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Effect of “Garbha Cintamani Rasa”, an Ayurvedic Formulation on Lipid Profile, Liver Function and Kidney Function Parameters of Rat Plasma after Chronic Administration

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Abstract

Garbha Cintamani Rasa (GCM), a classical Ayurvedic preparation which is used in puerperal complications, was studied for its effect on liver function, kidney function and lipid profile after chronic administrations into the biological system. The experimental animal model was rat of both sexes. Triglycerides (TG) content was observed to be increased very high significantly in male as well as female group. Statistically, irrespective of sexes, high significant increase was found in very low density lipoprotein (VLDL). Decrease of total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) were negligible in male rats. Similar results were shown by female rats also. In both of the sexes, total protein and albumin content of plasma were increased very high significantly. In case of bilirubin, the increase was negligible for all sexes. The serum

glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and alkaline phosphatase (ALP) content in the plasma were decreased very high significantly in both of the experimental groups. Creatinine, urea and uric acid were decreased in male and female where only change of creatinine level was significant for both of the sexes. The changes of plasma parameters of both sexes were in similar fashion when one sex group is compared to opposite sex group.

Keywords: Garbha Cintamani Rasa, GCM, Ayurvedic, Lipid profile, Kidney function and Liver function.

1. Introduction

Ayurvedic metallic preparations with herbal liquids are known in the Indian subcontinent since the seventh century BC and widely recommended for treatment of a variety of chronic ailments. “Garbha Cintamani Rasa” (GCM), used in puerperal complications, is one of unique metallic-herbal Ayurvedic preparation. In this preparation, various roasted metals (bhasma) are used with other medicinal plants. These roasted metals are found to be chelated with organic ligands derived from these plants liquids. These bhasmas are biologically produced nanoparticles and are taken along with herbal liquids. Thus, this makes these elements easily assimilable, eliminating their harmful effects and enhancing their biocompatibility (kumar et al, 2006).

The metals used in this preparation are Mercury, Silver, Iron, Tin and Copper but non-metal includes Silicone and Camphor. All these were used in roasted form which is produced by a special type of Ayurvedic technique. Among these, Iron and Copper are found to have some pharmacological activities. Sarkar et al (2007) reported the hematinic and cytoprotective activity of ‘Lauha bhasma’ (roasted Iron). The hepatoprotective activity of ‘Tamra bhasma’ (roasted Copper) was studied by Tripathi and Singh (1996) and suggested that it has a strong antioxidant potential and could be used in the management of lipid peroxidation. Abhra bhasma (Mica/ Siliceous encrustation) was believed to use as a rejuvenator and increase vitality. In case of ‘Rasa’ (Mercury) ‘Tara bhasma’ (roasted Silver) and ‘Vanga bhasma’ (roasted Tin), no individual report of medicinal uses was found but they are used in several Ayurvedic formulation along with medicinal herbs.

Different parts of five medicinal plants are also used in GCM where plants are reported for their medicinal and therapeutic uses. The anti-fungal, antibacterial and antioxidant potentials of essential oil and acetone extract of *M. fragrance* were reported by Singh et al (2004). Olaleye et al (2006) confirmed the presence of alkaloids, saponins, anthraquinones, cardiac glycosides, flavonoids and phlobatanins in *M. fragrance*. Pro-erectile pharmacological effects of *T. terrestris* extract on the rabbit corpus cavernosum were studied and significant result was found by Adaikan et al (2000) and its aphrodisiac properties were unfolded by Gauthaman et al (2002). Six new steroidal saponins were isolated from *T. terrestris* (Wang et al, 1997). *A. racemosus* is a popular medicinal plant and it is found for several traditional uses. Aqueous extract of *A. racemosus* root is a potential immuno-adjuvant (Gautam et al, 2004). The ulcer protective effect of methanolic extract of fresh roots of this plant was studied by Sairam et al (2003) and it was found to increase mucosal defensive factors like mucus secretion, cellular mucus, and life span of cells and it also possessed significant anti oxidant effect. The antioxidant potential of *A. racemosus* extract in vitro in mitochondrial membranes of rat liver is reported by Jayashree et al (2000) and against DPPH is reported by Wiboopun et al (2004). The anti-inflammatory and analgesic effects of *S. cordifolia* is confirmed by Franzotti et al (2000). Significant analgesic activity (Ahmed et al, 2000), hepato-protective potentials of aqueous extract (Porchezian and Ansari, 2005) and hypoglycemic properties of alcohol and water extract (Seetharam et al, 2002) of *A. indicum* were reported. Present study was undertaken to evaluate the effect of GCM on various biochemical markers which includes lipid profile, kidney function and liver function parameters.

2. Materials and Methods

2.1. Drugs, Chemicals and Reagents

For the biochemical study, Garbha Cintamani Rasa (GCM) was collected from Sree Durga Aushadhalaya Ltd, Chittagong. All other reagents and chemicals that were used in this work were purchased from Sigma Chemical Co. and were prepared in all glass-distilled water.

2.2. Experimental Animals

Albino rats (*Rattus norvegicus*: Sprague-Dawley strain,) of both sexes, bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University were used in the toxicological experiment. These animals were 48 weeks old, apparently healthy and weighed 50-70 g. Throughout the period of the experiment, the animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions. All of the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animals was done *ad libitum*, along with drinking water and maintained at natural day night cycle. They were fed with “mouse chow” (prepared according to the formula developed at BCSIR, Dhaka). Absolute compliance and ethical guide lines are followed for laboratory animal during all the experiments.

2.3. Experimental Design

In all the experiment, a total of forty rats of both sexes were used. The rats were divided into four groups of ten animals where two were male groups and other were female groups. For both of the sexes, one group was treated with GCM and another was used as a control. The control animals were administered with distilled water as placebo as par the same volume as the drug treated group for the same number of days. Before starting an experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular rat prior to and after the administration could be noted separately. For all the pharmacological studies the drugs were administered per oral route at a dose of 100mg/kg body weight. After acclimatization, administration of GCM was done by intra-gastric syringe between the hours of 10 am and noon. At the due of the 46-day treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration, the animals were anaesthetized using i.p. Ketamine (500 mg/kg i.p.).

2.4. Blood Samples Collection and Preparation of Plasma

Blood samples were collected from post vena cava and transferred into heparinised tubes immediately. Blood was then centrifuged at 4,000 g for 10 min using bench top centrifuge (MSE Minor, England) to remove red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteur pipette and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection.

2.5. Determination of Biochemical Parameters

To assess the state of the liver and kidney, biochemical studies involved analysis of parameters such as total protein, serum albumin, blood urea nitrogen (BUN), bilirubin (total and direct), creatinine, and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). For lipid profile study, triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL) were determined but low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated.

Biuret method (Plummer, 1971) was followed to determine the Total protein and serum Albumin concentration was determined by using the method of Doumas *et al* (1971). TG, TC and HDL concentration were evaluated according to the instruction of manufacturer of assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). According to Friedewald’s formula (Friedewald *et al.*, 1972) VLDL and LDL were calculated as: VLDL cholesterol = TG/5 and LDL cholesterol = TC – (VLDL+HDL cholesterol). Serum bilirubin was determined according to the method of Evelyn and Malloy (1938). The procedure of Tietz *et al* (1994) was used to determine serum creatinine concentration while the serum urea concentration was determined by the method of Kaplan (1965). King and King (1954) method was employed to determine serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and alkaline phosphatase (ALP). The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC.).

2.6. Statistical Analysis

The data were analyzed using unpaired t-test as described by Glasnapp and Poggio (1985) and expressed as Mean \pm SEM (Standard Error of the Mean). SPSS for windows was applied for the analysis of data and $p < 0.05$ was taken as the level of significance.

3. Result and Discussion

3.1. Lipid Profile

Statistically very high significant decrease of TG (male: $p = 0.001$; female: $p = 0.001$) was found in the plasma of both sexes. The decrease of VLDL (male: $p = 0.005$; female: $p = 0.005$) content in plasma of male and female was significant. On the contrary, the changes in TC, LDL and HDL were not significant in none of the sexes. Like other plants constituents (Lee *et al.*, 2000) GCM reduced TG level and it could be suggested that GCM increased lipase activity which hydrolyzed TG. Among the lipids, increased blood level of TC, LDL and VLDL as well as lowered level of HDL has been identified as contributors in the development of hyperlipidemia (Ross, 1999) which is the consequences of, in majority of the cases, diabetes mellitus (Pushparaj *et al.*, 2000; Pepato *et al.*, 2003; Sharma *et al.*, 1983). The elevation of lipid components is a risk factor for coronary heart disease (Mironova *et al.*, 2000). GCM may act as inhibitor for enzyme such as hydroxyl-methyl-glutaryl-CoA reductase, which is the key enzyme in de novo cholesterol biosynthesis as has been suggested for some plants earlier (Gebhardt and Beck, 1996; Eidi *et al.* 2006). This reduction could be beneficial in improving lipid metabolism and complications in diabetes (Cho *et al.*, 2002) (Table: 2).

Table 1: Name of the plants/ ingredients used in the preparation of “Garbha Cintamani Rasa” (GCM).

Name of Plants / Ingredients	Used Parts	Botanical/ Scientific Name	Family	Amount Used
Rasa (suddha parada)		Mercury	--	12 g
Tara (Rajata bhasma)		Roasted Silver	--	12 g
Lauha bhasma		Roasted Iron	--	12 g
Abhra (abhraka) bhasma		Mica or Siliceous encrustation	--	24 g
Karpura		Camphor	--	24 g
Vanga bhasma		Roasted Tin	--	24 g
Tamra bhasma		Roasted Copper	--	24 g
Jati phala	Seed	<i>Myristica fragrance</i>	Myristicaceae	12 g
Jatikosa (jatiphala)	Arillus	<i>Myristica fragrance</i>	Myristicaceae	12 g
Goksura	Flower	<i>Tribulus terrestris</i>	Zygophyllaceae	12 g
Satavari	Root	<i>Asparagus racemosus</i>	Litiaceae	12 g
Balamula	Root	<i>Sida cordifolia</i>	Malvaceae	12 g
Atibala mula	Root	<i>Abutilon indicum</i>	Malvaceae	12 g
Jala		<i>Water</i>	--	Q.S.

Table 2: Effect of chronic administration of GCM (100 mg/ kg body weight) on Lipid Profile of rats' plasma.

Parameters	Male Rats			Female Rats		
	Control (n= 10)	Test (n= 10)	P values	Control (n= 10)	Test (n= 10)	P values
Triglycerides	96.47 ± 1.1113	51.6964 ± 0.9195	0.001***	97.9667 ± 3.5946	68.1957 ± 0.5698	0.001***
Total cholesterol	68.18 ± 1.6964	60.93 ± 1.9398	0.07	75.2444 ± 1.6415	66.6217 ± 3.9706	0.073
VLDL	14.75 ± 0.7606	12.5554 ± 0.4467	0.005**	17.7444 ± 0.4385	15.9323 ± 0.4821	0.002**
LDL	19.1 ± 0.7734	18.1118 ± 0.7304	0.389	19.6556 ± 0.6976	18.9726 ± 0.9627	0.498
HDL	33.01 ± 0.8822	31.232 ± 1.053	0.742	34.3778 ± 1.0118	33.3920 ± 1.8246	0.711

3.2. Liver Function

In the male as well as female rats there was statistically very highly significant increase in the total protein (male: $p = 0.001$; female: $p = 0.001$) and the albumin (male: $p = 0.001$; female: $p = 0.001$) content of the plasma. These proteins are important liver function marker. According to Naganna (1989), increase in bilirubin is indicating the abnormal liver function which may be the results of higher synthetic function of the liver. Statistically no important data of bilirubin, another liver function indicator, was found in none of the sexes. This is indicating the normal liver function which is contradictory with the total protein and albumin observation. sGPT (male: $p = 0.001$; female: $p = 0.001$), sGOT (male: $p = 0.001$; female: $p = 0.001$) and ALP (male: $p = 0.001$; female: $p = 0.001$) content in the plasma, irrespective of sexes, were decreased very high significantly. Alkaline phosphatase is the marker enzyme for plasma and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan *et al.*, 2004) and its decrease indicates the improved synthetic activity of liver. From the bilirubin and serum enzyme observations, it seems that GCM increases the liver function significantly (Table: 3)

Table 3: Effect of chronic administration of GCM (100 mg/ kg body weight) on various parameters of liver functions of rats' plasma.

Parameters	Male Rats			Female Rats		
	Control (n= 10)	Test (n= 10)	P values	Control (n= 10)	Test (n= 10)	P values
Total protein	5629.099 ± 65.8914	6801.9213 ± 98.1568	0.001***	5384.6644 ± 160.4354	6403.9275 ± 56.9730	0.001***
Albumin	4517.12 ± 117.6067	5226.4088 ± 68.6375	0.001***	4221.3044 ± 75.5618	6082.0084 ± 23.9830	0.001***
Bilirubin	0.1237 ± 0.002463	0.1248 ± 0.002474	0.901	0.07222 ± 0.004006	0.07298 ± 0.0009913	0.901
sGPT	60.27 ± 0.1257	55.5366 ± 0.1122	0.001***	50.1667 ± 0.1434	46.9768 ± 0.1098	0.001***
sGOT	101.73 ± 0.3015	93.7411 ± 0.2309	0.001***	82.500 ± 0.2041	77.8568 ± 0.1842	0.001***
ALP	43.56 ± 0.1087	40.1266 ± 0.07816	0.001***	35.4556 ± 0.1042	32.2017 ± 0.05198	0.001***

3.3. Kidney Function

Creatinine content, major kidney function parameter, in the male and female plasma was decreased significantly (male: $p = 0.018$; female: $p = 0.015$) but the content of urea and uric acid were not changed in significant manner. This reduced creatinine level might have results from the decreased synthesis or increased functional capacity of tubular excretion (Mitchell et al., 1972; Zilva et al., 1991) (Table: 4).

Table 4: Effect of chronic administration of GCM (100 mg/ kg body weight) on various parameters of kidney functions of rats’ plasma.

Parameters	Male Rats			Female Rats		
	Control (n= 10)	Test (n= 10)	P values	Control (n= 10)	Test (n= 10)	P values
Creatinine	0.9487 ± 0.01214	0.8042 ± 0.03555	0.018*	0.9778 ± 0.04134	0.8295 ± 0.03181	0.015*
Urea	65.862 ± 1.0452	65.085 ± 0.6941	0.618	57.5333 ± 1.2423	57.1030 ± 1.0294	0.865
Uric acid	2.578 ± 0.05481	2.5335 ± 0.03323	0.872	2.7967 ± 0.0944	2.7297 ± 0.0839	0.598

Note: In the tables the statistical data are shown as: * = $p < 0.05$ = Significant, ** = $p < 0.01$ = High Significant, *** = $p < 0.001$ = Very High Significant

4. Conclusion

The present investigation has shown that GCM improved liver synthetic activity, reduced lipids level and increased kidney function parameters. Diabetic condition alters these parameters specially lipid profile. Abnormalities in serum lipids are associated with diabetes (Virella-Lopes and Virella, 2003; NCEP, 2002). GCM may be used to improve the complications in diabetic condition. If further investigations show hypoglycemic activity then GCM could be a safe Complimentary and Alternative Medicine (CAM) in diabetic treatment.

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