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Research Article

EFFECT OF *GUDUCHI TRIPHALA KWATHA* WITH *LOHABHASMA* AS *PRAKSHEPA CHURNA* IN THE MANAGEMENT OF OBESITY

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ABSTRACT

Obesity a complex metabolic disorder, involves the excessive accumulation of adipose tissue and it is recognised as a major global health challenge. Preclinical study conducted on obesity provides valuable insights into the revalidation of the treatment approach of *Sthoulya*. **Methods:** In this study the efficacy of *Guduchi Triphala Kwatha* added with *Lohabhasma,* as *Prakshepa Churna* evaluated in High fat diet (HFD) fed, obese Wistar albino rats. A Control group containing 6 rats were administered with distilled water (9.86ml/kg), another group received standard drug Orlistat (30mg/kg) and the trial group was given *Kwatha* (9.86ml/kg) added with *Lohabhasma* (25.65mg/kg). High fat diet was continued throughout the study period. All the drugs were administered orally once a day for 30 days. At the end of the experimental period various physical, biochemical and histopathological observations were made. **Results:** Data of in-vivo studies revealed that significant reduction in body weight, body mass index (BMI), waist circumference (WC), upon trial drug treatment. Elevated levels of, insulin, leptin, lipid profile, liver function, kidney function and antioxidant status were also showed significant results. Milder changes were observed in histopathological examination of liver, kidney and adipose tissue even though HFD was continuing throughout the study period. **Conclusion:** The trial drug showed significant result in the assessment parameters after treatment.

INTRODUCTION

Obesity is considered as the fat accumulation due to the intake of high caloric food and less physical activity. The pathological features and consequences of Obesity might be correlated to the sequences of pathology explained under *Sthoulya* by different *Acharyas*. *Kapha Vridhi, Medho Dhathu Dushti, Dhatvagnimandya, Srothorodha*, and *Vatavilomata* are the major factors in *Samprapti*.[1] *Guduchi Triphala Kwatha* with *Lohabhasma* as *Prakshepa Churna* as mentioned by *Acharya Vangasena* in *Sthoulya Chikitsa* was selected.[2]

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The present study was conducted in Obesity induced Wistar rats with the view that the combination have action in obesity and its related pathology. The trial drug was compared with a HFD fed control group and standard group.

METHODOLOGY

The study has adhered to the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and obtained approval from the Institutional Ethical Committee of Pankajakasthuri Ayurveda Medical College and Post Graduate Centre, under the reference number PKAMC/IEC/63/2020.It was also approved by the Institutional Animal Ethical Committee of Pankajakasthuri Herbal Research Foundation, located in Killy, Kattakada, Thiruvananthapuram, Kerala, India (Reg.No.2093/PO/ReRcBi/S/20/CPCSEA), with the reference number PKAMC/IAEC/NOC/03/2021.

Experimental Animals: Wistar rats of either sex weighing 200- 225g were used for the study. The animals were obtained from Animal house,

Pankajakasthuri Ayurveda Medical College and PG Centre, Thiruvananthapuram. The animals were maintained under normal laboratory condition of humidity (50%), temperature (23±2°C) and 12-hour light /dark cycle.

METHODS

Induction of Obesity: Wistar rats weighing 200-225gm were selected and obesity was induced in them by feeding them with standard high fat diet.[3,4,5] When the animals weigh around 325-350gm (considered obese), they were selected for the study. High Fat Diet was continued during the study period also.[5,6]

Grouping Technique

Oral administration of drugs was done by using oral gavaging technique.

- Control group (n=6) was administered with distilled water (9.86ml/kg).
- Standard drug (n=6) was administered with Orlistat 30mg/kg.
- Trial group (n=6) was administered with *Guduchi Triphala Kwatha* (9.86ml/kg) added with *Loha Bhasma* (25.65mg/kg).

Determination of Anti-Obesity Potential[5,6,7]

1. **Measurement of food intake:** Food intake was measured every day from day 1. It was determined by measuring the difference between the pre-weighed pellets and weight of the pellets that remained every 24hr per cage basis.
2. **Body weight of the animals:** Body weight of the experimental animals was recorded daily using rough table top balance.
3. Determination of Body Mass Index (BMI) and Waist Circumference (WC)

The BMI was calculated using the formula, BMI =Body Weight (g)/Length (cm^2). Body length (nose-to-anus length) of the experimental animals were measured on 1st and 30th day of the study period. WC was also measured on the 1st and 30th day of the study period on the largest zone of the rat abdomen using a plastic non extensible measuring tape by keeping the rats in ventral position.

4. **Determination of biochemical parameters**
   Biochemical parameters such as Glucose, Insulin, Lipid profile, LFT, RFT, Leptin, Lipid Peroxides (LPO), Glutathione (GSH), Superoxide Dismutase (SOD), Catalase were assessed on 1st and 30th day of the study period. Blood was withdrawn from lateral tail vein under mild anaesthesia on 1st day and by cardiac puncture method at the end of the study.
5. Histopathological analysis of liver, kidney and adipose tissue were done by sacrificing the rats after 24 h observation period of the last dose of administration of the drug.[8,9,10]

Statistical Methods

All the values were expressed as MEAN ± SEM (Standard error of the mean). The statistical test used was one way ANOVA method. P-value <0.05 was considered statistically significant. The level of significance was noted and interpreted accordingly.

RESULTS

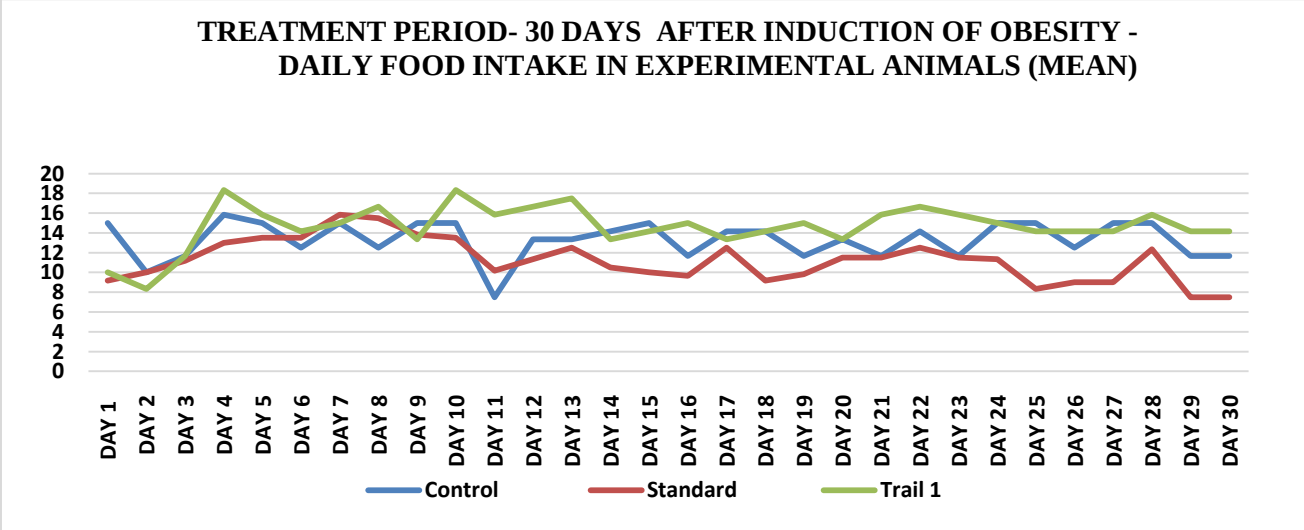
Data Related to Induction of Obesity

For the induction of obesity, HFD given for each group and weekly assessment of body weight was done. There was a 50% increase in body weight of all groups. The results of mean body weight of rats were shown in table.

Table 1: Data related to induction of obesity

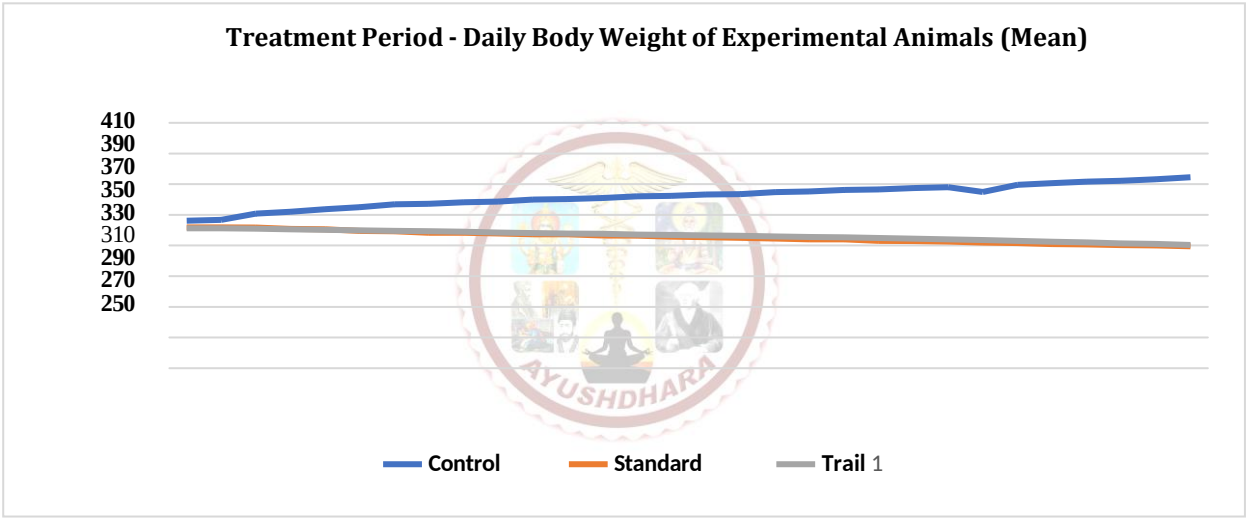
Groups		Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	Mean ± SD	215 ± 10	231.7 ± 16	255.8± 14.6	284.2 ± 16.8	298.3 ± 15	312.5 ± 19.2	324.2± 21
Standard	Mean ± SD	211.7 ± 8.2	224.2 ± 7.4	252.5 ± 6.12	278.3 ± 10.8	293.3 ± 12.5	307.5 ± 11.7	318.3 ± 11.6
Trial	Mean	210.8 ± 6.6	226.6 ±10.3	247.5 ± 10.3	275.8 ± 16.2	294.2 ± 10.6	310 ± 13	317.5 ± 13.6

Figure 1: Effect of Treatment in Daily Food Intake



The daily food intake of experimental rats was measured and the obtained results were presented in form of average food intake. The percentage increase in daily food intake of the control group is 18%. The percentage decrease in daily food intake of standard was 41% and 22% increase in food intake obtained in trial group.

Figure 2: Effect of treatment on bodyweight



Statistically significant result was obtained in bodyweight of trial group when compared to control and standard group.

Table 2: Effect of treatment on Body Mass Index (g/cm²)

Groups	BT			AT		
	Mean± SD	SEM	% Change	Mean ± SD	SEM	% Change
Control	0.79 ±0.04	0.02	0	0.82 ± 0.04	0.02	3.59
Standard	0.82 ± 0.1	0.04	0	0.73 ± 0.07	0.03	-11.16
Trial	0.73 ±0.06	0.02	0	0.58 ± 0.05	0.02	-20.59

Statistically significant result was obtained in BMI of trial group when compared to control and standard group.

Table 3: Effect on Waist circumference (cm)

Groups	BT			AT		
	Mean± SD	SEM	% Change	Mean ± SD	SEM	% Change
Control	18.17± 1.4	0.57	0	18.8 ± 1.37	0.56	3.67
Standard	17.33±1.47	0.6	0	18.9 ± 1.48	0.61	9.04
Trial	20.18±1.72	0.7	0	19 ± 1.9	0.77	-5.86

Waist circumference of all groups were within normal limits before and after treatment when compared to normal reference rats.

Table 4: Effect on blood glucose level (mg/dl)

Groups	BT			AT		
	Mean± SD	**SEM (±)**	**% Change**	**Mean ± SD**	**SEM (±)**	**% Change**
Control	101.2±5.63	2.3	0	106.83±6.49	2.65	5.57
Standard	114.33±13.8	5.62	0	103.27±13.7	5.61	-9.68
Trial	119.28±24.5	10	0	78.83±16.07	6.56	-33.91

The blood glucose level of all groups were within normal limits before and after treatment.

Table 5: Effect on blood insulin level (pg/ml)

Groups	BT			AT		
	Mean± SD	**SEM**	**% Change**	**Mean ± SD**	**SEM**	**% Change**
Control	125.75±39.9	16.28	0	112.17±55.7	22.77	-10.8
Standard	130.65±30.5	12.45	0	121.53±33.5	13.69	-6.98
Trial	175.72±44.3	18.07	0	60.23±2.82	6.9	-65.72

Statistically significant result was obtained in blood insulin levels of trial group when compared to control and standard group but not within normal limits.

Table 6: Effect on Lipid profile (mg/dl)

Lipid Profile											
		TC (mg/dl)		**TG (mg/dl)**		**HDL (mg/dl)**		**LDL (mg/dl)**		**VLDL (mg/dl)**	
		BT	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**
Control	Mean ± SD	145.8 ± 16.7	146.1 ± 14.4	134.8 ± 14.4	128.6 ± 26.4	26.6 ± 2.9	23.9 ± 2.01	92.2 ± 12.9	96.4 ± 11.0	26.9 ± 2.9	25.6 ± 5.3
Standard	Mean ± SD	136.0 ± 6.01	106.1 ± 4.8	279.8 ± 10.5	112.3 ± 22.9	27.1 ± 0.68	24.2 ± 0.05	52.9 ± 15.7	59.5 ± 0.34	55.9 ± 21.0	22.5 ± 4.6
Trial	Mean ± SD	157.9 ± 21.6	110.3 ± 5.26	194.2 ± 5.8	92.2 ± 17.2	30.4 ± 4.7	20.4 ± 1.2	88.7 ± 15	71.4 ± 0.7	38.7 ± 1.1	18.4 ± 3.4

Statistically significant result was obtained in Lipid profile of trial group when compared to control and standard group but not within normal limits.

Table 7: Effect on Liver function test

	Albumin g/dl		**TP g/dl**		**T.Bilirubin mg/dl**		**D.Bilirubin mg/dl**	
	BT	**AT**	**B T**	**AT**	**BT**	**AT**	**BT**	**AT**
Control								
Mean ± SD	4.42± 0.1	4.32± 0.1	6.97± 0.3	7.43± 0.04	0.4± 0.09	0.25± 0.05	0.15 ± 0.05	0.1± 0
Standard								
Mean ± SD	4.35± 0.12	4.8± 0	7.67± 0.2	7.1± 0.4	0.3 ± 0	0.2 ± 0	0.32 ± 0.2	0.15± 0.05
Trial								
Mean ± SD	4.43± 0.4	4.3 ± 0.06	6.9 ± 0.05	6.14 ± 2.9	0.6 ± 0.05	0.25 ± 0.05	0.4 ± 0.05	0.25 ± 0.05

SGOT and SGPT level was increased in all groups after induction of obesity. All other parameters were within normal limits. Trial group showed statistically significant result in SGOT and SGPT level, after treatment when compared to control and standard group.

Table 8: Effect of treatment on Renal function test

Renal Function Test							
		Uric acid (mg/dl)		Urea (mg/dl)		Creatinine (mg/dl)	
		BT	AT	BT	AT	BT	AT
Control	Mean ± SD	2.91 ± 0.2	1.98 ± 0.03	29.88 ± 2.66	35.68 ± 0.18	0.43 ± 0.02	0.44 ± 0.02
Standard	Mean	4.63 ± 2.18	2.12 ± 0.06	31.17 ± 3.51	39 ± 1.83	0.46 ± 0.06	0.41 ± 0.05
Trial	Mean	3.31 ± 0.06	1.95 ± 0.11	32.77 ± 2.85	32.3 ± 0.24	0.46 ± 0.02	0.41 ± 0.02

Statistically significant result was obtained in Uric acid, Urea levels of trial group when compared to control and standard group. Creatinine levels were within normal limits in all groups before and after treatment.

Table 9: Effect of treatment on Antioxidant markers

		LPO		ANTIOXIDANTS					
		MDA (nmols/mg protein)		GSH (nmols/ mg protein)		SOD (U/mg protein)		CAT (U/mg protein)	
		BT	AT	BT	AT	BT	AT	BT	AT
Control	Mean	14.41 ± 1.9	14.79 ± 1.8	6.37 ± 0.2	6.1 ± 0.03	0.09 ± 0.02	0.1 ± 0.02	3.65 ± 1.23	2.93 ± 3.51
Standard	Mean	14.5 ± 0.55	2.03 ± 0.8	9.5 ± 2.03	13.09 ± 4.9	0.09 ± 0	0.18 ± 0.04	5.5 ± 4.5	2.55 ± 0.5
Trial	Mean ± SD	10.43 ± 6.07	5.19 ± 0.53	4.9 ± 1.74	17.1 ± 15.32	0.05 ± 0.03	0.15 ± 0.03	2.7 ± 0.9	6.2 ± 0.2

Statistically significant result was obtained in lipid peroxidation levels and antioxidant enzyme activity levels of trial group when compared to control and standard group.

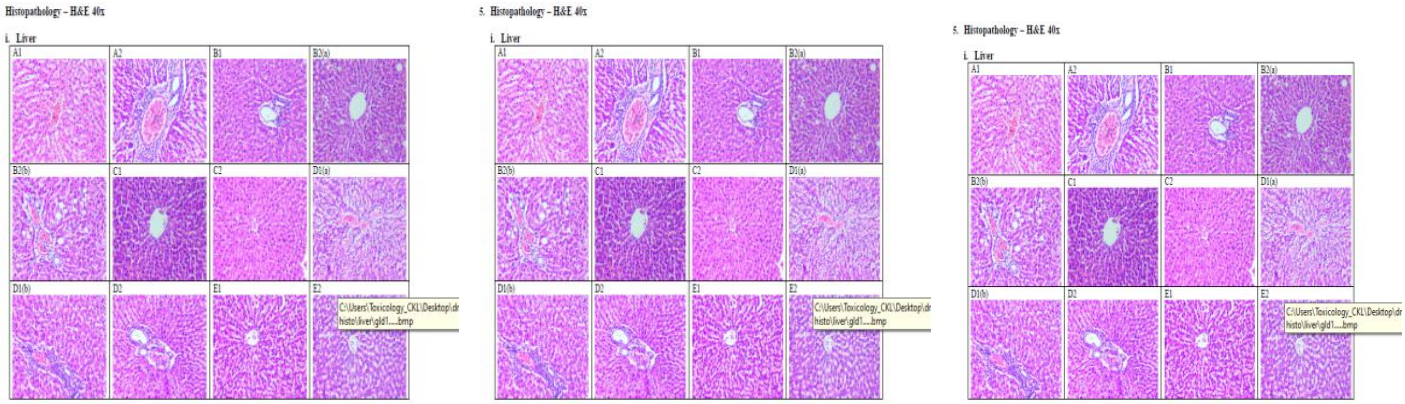
Table 10: Effect on blood leptin levels (pg/ml)

Blood Leptin Levels (pg/ml)			
		BT	AT
Control	Mean	1318.5± 9.79	1249.07±81.68
Standard	Mean	391.24±1.21	333.78±1.22
Trial	Mean	308.76	302.53

Statistically significant result was obtained in blood leptin levels of trial group when compared to control and standard group

Histopathological Evaluation of Organs

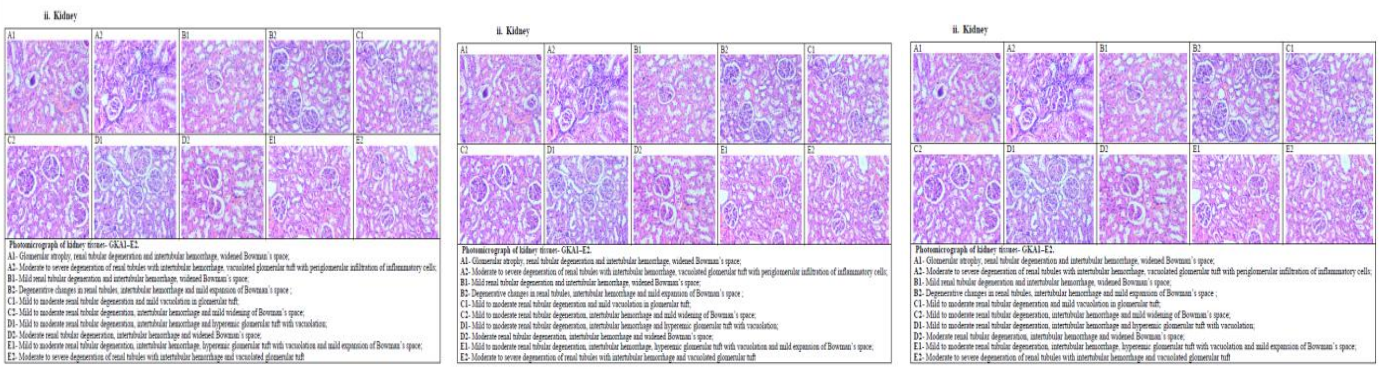
Liver



Control group **Standard group** **Trial group**

Control: Central venous congestion, severe sinusoidal dilatation and congestion, hepatocyte degeneration and Kupffer cell hyperplasia; Standard: Mild hepatocyte degeneration with mild sinusoidal dilatation and congestion, mild periportal edema: Trial: Mild cytoplasmic vacuolations in hepatocytes, mild hepatocyte degeneration and congestion of central vein and sinusoids

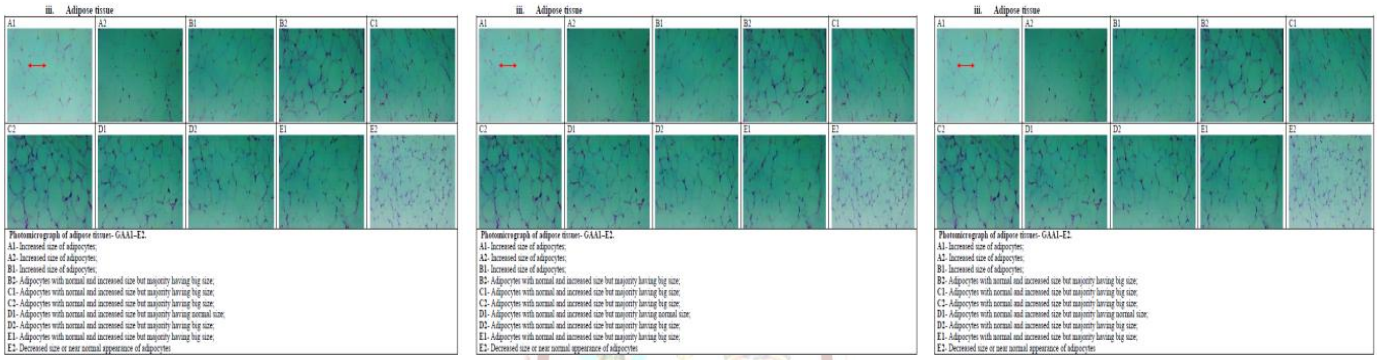
Kidney



Control group **Standard group** **Trial group**

Control: Glomerular atrophy, renal tubular degeneration and intertubular hemorrhage, widened Bowman's space. Standard: Mild renal tubular degeneration and intertubular hemorrhage, widened Bowman's space; Trial: Mild to moderate renal tubular degeneration and mild vacuolation in glomerular tuft;

Adipose Tissue



Control group **Standard group** **Trial group**

Control: Increased size of adipocytes; Standard: Increased size of adipocyte: Trial: Adipocytes with normal and increased size but majority having big size

DISCUSSION

The pathogenesis of Obesity, or *Sthoulya* in Ayurveda, can be understood through various processes involving adipose tissue dysfunction, impairment in brown fat oxidation free radical formation, oxidative stress and lipid peroxidation. Administration of HFD containing *Snehana dravya* may nourish the *Medodhathu* resulting in the *Medodhathu vridhi*. This may further result in accumulation of *Medodhathu* in *Srothomukha* causing *Srothorodha*. This *Margavarodha* may cause *Vilomata* of *Samana vayu* in *Koshta* leads to *Tikshna Agni*. Leptin hormone helps in Controlling food intake and satiety via the neurohormonal pathway. Involvement of *Prana Vayu* and *Samana Vayu* can be seen in the pathogenesis which leads to increased food intake and leptin levels. Excessive accumulation of fat in their depots can be seen as the direct transformation of *Ahararasa* into *Medodhatu*. Impairment in brown adipose tissue function reduces fat oxidation, leading to further fat accumulation, which can be considered as *Medodhatvagnimandhya* resulting in *Medovridhi*. Impairment in glucose and lipid metabolism at the cellular level leads to the formation of free radicals and oxidative stress. This can be viewed as the development of *Ama* at the level of *Dhatus*. Oxidative stress further promotes lipid peroxidation, causing structural and functional abnormalities in lipids. This contributes to the development of Atherosclerosis and its related diseases. This can be understood as *Medhodhathudushti* leading to the *Samprapti* of *Dhamani Pratichaya*. Excessive lipid accumulation also leads to the excess formation of circulating free fatty acids, which can result in insulin intolerance. As Type 2 diabetes mellitus is a common consequence of Obesity. This aspect can be observed as development of *Prameha Purvarupa* due to *Sthoulya*. In the present study the Trial group resulted in reduction of Leptin levels and food intake. Increase in body weight, BMI, triglycerides and other lipids might be considered as *Medodhathu Vridhi* in *Srothus*. As the treatment principle is *Virookshana* which means *Medoharatva* and *Chedaniya* means *Srothosodhana*. Here the combination of *Guduchi* and *Triphala* may act at the *Srothorodha* as *Virookshaniya* and *Chedaniya* with the help of *Lohabhasma* as it possess *Lekhana karma.* The results of the study suggest that *Guduchi Triphala Kwatha* with *Lohabhasma* was effective in reducing body weight and BMI. It also showed significant

improvement in elevated insulin levels. This indicates that this particular combination may be addressing obesity by rectifying *Malarupa Kapha* from the *Rasa dhathu* onwards. The presence of *Loha Bhasma,* with its *Lekhana karma* might aid in this process by removing *Mala* from each *Dhathu* and helps in proper *Dhathu Parinama.* Additionally, *Lohabhasma* is a known drug in treating *Pandu Roga* which might helps in maintaining adequate oxygen levels in the circulation, which in turn may facilitate effective brown fat oxidation. This action is thought to alleviate *Medodhatvagnimandya*, leading to the removal of excess *Medas* and the proper formation of *Medodhatu*. Consequently, it appears to target the *Abadha Medas*, correcting *Agni* at the *Dhathu* level, particularly insulin resistance, which is linked to impaired lipid accumulation. *Bhasma* form is *Yogavahi* and *Seegravyapthi* which aids the action of *Guduchi Triphala Kwatha* in deeper *Dhathus*. The *Deepana*, *Rasayana* and *Laghu Ruksha guna* of the *Guduchi Triphala Kwatha* with *Loha Bhasma* play a crucial role in promoting *Medoharatva* and *Tikshna Agni* by supporting the normal functioning of *Prana* and *Samana vayu* which is evident from the significance in blood Leptin levels. Even though *Lohabhasma* was a *Rasa* preparation, *Guduchi Triphala Kwatha* with *Lohabhasma* treated group possessed significance in liver function. From these findings we can assume that the drug is safe in this prescribed dose. The histopathological examination and biochemical examination of liver and kidney function demonstrate that the trial drug was safe when in the prescribed dose, even when the high-fat diet is continued throughout the study. The minimal changes observed in some parameters may be attributed to the persistent HFD feeding.

CONCLUSION

Based on the observations and result, the use of *Guduchi Triphala Kwatha* with *Lohabhasma* as *Prakshepa Churnas* has a significant effect in the management of obesity.

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