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Studies of Lipid Profile, Liver Function and Kidney Function Parameters of Rat Plasma after Chromic Admimistration of "Sulavajrini Vatika"

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Abstract: The successful use of Ayurvedic medicines is for many years but there is no guideline for studying the toxicity of these preparations through preclinical or clinical investigations. The present study was conducted to evaluate the effect of conventionally prepared Sulavajrini Vatika (SBB), an Ayurvedic formulation on various biochemical parameters of experimental animals after chronic administration. The animal used was albino rats (*Rattus norvegicus*: Sprague-Dawley strain) and SBB was administered orally at a single dose of 100 mg kg^{-1} b.wt. day^{-1}, up to 62 days. During the study, forty rats, equally of both sexes, were randomly grouped into four where one male and one female group were used as control and other groups were used as test. Among the lipid components, Triglyceride (TG) was decreased very high significantly in both sexes of animal. The decrease of Total Cholesterol (TC), Very Low Density Lipoprotein (VLDL) and high-density lipoprotein (HDL) were also highly significant. Low Density Lipoprotein (LDL) decreased in all SBB treated group. In the liver function parameters, the total protein and albumin content were increased very high significantly in both sexes of rat. But the bilirubin was decreased insignificantly in male and female rats. Serum Glutamic Pyruvic Transaminase (GPT), Glutamic Oxaloacetic Transaminase (GOT) and Alkaline Phosphatase (ALP) were decreased in all treated animals and it was very high significant. In case of kidney function parameters, creatinine was increased very high significantly but the urea was decreased very high significantly in both sexes of rat. The decrease in uric acid was not significant in none of the sexes of rat. The present study confirms that SBB can be contributory for the complications in diabetics with hyperlipidemia and nephropathy as it lowers most of the lipids components and improves liver function and kidney function parameters.

Key words: Sulavajrini vatika, Ayurvedic, lipid profile, kidney function, liver function

INTRODUCTION

Roasted and non-roasted metals are used in the some formulation of Ayurvedic medicinal system. "Sulavajrini Vatika" (SBB) is one of the formulations, where four metals and eighteen herbs are used (Table 1). All the herbs and Tankana (Sodium bicarbonate) were used in equal amount of 12 g, metals are used as 24 g except Sulva (tamra) bhasma 12 g whereas, Chagi dugdha (goats milk) was used as required for the formulation. Because of having chelating ability with organic-liquids, these metals (roasted and non-roasted) are easily assimilable, eliminate harmful effect and improve the biocompatibility of the formulations (Kumar *et al*., 2006). Among the four metals used in the studied preparation, iron and copper has been reported as hematinic as well as hepatoprotective (Tripathi and Singh, 1996; Sarkar *et al*., 2007).

Individual plants used in this formulation have several medicinal and therapeutic uses. The equi-proportional combination of *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinallis* known as "Triphala" is antioxidant and radioprotective (Jagetia *et al*., 2002). Gallic acid and other phenolics isolated from *T. chebula and T. bellerica* are antioxidant (Aqil *et al*., 2006). Several cytotoxic compounds are isolated from *T. chebula* (Lee *et al*., 1995). Antibacterial activity of *T. bellerica* was also confirmed by Elizabeth (2005). The fruit extract of *E. officinalis* inhibit

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Table 1: Name of different plants and ingredients use in the formulation of Sulavajrini Vatika (SBB)

Name of the plants/ingredients	Used parts	Botanical/Scientific name	Family	Amount used
Rasa (parada)		Mercury		24 g
Gandhaka		Sulphur		24 g
Lauha bhasma		Roasted Iron		24 g
Sulva (tamra) bhasma		Roasted Copper		12 g
Tankana		Sodium bicarbonate		12 g
Haritaki	Fruit pulp	*Terminalia chebula*	Combretaceae	12 g
Bibhitaka (Belleric Myrobalan)	Fruit pulp	*Terminalia bellerica*	Combretaceae	12 g
Amalaki (Gooseberry)	Fruit pulp	*Emblica officinalis*	Euphorbiaceae	12 g
Ramatha (Asafotida)	Exudates	*Ferula asafoetida*	Umbelliferae	12 g
Sathi (kaempferia)	Rhizome	*Hedychium spicatum*	Zingiberaceae	12 g
Sunthi (ginger)	Rhizome	*Zingiber officinale*	Zingiberaceae	12 g
Marica (black pepper)	Fruit	*Piper nigrum*	Piperaceae	12 g
Pippali (Long pepper)	Fruit	*Piper longum*	Piperaceae	12 g
Tejapatra (dry bay leaf)	Leaf	*Cinnamomum macrocarpum*	Lauraceae	12 g
Tvak (Ciunamon)	Stem bark	*Cinnamomum zeylanicum*	Lauraceae	12 g
Ela (cardamon)	Seed	*Eletaria cardamomum*	Zingiberaceae	12 g
Talisa (holy basil)	Leaf	*Abies webbiana*	Pinaceae	12 g
Jatiphala (nutmeg)	Seed	*Myristica fragrans*	Myristicaceae	12 g
Lavanga (clove)	Flower	*Syzygium aromaticum*	Myrtaceae	12 g
Yamani (henbane)	Fruit	*Hyoscyamus niger*	Solanaceae	12 g
Jiraka (cumin)	Fruit	*Cuminum cyminum*	Apiaceae	12 g
Dhanya (coriander)	Fruit	*Coriandrum sativum*	Apiaceae	12 g
Chagi dugdha (goats milk)				Q. S (quantity sufficient) for mardana

micronuclei formation, sister chromatid exchanges, clastogenesis and mutagenesis induced by metals and other clastogens; protect from radiations (Scartezzini and Speroni, 2000; Haque *et al*., 2001) possess antidiabetic property (Sabu and Kuttan, 2002). It is gastroprotective (Al-Rehaily *et al*., 2002), cytoprotective and immunomodulative (Sai Ram *et al*., 2002, 2003). Recent research shows that *Emblica* is antioxidant (Rajak *et al*., 2004), antivenomic (Alam and Gomes, 2003), antiproliferative (Lambertini *et al*., 2003), antitussive (Nosal'ova *et al*., 2003) and ameliorates hyperthyroidism and hepatic lipid peroxidation (Panda and Kar, 2003) as well as induces apoptosis (Rajeshkumar *et al*., 2003). Anticoagulant and muscle relaxant activity of *Ferula asafoetida* is explored by Leung (1980). Different compounds isolated from *Zingiber officinale* are used as antiemetic, abortifacient, antibacterial, anti-inflammatory (Verma *et al*., 1993), antioxidant (Shobana and Naidu, 2000), anticoagulant, antihyperlipidemic, antihypertensive, antihyperglycaemic, anti-spasmodic, aperient alexeteric, circulatory stimulant, counter irritant, sialagogue and vasodilator. The medicinal value of *P. nigrum* has been unfolded for its use against the treatment of cholera, malaria, bacterial infection, paraplegia and arthritic diseases weakness following fevers, vertigo coma, sore throat, piles and skin disease (Chopra *et al*., 1956; Nosal'ova *et al*., 2003).

Analgesic and diuretic effect, relaxation of muscle tension and alleviation of anxiety activity has been reported by Singh (1992). Antioxidant, antimicrobial and fungicidal activity of the oil of *Cinnamomum zeylanicum* has been published (Baratta *et al*., 1998; Ranasinghe *et al*., 2002). *Elettaria cardamomum* (Linn.) is stomachic, carminative (Khory and Katrak, 1985), tonic (Chopra *et al*., 1958). *Syzygium aromaticum* is used in dyspepsia, gastric irritation and analgesia (Shyamala *et al*., 2003). The seed extracts from *Coriandrum sativum* has been confirmed for anti-fertility, anti-diabetic, antihyperlipidemic, antioxidant, and hypotensive activities (Al-Said *et al*., 1987; Chithra and Leelamma, 1997; Gray and Flatt, 1999; Melo *et al*., 2003). The essential oil of *Cuminum cyminum* is antiepileptic (Janahmadi *et al*., 2006). It is necessary to have the experimental evidence for the medicinal value of this formulation apart from its traditional uses. In the recent study, the effects of SBB on the lipid profile, liver function as well as kidney function parameters of rats' plasma after its chronic administration was evaluated.

MATERIALS AND METHODS

Dose and route of administration: To accomplish the study of intended biochemical parameters, SBB was collected from Sree Durga Aushadhalaya Ltd., Chittagong. The liquid was administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. The drugs were administered per oral route at a single dose of 100 mg kg^{-1} b.wt. day^{-1}.

Experimental animal: For the study, forty eight-week old albino rats (*Rattus norvegicus*: Sprague-Dawley strain) of both sexes, bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University. These animals were apparently healthy and weighed 450-500 g.

The animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. All of the rats were kept in plastic cages having dimensions of 30×20×13 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). All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals.

Controls: A group of equal number of rat as the drug treated group was simultaneously used in the experiment. They were administered with distilled water as placebo as par the same volume as the drug treated group for the same number of days and this group served as the control. A total of 40 rats were taken for experiment and prior to the experiment, they were randomly divided into 4 groups of 10 animals/sex. Thus, ten rats were taken for each group for both control and the experimental group. After acclimatization, administration of the Ayurvedic medicinal preparation was done by intra-gastric syringe. Administration of the extract was between the hours of 10 am and 12.00 am.

Blood sample preparation: At the due of the 62-day treatment period, the animals were fasted for 18 -24 h after the last administration, the animals were anaesthetized using i.p. Ketamine (500 mg kg^{-1} i.p.). Blood samples were collected from post vena cava and transferred into heparinized tubes immediately. Blood was then centrifuged 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.

Determination biochemical parameters: To assess the state of the liver and kidney function, measure the lipid profile, biochemical analysis was carried out on plasma. These 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 Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (ALP).

Total protein content of the samples was assayed by the Biuret method (Plummer, 1971). The method of Evelyn and Malloy (1938) was employed to determine the serum bilirubin concentration of the samples. 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). Alkaline phosphatase activities were determined using the method as described by Kind and King (1954). The absorbance of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer). The obtained data was analyzed using unpaired t test according to Glasnapp and Poggio (1985) and presented as Mean±Standard Error of the Mean (SEM). Statistical Package for Social Science (SPSS) for windows was applied for the analysis of the data. The $p = 0.05$ was taken to be level of significance.

RESULTS

Lipid profile: The analysis of various lipid components is shown in Fig. 1. In the lipid profile study, similar pattern of changes of different lipid components was found in both of sexes of animal. The Triglycerides (TG) was decreased very high significantly ($p = 0.001$) both of the male and female rats plasma. In case of Total Cholesterol (TC), Very Low Density Lipoprotein (VLDL) and High-Density Lipoprotein (HDL) the decrease were highly significant ($p = 0.001$) for each of these component for all SBB treated animals. Low Density Lipoprotein (LDL) also followed similar trend of increase but the result was not highly significant.

Liver function parameters: The studies of various liver function parameters are given in Table 2. Like the trend of lipid profile changes, the liver function parameters were changed in both of the male and female animal. Irrespective of sexes, the total protein and albumin content were increased very high significantly ($p = 0.001$). For total protein, the value of control was 5629.099±65.8914 and SBB treated male rats was 6128.1734±98.4029. In case of albumin, the value of control was 4517.12±117.6067 and SBB treated male rats was 5183.37±96.5843. For female rats, the values for total protein were 5384.66±160.4354 (control) and 6179.15±104.3606 (SBB treated rats). For albumin, the values were 4221.3044±75.5618 (control) and 4835.1790±68.6604 (SBB treated). But bilirubin was changed reversely. The decrease of bilirubin was not

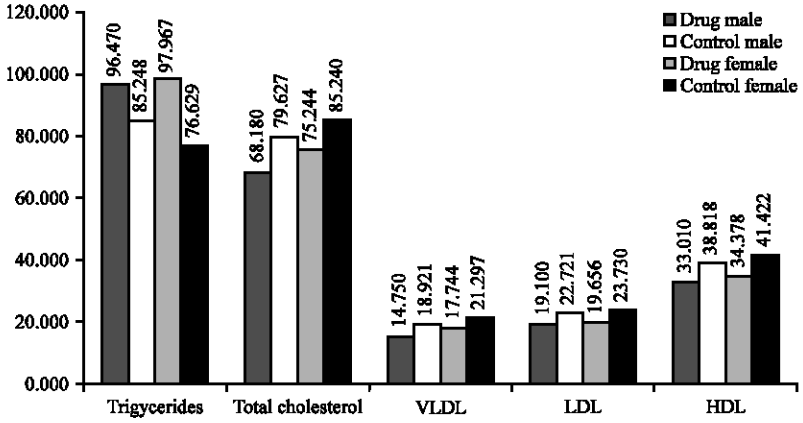

Fig. 1: Effect of Sulavajrini Vatika (SBB) on lipid profile of rat plasma after chronic administration at a single dose of 100 mg kg^{-1} b.wt. day^{-1}. Values are Mean±SEM, The mean of each lipid component is expressed on bar top where $p \leq 0.05$ was taken to be level of significance

Table 2: Study of the liver function parameters of rat plasma after chronic administration of Sulavajrini Vatika (SBB)

	Male rats			Female rats		
Parameters	Control	Test	p-value	Control	Test	p-value
Total protein	5629.099±65.8914	6128.1734±98.4029	0.001***	5384.66±160.4354	6179.15±104.3606	0.001***
Albumin	4517.12±117.6067	5183.37±96.5843	0.001***	4221.3044±75.5618	4835.1790±68.6604	0.001***
Bilirubin	0.1237±0.002463	0.1226±0.002674	0.701	0.07222±0.004006	0.07032±0.003978	0.698
GPT	60.9813±0.09534	60.27±0.1257	0.001***	55.1485±0.1352	50.1667±0.1434	0.001***
GOT	115.5739±0.9864	101.73±0.3015	0.001***	20.9778±0.2278	17.7444±0.4385	0.001***
ALP	46.3561±0.1563	43.56±0.1087	0.001***	43.4869±0.1069	35.4556±0.1042	0.001***

Values are Mean±SEM, $p \leq 0.05$ was taken to be level of significance, ***Very highly significant at $p \leq 0.001$, n = 10

Table 3: Study of the kidney function parameters of rats plasma after chronic administration of Sulavajrini Vatika (SBB)

	Male rats			Female rats		
Parameters	Control	Test	p-value	Control	Test	p-value
Creatinine	0.9487±0.01214	1.02868±0.01354	0.001***	0.9778±0.04134	1.2109±0.01971	0.001***
Urea	65.862±1.0452	58.1972±1.1824	0.001***	57.5333±1.2423	49.1563±1.0976	0.001***
Uric acid	2.578±0.05481	2.5481±0.06893	0.843	2.7967±0.0944	2.7728±0.08642	0.928

Values are Mean±SEM, $p \leq 0.05$ was taken to be level of significance, ***Very highly significant at $p \leq 0.001$, n = 10

statistically significant (p = 0.701) in none of the SBB treated group. Marker enzyme of liver i.e., serum Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Alkaline Phosphatase (ALP) were decreased very high significantly (p = 0.001) in both of the sexes.

Kidney function parameters: Kidney function analysis includes the study of creatinine, urea and uric acid. Creatinine is increased very high significantly (p = 0.001) in both of the sexes of rats (for male, control, 0.9487±0.01214, SBB treated rats, 1.02868±0.01354 and for female rats, control; 0.9778±0.04134, SBB treated; 1.2109±0.01971 but the urea was decreased (male, control; 65.862±1.0452 , SBB treated rats; 58.1972±1.1824 and for female rats, control; 57.5333±1.2423 , SBB treated rats; 49.1563±1.0976) very high significantly (p = 0.001). These results are supporting the results of other parameters. The decrease of uric acid was not significant in any of the treated group (Table 3).

DISCUSSION

As TG is decreased very high significantly and HDL is increased it could play an important role in the treatment of hyperlipidemic patient (Sharma *et al*., 1983). SBB reduced TG which may interpret that it increases lipase activity which hydrolyzes lipids. Increased in TC, VLDL and LDL level as well as decreased in HDL level are the contributory factors for the development of hyperlipidemia (Ross, 1999), which is vulnerable for coronary heart disease (Mironova *et al*., 2000). This

elevated level of lipids, in majority of the cases, is the consequences of diabetic mellitus (Sharma *et al*., 1983; Pushparaj *et al*., 2000; Pepato *et al*., 2003). High blood glucose level potentiate the Hormone Sensitive Lipase (HSL), which enhances the release of free fatty acid from adipose tissue (Al-Shamaony *et al*., 1994). Later on these fatty acids are converted to phospholipids and cholesterol in the liver. These components are discharged into the blood in the form of lipoproteins (Bopanna *et al*., 1997). The polysaccharides present in the plants used in this formulation may be responsible for hypocholesterolemic effect as some these polysaccharides are identified as bioactive compound (Yuan *et al*., 1998; Wang and Ng, 1999). Some of the component of SBB may also reduce de novo cholesterol biosynthesis by antagonizing hydroxy-methyl-glutaryl-CoA reductase which has been suggested for some plants earlier (Gebhardt and Beck, 1996; Eidi *et al*., 2006). The lowering of lipids could be contributory for the complications in diabetics (Cho *et al*., 2002). Elevation of plasma bilirubin indicates the abnormal liver function, which may be the result of higher synthetic function of liver (Naganna, 1989). In our present study the decreased bilirubin is, although it is not significant, contradictory with the changes of total protein and albumin. The decrease of serum GPT, GOT and ALP are supporting the changes of lipid component in the SBB treated rats plasma. The increase GPT activity is always due to hepatocellular and tissue damage which usually accompany the increase of GOT during diabetic condition (Sekar *et al*., 1990). Our study shown the trends toward the decreased activity of transaminases, used as indicator of liver function (Hearse, 1979), which indicates the improved synthetic activity of liver. This reduction may result the increase clearance and decreased production of cholesterol and triglycerides (Rajasekaran *et al*., 2006). This formulation may be used for diabetic condition because liver play a vital role in glucose and lipid homeostasis, which is severely affected in diabetic (Seifter and England, 1982). Liver performs the uptake, oxidation and metabolic conversion of fatty acid, generation of cholesterol and phospholipids and the secretion of specific classes of serum lipoprotein (Rajasekaran *et al*., 2006).

The observed creatinine, major kidney function parameter, high level might have result from the decrease synthesis or increased functional capacity of tubular excretion (Mitchell *et al*., 1972; Zilva *et al*., 1991). As this formulation increase the kidney function it may be used in diabetic nephropathy because nephropathy is accompanied by oxidative stress, advanced glycation end products, abnormal lipid metabolism and renal accumulation of lipids (Rajasekaran *et al*., 2006).

CONCLUSION

The present study confirms that SBB alters the different biochemical parameters of rat plasma after chronic administration. The results of the present study guide us that SBB could be contributory for the complications in diabetics with hyperlipidemia and nephropathy as it lowers most of the lipids components and improves liver function and kidney function.

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