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EFFECT OF FORMULATION OF *EMBLICA OFFICINALIS* ON BIOCHEMICAL PARAMETERS IN WISTAR RATS

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ABSTRACT
Emblica officnalis formulation with ghee and honey is in wider practice in the Kerala, India as a rejuvenating agent. The objective of this paper is to study the effect of *Emblica officinalis* formulation on the following biochemical parameters like glucose, alkaline phosphotase, alkaline amino transferase, aspartate transferase, albumin, bilirubin, blood urea nitrogen, creatinine and total protein. The results indicate that there is a beneficial trend in some of the biochemical parameters within the normal ranges.

INTRODUCTION

World Health Organization has approved the use of traditional medicines as a part of the health program for the treatment of various diseases. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for the primary health care needs (WHO, 1993; Goyal, 2005). Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. Among the all estimated plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically (verpoorte *et al.* 1999). India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Numerous drugs have entered the international market through exploration of ethno pharmacology and traditional medicine (Goyal and Patel, 2010; Cooper, 2004).

Amla fruit is a rich natural source of vitamin C. It also contains cytokinine like substances identified as zeatin, zeatin riboside and zeatin nucleotide. The seeds yield 16% fixed oil, brownish yellow in colour. The plant contains tannins like glucogallia, corilagin, chebulagic acid and 3, 6-digalloyl glucose. Root yields ellagic acid, lupeol, quercetin and β-sitosterol (Thakur *et al.*, 1989). At present, investigation studies have been carried out on potential of *E. officinalis* formulation through exploration of phyto pharmacology and ethno medicinal use. The fruits of Amla are used in many medicinal preparations of Ayurvedic and Unani systems of medicine as well as food supplement (Kirtikar and Basu, 1935; Mishra *et al.* 2011). Many polyherbal formulations like Trifala, Cogent db, Diasulin contains *Emblica officinalis* as one of the ingredient (Pari and Saravanan, 2002; Ramalingam and Pari, 2005; Naik *et al.* 2006; Patel *et al.* 2009).

Ayurveda recommends that those who want to prolong their life should take the youth elixir, consisting of honey, milk and follow a diet. Honey is sweet and viscous fluid produce by honey bees (genus Apis) and other insects from the nectar of flowers. Modern system of medicine is also finding the honey efficacious in various medicinal and surgical conditions (Frankel *et al.* 1998; Lubsy *et al.* 2003). Antimicrobial, antioxidant and wound healing properties of honey are being evaluated with successful outcome. Prevention and treatment of various infections due to wide variety of organisms and promoting surgical wound healing are some of the areas where honey is making its mark (Bansal *et al.* 2005).

Ghee contains 8% of short chain saturated fatty acids, which helps in digestion (swern, 1979). Ghee is lipophilic and thus facilitates transportation of ingredients of formulation to target organ and final delivary inside the cell. Traditional cooking fat is healthier due to an ideal ratio of omega 6 to omega 3 fatty acids. Hyper cholemsterolemic effect of ghee is mediated by increasing the secretion of biliary lipids (Kumar, 2000).

As many supplements or drugs alter the biochemical status in the body of any animal including human beings, it is mandatory to study biochemical parameters. In the present investigations the impact of *Emblica officinalis* formulation on the biochemical parameters have been undertaken.

MATERIALS AND METHODS

Collection of *Emblica officinalis* formulation

Emblica officinalis formulation was procured from kottakkal ayurvedic vaidya Sala, Kerala. *Emblica officinalis* formulation

is available as a rejuvenating agent in ayurvedic practice and is prepared in combination of amla, ghee and honey. The rasayana is a mix of amalaki powder (12g), ghee (12g) and honey (24g) - together packaged as 45g/day. As per the ayurvedic literature, it should be one thola (about 11.66g) to be taken for one mandala (45 days). Actual amalaki (Processed amla powder) would then be about 0.2g/kg/day assuming they are 60kg in weight.

Procurement of animals for study

Animals were procured from National Institute of Nutrition (NIN), Hyderabad and cohorts of Wistar strain rats in-bred over generation and maintained in animal house and they were used for the following studies.

Study design

In this study, 48 animals were subjected for additional supplement of Rasayana and 48 were on normal diet. These two groups were designated as 'Treated' and 'Control', respectively. Rats were maintained in a pathogen free environment with a 12h light-dark cycle. Food and water were provided ad libitum.

Randomization and numbering of animals

After randomization, all the animals were acclimatized to laboratory conditions for a minimum of five days, and assigned to four groups of 24 animals consisting 12 males and 12 females. The females were nulliparous and non-pregnant.

Study consists of 96 rats (48 males + 48 females) which were divided into four groups and each with 24 rats. Among the four groups first was sacrificed on 3rd month, second, third and fourth groups were sacrificed in 6^{th}, 9^{th} and 18^{th} months respectively.

Randomization ensured that the allocation of treatments to animals/groups was independent of their characteristics and were similar in all the groups. It was also taken care while randomization, base variables were homogenized and were allotted to different groups. The need for randomization applies not only to the allocation of the animals to the different (control as well as treatment) groups but also anything that can materially affect the recorded response.

Dosage and route of administration

Administration of *Emblica officinalis* formulation is within the standard volume of 10 ml/kg body weight. *Emblica officinalis* formulation was administered at the concentrations of 0.45g/ 1mL per wistar rat which is 6 times greater than the human dosage and reason behind this is the higher metabolic rate of wistar rats is 6 times greater than human, and this dosage has been determined keeping the concentration prescribed for human dosage as 0.75g/kg body weight. Wistar rats 6-8 weeks of age, weighing 120 to 165g of both sexes have been used and the animals used in this study were obtained from NIN.

Route of administration and reason for choice

Oral route has been chosen because it is the proposed route for administration to humans. The dosage can be accurately administered. The oral route is one of the proposed routes for toxicity testing. The test item was administered by oral route.

Biochemical procedures

The blood samples drawn from the orbital plexus were collected in plain vacuette tubes and centrifuged at 3000 rpm for about 10 minutes to separate the serum for biochemical analysis using fully automated random access biochemical analyzer (ERBA XL 300). Following are the principles used to study various parameters.

Albumin

Albumin present in the serum binds specifically with Bromo Cresol Green to form a coloured complex. Albumin binds with Bromo Cresol Green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye. The intensity of blue-green color formed is proportional to concentration of albumin present, when measured photo metrically between 580-630nm (Spencer and Price, 1997).

Alkalaline phosphotase (ALP)

Para-Nitro phenyl Phosphate reacts with water in the presence of ALP and Magnesium Para nitro phenol and phosphate (Wenger *et al*. 1984). The intensity of yellow colour is directly proportional to the enzyme and can be measured at 450nm using spectrophotometer.

Bilirubin

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water-soluble directly reacts in acidic medium. However indirect or unconjugated bilirubin is solubilised using a surfactant and then it reacts similar to Direct Bilirubin (Jendrassik and Grof, 1938; Powell, 1994).

Creatinine

Creatinine is a waste product of creatine and is excreted by kidneys. Creatinine is synthesized from the amino acids glycine, arginine, and methionine by liver and pancreas. Glomerular filtration is responsible for removing creatinine from extracellular fluid (serum) Creatinine reacts with picric acid in alkaline medium to form reddish yellow complex, the intensity of which is directly proportional to the concentration of creatinine in the sample and can be measured at 520nm spectrophometrically (picric acid method) (Seation and Ali, 1984; Slot, 1965).

Creatinine + NaOH + Picric acid $\rightarrow$ Creatinine picric acid

Glucose

Glucose oxidase is an enzyme; it converts glucose to gluconic acid and hydrogen peroxide in the presence of water and oxygen. Hydrogen peroxide reacts with 4 – amino anti pyrine and 4 – hydroxy benzoic acid in the presence of enzyme peroxidase, it is converted into ounioneimine dye (pink colour) and water. Pink colour is proportional to the glucose concentration and can be measured photo metrically between 500 to 540 nm.

Methodology for AST and ALT is adopted from Bergmeyer method (Bergmeyer and Bernt, 1974).

Aspartate amino transferases (AST)

Aspartate aminotransferase was once referred to as glutamate oxaloacetate transaminase (GOT). This enzyme catalyses the transfer of an amino group from the aminoacid aspartate to oxalobutarate to form L-glutamate. As with ALT, pyridoxal pyridoxamine-5′ phosphate function as coenzyme which

favours the production of aspartate invivo which can be measured at 520nm.

Alanine amino transferases (ALT)

The end product, pyruvate, undergoes oxidation in the presence of LDH to develop a coloured complex which can be measured at 520 nm.

Total protein

According to biuret method, proteins react with cupric ions in alkaline medium to form violet complex. The intensity of colour produced is directly proportional to proteins present in the sample and can be measured at 530 nm using a spectrophotometer.

Blood urea nitrogen

The procedure is based on the Berthelot reaction. Urease splits urea into ammonia and carbon dioxide. The ammonia reacts with phenol in the presence of hypochlorite to form indophenols, which in alkaline medium gives blue colour, the intensity of which can be measured at 620nm.

Data analysis

The data of the above biochemical parameters was subjected for statistical analysis using MS Excel 2010. The difference between the groups was tested.

RESULTS AND DISCUSSION

Amla is a rich source of several flavonoids, tannins, saponinins etc. Earlier Natural medicinal plant reports reveals the alterations of various physiological parameters with improved health status in wistar rats. Ahmed *et al*. (2005) reported that the leaves of *T. Catappa* contained several flavonoids, tannins, saponins, triterpinoid and phytosterols.

Due to the above chemical richness, the leaves and bark are used in different traditional medicines for various purposes worldwide. They also reported the biochemical effects of administrating *T. catappa* Linn. aqueous leaf extracts intraperitoneally showed the regeneration of â-cells of the islets of Langerhans, decreased blood sugar, serum cholesterol, triglycerides, low density lipoprotein (LDL), creatinine, urea and alkaline phosphatase levels, while increasing the high density lipoprotein (HDL) level in diabetes mellitus (DM). Ram *et al*. (1997) who had earlier worked on *Terminalia arguna* reported that the oral administration of its aqueous tree bark extract did not have any effects on the liver, kidneys, lipid profile and haematological parameters of rats. The aqueous extracts of *T. catappa* leaves have been reported to have strong free radical scavenging activities (Kinoshita *et al*. 2007). Moody *et al*. (2003) and Ibegbulem *et al*. (2010), reported the *in vitro* anti sickling property of the extracts and decoction of *T. catappa*. The decoction is administered in folklore medicinal practice as a homemade prophylaxis against sickle-cell crises; without any reported side effect. Most of our traditional medicines are in the form of *chyawanprash*.

The results of the biochemical parameters analyzed are shown in Table 1. There is no significant difference between levels of albumin in female control and treated groups. The difference in bilirubin, creatinine and total protein levels of all groups is insignificant. Enhancement in glucose, ALP, ALT and AST in both sexes and albumin in males was observed following the

Table 1: Measured levels of various biochemical parameters for control and treated males and females

Males						Females				
Parameter	Control and treated	Months				Control and treated	Months			
		3	6	9	18		3	6	9	18
Glucose(mg/dL)	C-M	63.8±8.1	103.3±7.2	91.8±9.8	100.7±9.7	C-F	97.0±9.5	94.0±5.7	81.6±7.6	98.5±8.6
	T-M	97.2±10.4	99.0±6.9	104.0±11.9	89.0±8.0	T-F	95.0±4.7	98.8±11.8	102.2±9.7	105.0±11.2
Albumin(g/dl)	C-M	3.5±0.2	3.6±0.1	3.0±0.1	3.5±0.12	C-F	3.6±0.3	3.6±0.1	3.2±0.1	3.3±0.0
	T-M	3.3±0.1	4.8±0.1	6.0±0.0	10.7±0.1	T-F	3.7±0.1	3.6±0.2	3.3±0.1	3.3±0.1
ALP(U/L)	C-M	184.8±48.2	234.7±29.7	395.5±52.3	414.3±176.8	C-F	166.7±38.7	222.8±41.7	484.6±135.0	287.3±74.8
	T-M	230.0±22.7	249.2±15.4	330.2±40.0	367.3±90.8	T-F	211.3±36.8	210.2±46.0	380.0±86.6	333.7±73.3
ALT(U/L)	C-M	48.5±6.0	52.5±7.1	56.0±7.3	87.7±21.8	C-F	41.8±1.7	48.2±7.7	66.2±9.4	73.3±7.6
	T-M	54.3±6.4	56.0±5.6	71.7±14.9	99.7±15.5	T-F	53.5±5.4	58.0±4.0	65.5±6.8	76.3±13.0
Bilirubin(mg/dL)	C-M	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	C-F	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
	T-M	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	T-F	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
BUN(mg/dL)	C-M	15.7±2.1	15.2±2.1	20.3±1.8	23.0±1.0	C-F	16.2±1.3	15.0±1.7	20.8±1.7	23.0±1.6
	T-M	15.5±0.5	14.7±0.8	21.0±1.2	21.0±0.8	T-F	14.7±0.8	18.2±2.4	21.3±1.8	18.5±2.1
Creatinine(mg/dL)	C-M	0.5±0.0	0.4±0.0	0.5±0.0	0.4±0.0	C-F	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0
	T-M	0.4±0.0	0.4±0..0	0.4±0.0	0.5±0.0	T-F	0.4±0.0	0.4±0.0	0.5±0.0	0.4±0.0
Total protein(g/dl)		C-M	7.3±0.2	7.5±0.3	5.8±0.2	6.4±0.2	C-F	7.0±0.1	7.6±0.4	6.1±0.36.2±0.2
	T-M	7.4±0.2	7.3±0.2	6.1±0.1	6.4±0.3	T-F	7.0±0.1	7.5±0.3	6.3±0.3	5.9±0.3
AST(U/L)	C-M	166.3±12.4	185.5±25.9	174.5±26.2	188.8±45.0	C-F	143.2±16.4	175.7±23.3	196.4±15.2	171.0±15.3
	T-M	169.2±21.5	154.7±17.6	171.2±22.5	206.5±40.1	T-F	148.5±17.4	183.0±15.1	167.0±13.1	172.0±25.9

Values are presented as mean ± Standard Deviation ALP - Alkalaline Phosphotases, ALT - Alkaline Amino Transferases, AST - Aspartate Amino Transferases, BUN-Blood Urea Nitrogen CM-Control Males CF - Control females

Emblica officinalis formulation treatment within the normal levels. Table 1 shows the biochemical data for males and females for all the 4 groups *i.e.* control vs. treated Males and Females during various life stages. *i.e.* after 3, 6, 9 and 18 months of dosage .

The results reveal that there is a positive biochemical profile in all the parameters of both males and females. The data of all the parameters were comparatively normal and were near to the data of the control group. Presently formulations are in agricultural practice to reduce the toxicity and to have better efficacy due to the synergetic effect of its constituents. Hence a 18 month study was designed by administering dosage daily through oral gavage. The Blood samples were collected from both experimental and control Rats to assess the biochemical markers like Creatinine, glucose, blood urea nitrogen, Alkaline phosphatase, ALT and AST. The results are quite interesting to note that both male and female experimental animals showed a significant increase in glucose, creatinine and ALP was observed in treated groups when compared to untreated control whereas other biochemical markers did not show any significance. From the results it is evident that the organs like pancreas [glucose], kidney[creatinine] and bone [ALP] showed marginal change in their biochemical markers only in the treated without showing any significant change in the controls.

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