weeks. Blood samples in vials without anticoagulant were collected aseptically, allowed to clot for two hours and centrifuged at 3000 rpm for 10 minutes for separation of serum. Serum was aspirated and stored at -80°C till analysis. Alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), Total Bilirubin (BIT), Creatinine (CREA), Glucose (GLU), Triglycerides (TRIG), Cholesterol (CHOL), High density lipoproteins (HDL), Low density lipoproteins (LDL), Calcium (CAA), Albumin (ALB), Protein (PRO) were measured using biochemistry analyzer. The means and standard errors, and multivariate analysis of variance were calculated, using SPSS/PC student were computer software (Norusis, 1988).

The biochemical and enzymatic profile of rabbits of different experimental groups is presented in table 1. The results depicted that the level of ALT and AST showed an increasing trend in rabbits kept at high temperature (group II) as compared to rabbits kept at normal room temperature (group I). This might be due to the increase in stimulation of gluconeogenesis due to increased levels of cortisone, cortisol or adrenocorticotrophic hormones during heat stress (Thompson, 1973). ALT and AST levels decreased significantly following supplementation of *E. officinalis*.

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Table 1
Biochemical and Enzymatic profile of Rabbits supplemented with *Emblica officinalis*

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	BIT (mg/dl)	CREA (mg/dl)	GLU (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	CAA (mg/dl)	ALB (g/dl)	PRO (g/dl)
I	129.95 ^b ±4.89	101.58 ^a ±24.44	121.33 ±23.19	4.71 ±0.37	0.07 ±0.00	1.27 ±0.12	125.06 ±16.60	95.33 ±8.98	114.66 ^b ±21.18	16.20 ^a ±1.51	2.68 ±0.08	12.55 ^b ±0.45	5.16 ^b ±0.21	8.10 ^b ±1.50
II	150.86 ^b ±14.72	140.55 ^b ±20.46	77.00 ±6.07	7.40 ±1.87	0.43 ±0.30	1.35 ±0.07	132.20 ±8.44	99.33 ±14.37	104.77 ^b ±5.59	12.41 ^a ±0.85	2.47 ±0.16	10.48 ^a ±0.56	4.01 ^a ±0.16	5.94 ^a ±0.24
III	95.20 ^a ±12.50	109.01 ^a ±14.72	108.16 ±24.57	5.00 ±0.27	0.10 ±0.01	1.16 ±0.19	113.75 ±8.28	74.67 ±9.40	78.16 ^a ±11.53	17.06 ^{ab} ±1.34	2.63 ±0.09	12.96 ^b ±1.12	5.41 ^b ±0.34	6.48 ^a ±0.55
IV	85.05 ^a ±9.26	100.08 ^a ±19.42	75.08 ±5.51	7.71 ±0.68	0.27 ±0.18	0.95 ±0.12	90.60 ±10.57	85.50 ±8.32	74.50 ^a ±4.26	19.90 ^b ±2.50	2.32 ±0.15	9.40 ^a ±0.33	4.29 ^a ±0.19	6.98 ^a ±0.47

Values with different superscripts within a column differ significantly (p<0.05)

ALT- Alanine aminotransaminase, AST- Aspartate aminotransaminase, ALP- Alkaline transaminase, GGT- Gamma glutamyl transferase, BIT- Total Bilirubin, CREA- Creatinine, GLU- Glucose, TRIG- Triglycerides, CHOL- Cholesterol, HDL- High density lipoproteins, LDL- Low density lipoproteins, CAA- Calcium, ALB- Albumin, PRO- Protein

The levels of alkaline phosphatase (ALP) showed a non-significant decline in supplemented groups III and IV as compared to group I and II. This suggests the hepatic restorative effect of *E. officinalis* and its nontoxic effect on liver. The flavonoids and tannins present in *E. officinalis* offer protective effect by combating oxidative stress via decreasing lipid peroxidation and scavenging reactive nitrogen species (Reddy *et al.*, 2010).

GGT, BIT, creatinine and glucose levels differed non-significantly under hot conditions as well as after supplementation of *E. officinalis* in rabbits. Although there was slight increase in the levels of BIT, creatinine and glucose in group II animals which restored to normal in supplemented group IV. The environmental conditions and many factors of herd management viz., feeding practices and type of shelter influence the levels of enzymes in blood since they are intimately related to metabolism (Marai and Habeeb, 1998). However, studies on the effect of ambient temperature on glucose levels are conflicting. The variation in the level of glucose in the present study is in agreement with Webster (1976) who also reported increased concentration of glucose under heat stress conditions. Elevated plasma glucose after heat stress aids in “fight or flight” responses during thermal challenges, thus enhancing the survivability of animals (Jingjing *et al.*, 2015).

The unaltered levels of enzymes and biochemical parameters related to liver functions suggest that the *E. officinalis* powder is nontoxic to rabbits at this supplementation level and offers protection from stress due to high temperature. This is in agreement with the findings of Pramyothin *et al.* (2006) who also reported protective role of *E. officinalis* against ethanol induced liver injury in rats. *E. officinalis* exhibits hepatoprotective activity due to its membrane stabilizing, antioxidative and cytochrome P450 2E1 inhibitory roles.

Triglyceride levels showed a non significant increase in rabbits kept at high temperature (group II). Amici *et al.* (2000) also reported significant but transient increase in the level of triglyceride 30 hours at the end of stress. The level of triglycerides however, decreased in *E. officinalis* supplemented groups III and IV in the present study. The cholesterol level showed a significant decline in groups III and IV rabbits as compared to non supplemented groups I and II. The significant low level of cholesterol in *E. officinalis* supplemented rabbits is in agreement with the findings of Yokozawa *et al.* (2007). High density lipoproteins (HDL) differed non significantly in group I and II rabbits under normal as well as high temperature conditions, however HDL increased significantly in supplemented rabbits (group III and IV). Low density lipoproteins (LDL) showed a non significant variation among rabbits of different groups. Though, the exact mechanism of hypolipidemic action of *E. officinalis* is not known, favorable changes in lipid profile induced by amla may be due to several mechanisms such as an interference with cholesterol absorption (Mathur *et al.*, 1996), inhibition of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase activity and increase in Lecithin Cholesterol Acyltransferase (LCAT) activity (Anila and Vijyalaxmi, 2002). *E. officinalis* administration produces myocardial adaptation by augmenting endogenous antioxidants and protects heart from oxidative stress (Rajak *et al.*, 2004). Flavonoids derived from *E. officinalis* exhibit maximum beneficial action by eliciting highly potent hypolipidemic and hypoglycaemic activities.

Calcium levels were observed to be decreased significantly in rabbits kept at high temperature (group II) as compared to rabbits (group I) living under normal environmental temperature. However supplementation of *E. officinalis* had no effect on the levels of calcium in rabbits of group III as compare to the rabbits of group I and

also in rabbits of group IV as compared to the rabbits of group II. Total protein and albumin levels decreased significantly in animals when kept in hot conditions (group II) indicating inhibition of protein synthesis at high temperature (Mizzen and Welch, 1988). Supplementation of *E. officinalis* produced significant variation in protein concentration in group IV.

Thus, it can be concluded that *E. officinalis* has various medicinal applications, but it is the need of hour to explore its medicinal values at molecular level with help of various biotechnological tools and techniques. Further studies should be conducted to elucidate the molecular mechanism of interaction of various plant based drugs with animal body in different types of stresses.

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