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Anti-oxidant potential of herbal formulation (*Sahaj Vati*) modulating leptin, insulin activity

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Obesity increased at alarming rate is considered as a serious health risk and the World Health Organization wishes to halt the rise of obesity. Therefore, search for the treatment and dietary mediations is fundamental to lessen or potentially forestall the corpulence. *Sahaj Vati* was prepared using *Shilajeet, Curcuma longa, Plumbago zeylanica, Commiphora mukul* and might be helpful to reduce obesity. Twenty four albino rats were randomized into four equal groups and were selected as control, positive control, as standard (orlistat) and *Sahaj Vati* treated group, respectively. Monosodium glutamate (MSG) was administered at a dose of 10 mg/kg body weight for 10 days and *Sahaj Vati* & orlistat was regulated orally at portion of 200 and 10 mg/kg body weight individually for four week. After four week, the body weight of animals, serum leptin, insulin, total oxidant, total antioxidant and oxidative stress index were estimated. It was observed that body weight was significantly increased by MSG whereas body weight was significantly decreased by orlistat and *Sahaj Vati*. *Sahaj Vati* significantly increased insulin concentration, total anti-oxidant status and decreased total oxidant status & oxidative stress and significantly decreased leptin concentration. Thus, *Sahaj Vati* decreases body weight by modulating leptin & insulin concentration as well as total oxidant status, total anti-oxidant status & oxidative stress.

Keywords: Insulin, Leptin, Obesity, Oxidative stress, *Sahaj Vati*
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Obesity is a multifactorial condition influencing youngsters and grown-ups, considered as the most preventable wholesome pathology[1] and characterized by the consolidated consequence of satiety center dysfunction at the cerebral level, imbalance of intake and utilization of energy and hereditary varieties[2,3]. It is a hazard factor for a wide scope of illnesses, including insulin opposition (IR), Type 2 diabetes mellitus (T2DM), dyslipidemia and cardiovascular malady (CVD)[4,5] and has risen tenfold in past four decades[6] and predicted that 57.8% of the total populace could be either overweight or obese by 2030[7]. Obesity increases the burden of diabetes, cancer, arthritis and many more diseases, so the World Health Organization wishes to halt the rise of obesity[8]. Chronic low grade inflammation and leptin is positively correlated with obesity[9,10], furthermore, leptin is positively associated with accumulation and activation of inflammatory cells[11]. Moreover, obesity has been considered as a condition of incessant oxidative stress. That's why, we can assume anti-inflammatory and anti-oxidative stress potential of diet, herbs and metals & minerals may be recommended for prevention and control inflammation & obesity[13].

Herbal medicines are widely accepted as therapeutic agents for the treatment of varieties of disorders like diabetes, arthritis etc.[14] and the United States Food and Drug Administration (USFDA) suggested creating plant inferred drugs as an option in contrast to manufactured medications[15]. In the Ayurvedic system of medicine *Shilajeet, Haridra, Guggul and Chitrak* individually have anti-inflammatory and anti-obesity[16] and antioxidant activities[17]. So it may be assumed that the formulation prepared from it (coded as *Sahaj Vati*) may have anti-inflammatory, anti-oxidant and anti-obesity activity and thus be fruitful in the reduction of obesity.

Material & Methods

Preparation of *Sahaj Vati*

Sahaj Vati was prepared by *Suddha Shilajeet*, *Suddha* Guggul (*Commiphora mukul* (Hook. Ex

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stocks) and water extract of *Haridra* (*Curcuma longa* L.) & Chitrak (*Plumbago zeylanica* L.) in the ratio of 44.7%, 44.7%, 4.8% and 5.8% by weight by levigation (*bhavana*) of *Agnimantha* (*Premna mucronata* L.) *Kwatha* for seven times[17]

Animals

Albino rats of 140-180 g were housed in polypropylene confines at an encompassing temperature of 25°C±1°C and 45-55% relative stickiness, with a 12:12 h light/dark cycle in the animal room of laboratory of pharmacology, Institute of Medical Sciences, Banaras Hindu University. Standards of lab creature care (NIH distribution number # 85-23, updated in 1985) rules were constantly followed and earlier endorsement was taken before initiating tests. Rodents were given standard creature diet and faucet water. All the examinations were affirmed by the Institutional Creature Moral Panel (Dignitary/2015/CAEC/1269 dated 23.06.2015) of Banaras Hindu University.

Characterization of *Sahaj Vati*

Infra-Red & UV-Visible studies have been used for characterization of *Sahaj Vati*.

Dose determination

Sahaj Vati showed anti-obesity activity at the dose of 200 mg/kg body weight in monosodium glutamate induced obesity[18].

Drug administration

Sahaj Vati and orlistat (Standard anti-obese drug) was suspended in distilled water and directed orally through orogastric tube at portion of 200 mg and 10 mg/kg body weight separately for 28 days.

Experimental design

Twenty four albino rats were arbitrarily separated into four groups, six animals per group.

Group A (Control): fed with normal food and tap water

Group B (Positive control): treated with monosodium glutamate at 10 mg/kg body weight for 10 days with standard diet and tap water

Group C (Standard): treated with Orlistat at 10 mg/kg body weight + monosodium glutamate (10 mg/kg body weight for 10 days) with normal food and tap water

Group D (*Sahaj Vati*): treated with *Sahaj Vati* at 200 mg/kg body weight + monosodium glutamate (10 mg/kg body weight for 10 days) with standard diet and tap water

Plasma separation

Towards the end of the study, (28th day), blood samples of rodents were gathered in EDTA tubes through retro-orbital strategy for biochemical estimations. All creatures were abstained for the time being preceding blood assortment. Plasma was quickly isolated from the blood by centrifugation at 3000 rpm. Each sample was thawed at room temperature for performing every test; repeated thawing was avoided.

Chemicals

Monosodium glutamate (Batch No. CIBB2E001 manufactured by Titan Biotech Ltd. Bhiwadi, Rajsthan), Orlistat capsules USP 60 mg (manufactured by Acme formulation Pvt. Ltd. Solon H.P., marketed by Eris Life sciences Pvt. Ltd., batch no ERSA X5001). Ferrous ammonium sulfate, ortho-dianisidinedihydrochloride (3-3V-dimethoxy benzidine), Ascorbic acid, 5,5V-dithiobis-(2-nitrobenzoic acid) (DTNB), ethylenediaminetetra acetic acid (EDTA), 2,2V-azino-bis (3- ethylbenz-thiazoline-6-sulfonic acid) (ABTS), water soluble analogue of vitamin E (Trolox; 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), Xylenol orange [o-cresosulfonphthalein-3,3-bis (sodium methylimino-diacetate)], horseradish peroxidase, 3,5,3′,5′-tetramethylbenzidine (TMB), ortho-dianisidinedihydrochloride (3- 3′-dimethoxy benzidine), hydrogen peroxide (H_2O_2), hydrochloride acid, ferric chloride and sorbitol were purchased from Sigma- Aldrich Chemical Co.

Leptin estimation

Leptin was evaluated using the ELISA Kit-RD291001200R manufactured by Bio Vendor research and diagnostic product. All means followed according to producer carefully. The created shading force was corresponding to the convergence of leptin in the example. The absorbance was taken at 450 nm.

Insulin estimation

Insulin concentration was estimated with the use of ELISA Kit EIA-2935, purchased from DRG manufacturer (DRG instrument GmbH, division of DRG international, Inc Frauenbergstr, Marburg). In reaction, the amount of horse-radish peroxidase (HRP) complex was proportional to the developed colour intensity that was comparative to the concentration of insulin in the sample. The absorbance was taken at 450 nm.

Total anti-oxidant estimation

Plasma total antioxidant status (TAS) was estimated by the colorimetric technique (Erel, 2004) and absorbance was taken at 444 nm. Shading development was adjusted with Trolox and results were communicated in milli molar Trolox identical per liter (mmol Trolox Eq/L).

Total oxidant estimation

Plasma total oxidant status (TOS) was estimated by the colorimetric technique (Erel, 2005) and absorbance was taken as end point estimation, bi-chromatic at 560 nm (primary frequency) and 800 nm (optional frequency), first absorbance was taken in the wake of blending of reagent-1 and plasma (as clear) and last absorbance was taken following 4 min of blending of reagent. The measure was aligned with H_2O_2 and the outcomes were communicated in micromolar H_2O_2 proportional per liter (μmol H_2O_2 Eq/L).

Estimation of oxidative index

OSI was determined as the proportion of TOS (μmol H_2O_2 Eq/L) to TAS (mmol Trolox Eq/L)[19].

Statistical analysis

Standard measurable strategies one way ANOVA and student's' test were utilized for translation of significance was utilized. Information was portrayed as mean ± SD (standard deviation). Every single factual estimation were finished by utilization of SPSS 16.0 (IBM Organization) and worth under 0.05 was viewed as critical.

Results

Infra-Red & UV-Visible studies of *Sahaj Vati* have alkane/alkylase, alkyls, amine, amide, ketone, C=C of alkanes, carbon dioxide and hydroxyl functional group and also has humic acid & many organic compounds[20]

Following 10 days body weight and food utilization was seriously expanded in all gatherings and following 28 days weight and diet utilization in MSG bunch was essentially expanded in contrast with control gathering while body weight was altogether diminished by orlistat and *Sahaj Vati* with respect to control and MSG group (Table 1,2). Food admission was altogether diminished by orlistat and *Sahaj Vati* approached bunch in comparison to MSG gathering (Table 2).

Leptin concentration was significantly increased in MSG group with control group. It was significantly decreased by orlistat and *Sahaj Vati* whereas insulin concentration was significantly increased by orlistat and *Sahaj Vati* treated group with respect to control & MSG (Table 3). *Sahaj Vati* expressively decreased total oxidant status, oxidative stress and significantly increased total anti-oxidant status with respect to all groups (Table 4).

Discussion

Monosodium glutamate (MSG) is an appetite stimulant[21], thus higher food intake observed in B group may be due to appetite stimulation (Table 2). It is also responsible for higher body fat content and strong positive correlation exists between body fat content and leptin[22]. This might explain why body weight and leptin concentration increased in the MSG group (Table 1, 3). The increased concentration of leptin is associated with inflammation[23] which is a leading cause of obesity. Thus we can assume that obesity induced by MSG may be due to increasing food intake, leptin and inflammation.

Table 1 — Alteration in body weight of different animal groups with time of intervals

Groups	Initial weight (gram)	Weight after 10 days (gram)	Weight after 28 day (gram)
A	144.16 ± 7.58	150 ± 8.75	158.16 ± 9.06
B	144.66 ± 6.83	159.16^{*} ± 8.96	175.83^{*} ± 7.47
C	144.61 ± 6.56	$155.73^{*,\#}$± 7.98	$164.51^{*,\#}$ ± 5.71
D	144.83 ± 7.11	$152.35^{*,\#}$± 5.48	$160.91^{*,\#}$± 6.29

n; six animal in each group,*p < 0.001 as compared to group A, #p < 0.001 as compared to group B

Table 2 — Average food intake (in grams) by different animal groups with time interval

Groups	Food intake 0 days	Food intake after 10 days	Food intake after 28 days
A	128 ± 7.21	139 ± 3.76	138 ± 5.33
B	129 ± 5.31	188^{**} ± 4.06	166^{*} ± 2.11
C	130 ± 7.81	$158^{*,\#\#}$ ± 1.98	$146^{\#}$ ± 3.67
D	129 ± 5.21	$154^{*\ \#\#}$± 3.78	$136^{\#\#}$ ± 3.61

Six animal in each group, *p<0.05 as compared to group A, **p<0.01 as compared to group A, #p<0.05 as compared to group B, ##p<0.01 as compared to group B

Table 3 — Status of serum leptin, insulin in different groups

Group	Leptin (ng.mL^{-1}))	Insulin (ng.mL^{-1})
A	2.32±8.4	14.72± 4.90
B	2.63^{*} ±9.90	14.18 ±7.71
C	$1.81^{***,\ \#\#\#,}$ ±6.07	$26.98^{***,\ \#\#\#}$ ± 3.53
D	$1.47^{***,\#\#\#,\ \$\$}$±6.20	$33.92^{***,\#\#\#,\$\$}$±3.14

Six animal in each group, *p<0.05 as compared to group A, **p<0.01 as compared to group A, ***p<0.001 as compared to group A, ###p<0.001 as compared to group B, $$p<0.01 as compared to group C, $$$p<0.001 as compared to group C

Table 4 — Total oxidant status, total anti-oxidant status, oxidative stress index

Group	Total oxidant status (μmol H_2O_2 Eq/L)	Total anti-oxidant status (mmol Trolox Eq/L)	Oxidative stress index (Arbitrary unit)
A	19.51± 4.61	5.24 ± 1.36	3.89 ± 0.58
B	7.85^{***} ±1.31	4.17 ±0.46	1.92^{***} ± 0.53
C	$18.54^{\#\#\#}$±2.02	4.51 ± 0.70	$4.29^{\#\#\#}$ ±0.95
D	$5.28^{***,\#\#\#,\$\$\$}$±2.07	$10.18^{***,\#\#\#,\$\$\$}$±2.60	$0.58^{***,\#,\$\$\$}$±0.14

Six animal in each group, ***p<0.001 as compared to group A, # p<0.01 as compared to group B, ### p<0.001 as compared to group B, $$$p<0.001 as compared to group C

Oxidative stress (OSI) is an inequity between the production of free radicals and antioxidants and increased oxidative stress causes obesity[24]. Thus decline in antioxidant activity and increased generation of free radicals[25] is accountable for obesity[12]. *Sahaj Vati*, encompassed of *Shilajeet, Haridra, Guggul, Chitrak* increases the total anti-oxidant status and decreasing total oxidant status and also decreases oxidative stress (Table 4). Curcumin, the major component of *haridra* enhance glutathione and stabilize mitochondrial defense system against oxidative[26] and also protect toxic effects of hypoxia[27] ·Guggulsterone, a major component *Guggul*, have antioxidant activity[28] and inhibited oxidative degradation[29]. Furthermore, in *vitro* study of *Shilajeet, Haridra, Guggul, Chitrak and Sahaj Vati* showed antioxidant activity against 2,2-diphenyl-1-picrylhydrazil (DPPH)[17]. Thus decreased oxidant status, oxidative stress and increased anti-oxidant status by *Sahaj Vati* may be due to the synergistic nature of their parts and numerous activities. It is recognized that oxidative stress is positively associated with obesity, so decreased oxidative stress by *Sahaj Vati* is responsible for reduction in body weight of animals. Furthermore oxidative stress is one of the main source of Insulin resistance, metabolic syndrome (bunching of various conditions, including hyperglycemia, hyperlipidemia, hypertriglycerdemia, weight, hypertension and hepatic steatosis) and diabetes[30,31]

Leptin, satiety hormone is responsible for controlling body weight, homeostasis as well as glucose and lipid metabolism[32]. *Sahaj Vati* decreased leptin concentration in MSG induced obesity. After analyzing its components, we found that dietary iron intake negatively regulates leptin element binding protein activation (CREB activation)[33,44]. Furthermore, zinc also possesses negative correlation with leptin[35]. *Shilajeet* having elements like iron, copper, silver, zinc, iron, lead, etc.[36] Curcumin, suppress the leptin release in lipopolysaccharide[37,38]. Hence, decreased leptin concentration by *Sahaj Vati* may be due to its component such as Shilajeet and curcumin. Hyperleptinemia is one of the chief sources of obesity[34] and *Sahaj Vati* causes hypoleptinemia which in turn reduces body weight.

Leptin inhibit glucose-stimulated insulin secretion[39] by directly constraining insulin emission from pancreatic cells[40] and also increaseinsulin hepatic extraction[41,42]. Furthermore, leptin is responsible for 42% of hypoglycemic action, independent of weight reduction[43], so decrease serum leptin by standard and *Sahaj Vati* (Table 3,1) may produce hyperglycemia due to normal feedback mechanism thus to maintain the homeostasis. Thus body develop another mechanism i.e., increases insulin secretion to normalize hyperglycemia; this may be responsible for increasing concentration of insulin in orlistat and *Sahaj Vati*. This indicates that leptin & insulin concentration is negatively correlated. It is commonly acknowledged that some insulin enters the cerebrum from the flow and expands CSF insulin which give negative input signal in the guideline of food for example lessens food intake[44]. Thus we can assume that decreased food intake in standard and *Sahaj Vati* may be due to increase in cerebro-spinal fluid insulin concentration due to elevation of plasma insulin (Table 2).

Orlistat, a well-accepted anti-obesity drug, is a reversible inhibitor of gastrointestinal lipases and applies its helpful action by framing a covalent bond with the dynamic serine buildup site of gastric and pancreatic lipases[45], so this inactivated proteins are accordingly inaccessible to hydrolyze dietary fat and undigested triglycerides are not ingested, the subsequent caloric deficiency. This mimics a reduced calorie intake in obese patients and help in preventing weight gain. Orlistat and *Sahaj Vati* decreased body weight of animals which may be due to inhibition of gastrointestinal lipases (Table 1,3).

Conclusion

Sahaj Vati reversed MSG-induced obesitic conditions due to decreasing oxidative stress, leptin concentration, oxidant status and increasing insulin concentration and anti-oxidant status. Thus, it seems that *Sahaj Vati* might be a potential for treatment of obesity and metabolic disease.

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Conflict of Interests

Nil

Author Contributions

Concepts and design of article by K D Yadav and A Singh, written the article by K D Yadav and critically reviewed and put her suggestion for improvement of article by A K Chaudhary. All authors read and approved the final manuscripts

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