Ind. J. Pharm. Edu. Res, 2023; 57(1s):s126-s134.
https://www.ijper.org

Original Article

Preparation and *in-vitro* and *in-vivo* Evaluation of Ayurvedic Formulation "Amruthotharam" Formulated by Classical and Modern Technique

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ABSTRACT

Glucose intolerance, central obesity, hypertension, and dyslipidemia are all part of the metabolic syndrome. Thus, a number of theories have been put out to explain the development of the metabolic syndrome, including an initial condition of insulin resistance that progressed to the other elements, with obesity serving as the primary initiator of the metabolic syndrome. Thus, it can be understood that metabolic syndrome is multi related and the basic cause is inflammation. Thus, while treating the metabolic syndrome the root cause needs to be targeted and for this the ayurvedic formulation is the best solution. Amruthotharam is one of such preparation which takes care of metabolic syndrome through inflammation. "Amruthotharam" is the combination is known as Kashayam was made from three main herbs whose efficacy in treating indigestion and other stomach-related issues has been demonstrated in several cases. Three parts of *Tinospora cordifolia*, two parts of *Terminalia chebula*, and one part of *Zingiber officinale* are combined to create the mixture. The present study is focusing on the comparisons between the Amruthotharam a kerealian Ayurvedic medicine formulated by classical and modern technique which can be evaluated analytically and pharmacologically.

Keywords: Metabolic syndrome, Amruthotharam, Immunomodulatory.

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Received: 12-10-2022;
Revised: 02-12-2022;
Accepted: 15-01-2023.

INTRODUCTION

The multiple theories and hypothetical explanations have been given by the modern medical science, which helps to recognize the origin of metabolic disorders and the research is continued further till date. Few say that an advent of insulin resistance progresses to the subsequent factors, according to some generalized systemic obesity is the main precursor of the total syndrome.

Current recent data establishes that, low-grade inflammatory status that stay for a longer duration that often accompanies impaired metabolism and therefore is deemed as important causative factor for onset and establishment of the metabolic syndrome along with the subsequent Patho-physiological changes. The other causative factors for development of metabolic syndromes are diabetes and oxidative damage. Diabetes mellitus, a prominent and rapidly spreading illness in group of people all over the globe.[1]

Numerous factors that developed diabetes, includes glucose auto-oxidation resulting from formation of free radicals, tyrosine kinases receptor, like epidermal growth factor receptor, the enzyme glucosidase as well as insulin receptor may be regarded as a primary target to monitor for antioxidant also anti-inflammatory agents.[2]

Antioxidants have been widely acknowledged as having a significant impact on the biochemistry of living organisms in the last decade. An antioxidant is a chemical which inhibits oxidative damage of a target molecule.[3]

Metabolism is an example of a reaction that may produce free radicals. Although metabolism is an unavoidable process and oxygen is a need for living, the paradox is that oxygen is a highly reactive molecule that may be damaging when reactive oxygen species are produced. Free radicals have an unpaired electron, which makes them very reactive species. They may remove an electron from a stable molecule, making it unstable and starting a chain reaction. This is where antioxidants come into play,

DOI: 10.5530/ijper.57.1s.14

acting as a defense factor and reducing the negative effects of free radicals.[4]

Thus, it can be understood that in metabolic disorders is multi related and the basic cause is inflammation which is well discussed and addressed along with detailed solutions in the science of Indian Medicine, Ayurveda. While treating the metabolic disorders the root cause needs to be targeted and for this the Ayurvedic science is the best solution.

Ayurveda with new approach of disease management

Since ancient times India is practicing *Ayurveda*, approximately dates back to three thousand years. *Ayurveda, the science of life*, involves the incorporation of universal 5 elemental forces-namely Jala (Water), Prithvi (Earth), Vayu (Air), Aakash (space, ether) and Agni (Fire).

The medication is basically in the forms of various herbal, herbo-mineral, mineral drug formulations aimed at elimination of the root pathology of any kind of disorder in the body and by restoring balance, simultaneously creating apro-healthy life-style, thereby preventing the recur of any disorder. Cure and prevention is the two folded aim of Ayurveda.[5]

"Ama" may show to be a valid marker of early stage of inflammation in persons at risk for metabolic disorder or malignancies and thus management of these disorders will become easier even before the pathologies progress further. As in Ayurveda diagnoses of the Ama condition is laid down in elaborate and specific details and the process of physical examination can bypass the chemical examination of blood or urine samples thereby reducing the time and other investments in the diagnosis of early pathologies of metabolic syndrome.[6]

There are many treatment modalities in Ayurveda, to correct the status of Agni and metabolize or neutralize AMA based on the state and the stage of the disease. Dietary corrections or stipulated nutritional regimens work in congregation with carious oral formulations, Amrutottaram being one of them, which is the subject of study here.

Ayurvedic drug formulations like 'Amruttottaram', are extremely potential in reversing metabolical pathologies if given in intended dose and stage but they are still plagued by some loopholes in this era, affecting their ability and efficacy in treatments.

Clinical studies of individual components of *Amruttottaram Kashayam*

Zingiber officinalis (shunthi)

Studies related to Hepato protective and curative actions of ginger also gave promising results. The overall studies showed that rhizome of *Zingiber officinale* extracted in water displayed potent anti-inflammatory activity, hepato-protective and anti-viral activity.[7]

Another study to assess the anti-obesity action of ginger was conducted with significant positive findings. The ethanolic constituents, of *Zingiber officinale* in 'Shunthi' extract is known to have various properties like antidiabetic, antioxidant and antihyperlipidemic potential in animals under experimentation. Furthermore, there was improved HDL-C level was also observed, emphasizing the anti-obesity potential of Ginger.[8]

This study evaluated cancer opposing activity of ginger by studying its effect on 'Cholangiocarcinoma' (CCA) an adenocarcinoma of bile ducts. The results were very encouraging to discover further prospects about anti-carcinogenic actions of *Amrutottaram*.[9]

Tinospora cordifolia (Amruta)

This study showed Guduchi (*Tinospora cordifolia*) as a natural antioxidant, fibre source, and nutritional content in nutrition, fodder, also pharmaceutical sectors.[10]

Anti-inflammatory, antipyretic, antioxidant, anti-leprotic, anti-stress, antimalarial, anti-allergic, adaptogenic, anti-rheumatic, immunomodulatory, hepatoprotective, hypoglycemic, and anticancer characteristics have been observed.

The overall results of this study of guduchi bark imply that phytochemical evaluation may be liable for the high therapeutic efficacy.[11]

Tinospora cordifolia extracts were tested for *in vitro* phagocytosis immune system activation. Assay findings confirm this plant's immunomodulatory properties.[12]

This particular study was proven to give insightful information in determining the therapeutic efficiency of guduchi, for the controlling of depressive diseases.[13]

Terminalia chebula (Haritaki)

The researchers analyzed *Terminalia chebula* extracted in methanol for anti-ulcerogenic efficacy. The investigated extract inhibited gastrointestinal injuries generated by Pylorus ligation induced ulcer as well as Ethanol induced gastric ulcer, determining anti-ulcer also lesion healing capabilities of haritaki in the upper digestive tract also in stomach area.[14]

The previously reported analysis revealed that dried fruit of Haritaki shows antimicrobial activity on broad spectrum microbial flora and a prospective opportunity to examine active component required for various strains of bacteria.[15]

This study proved the immune-modulatory activity of fruits of Haritaki or *T. chebula* fruits depicted by increased concentrations of antioxidants, GSH, T and B cells, establishing its importance in effective immune response. This improves the melatonin concentration in the pineal gland as well as showed improved

cytokines level that play important roles in stress management and a normal immunity.[16]

Amrutottaram Kashayam

Amruthotharam/ Amrutottaram Kashayam a form of formulation used in metabolic disorder like inflammation. "Amruthotharam" Kashayam is the decoction, prepared from three herbal drugs that have proven to be extremely beneficial in many pathologies of ranging from indigestion to deeper cellular inflammation wherein cellular neutralization of AMA is expected.

The three ingredients are; Guduchi (*Tinospora cordifolia*),Haritaki (*Terminalia chebula*) and Shunthi (*Zingiber officinale*).[17]

Traditional Ayurvedic texts including Chikitsa Manjari and Sahasrayogam mention in it. It composed of three medicaments with the ratio 3:2:1. In terms of active ingredients, Amruta or Guduchi (*Tinospora cordifolia*) contains Tinosporaside, Tinsosporic acid, caridioside etc. Haritaki (*Terminalia chebula*) contains tannins, gallic acid, chebulagic acid, ellagicacid while Shunthi (*Zingiber officinale*) contains volatile oil 1%, 6-gingerol, 8-gingerol, 10-gingerol and shagol etc.[18]

MATERIALS AND METHODS

Drugs

The crude forms of herbal drugs *Tinospora cordifolia, Terminalia chebula, Zingiber officinale* were procured from local market of Ratnagiri, Maharashtra (India).

The aqueous extracts *Terminalia chebula* (Hareda), *Tinospora cordifolia* (Guduchi) and *Zingiber officinalis* (Ginger) were procured from authentic supplier, Amsar Pvt. Ltd., Goa with COA of each sample extract.

Methods

Methods for Preparation of amruthotharam Formulations (Classical methods)

The formulation F1 was prepared by mixing raw drugs such as guduchi, hareda and ginger in 3:2:1 by traditional techniques. 48 g of crude mixture of all the three herbs is taken and 768 mL (16 times to that of the churna) of water is added to it. The kashayam is then boiled on medium flame until the water quantity is reduced to 96 mL (1/ 8th of the original quantity). This is the standard method of Kashay preparation.[19]

Modern Method

The formulation F2 was prepared by mixing aqueous extracts of *Terminalia chebula* (Hareda), *Tinospora cordifolia* (Guduchi) and *Zingiber officinalis* (Ginger) in 3:2:1 by modern technique.

In-vitro antioxidant study

The antioxidant action of herbs was measured by various techniques. The methods are popular because of their ease, speed and sensitivity. In the present study antioxidant action was measured through DPPH, ABTS and lipid peroxidation inhibition assay, xanthine oxidase and superoxide radical scavenging assay.[20,21]

Calculation of 50% inhibition concentration (IC_{50})

The optical density at each concentration of the specified test chemicals and Butylated Hydroxyl Toluene (BHT). Percentage inhibition was estimated using equation.

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The IC_{50} value was determined by plotting graph taking concentration on X- axis and % inhibition on Y-axis.[22]

In- vivo studies

The experiment was done out on both sexes of Wister albino rats. The rats weighing 200-250 g were chosen with the committee's agreement. The experimental protocol was authorized by the Institutional Animal Ethics Committee (IAEC) of Indira Institute of Pharmacy, Sadavali, Maharashtra, (IIP/IAEC/07/2019-20) in accordance with the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

The animals were kept in a well-ventilated animal unit with a 12-hr light/dark cycle. For 28 days, the six experimental groups of six animals each were given a customized high cholesterol diet. There were 48 rats given a high cholesterol diet and 6 rats provided a regular diet. All administrations were done orally.[23]

Collection and Analysis of Blood Sample

After the induction of diabetic on 5th, 7th, 9rd week blood samples were collected and evaluated for blood glucose fasting, postprandial glucose level and blood lipid profile.

Histopathological study

The purpose of histopathology on the harvested pancreas and liver part was to see the effect of the formulations (F1 and F2). After completion of treatment i.e. after 4 weeks, the animals were sacrificed. The pancreas was collected from the group of animals I, III, IV, VII, VIII to evaluated for diabetic condition. The liver of animals (group I, II, V, VI) were collected and evaluated for liver condition due to high fat diet. This was done in accordance with established procedures. The staining of the kidney segment was done according to normal histological techniques. The examination of sections was carried out using microscopic techniques.[24]

Table 1: IC_{50} **in µg/mL for each activity.**

Sl. No.	Activity	IC_{50} value in µg/mL	
		F1	F2
1	DPPH	271.99	269.84
2	ABTS	293.07	225.04
3	Lipid Peroxidation	231.63	232.44

Table 2: Change in body weight during treatment (g).

Groups	Change in body weight during treatment (gm)			
	1st Week	2nd Week	3rd Week	4th Week
F1.1	254.52±5.90	247.58±5.35	240.07±4.66	230.77±4.30
F1.2	254.55±7.07	245.88±3.73	237.93±2.18	224.68±2.51
F2.1	254.03±1.67	243.58±2.96	236.53±2.68	223.72±3.10
F2.2	252.87±1.61	239.68±1.33	232.22±1.67	220.05±0.94
Standard	254.10±1.80	241.67±1.01	233.67±1.28	221.55±1.66
Standard	171.50±0.84	165.00±2.28	161.17±3.60	155.33±3.33

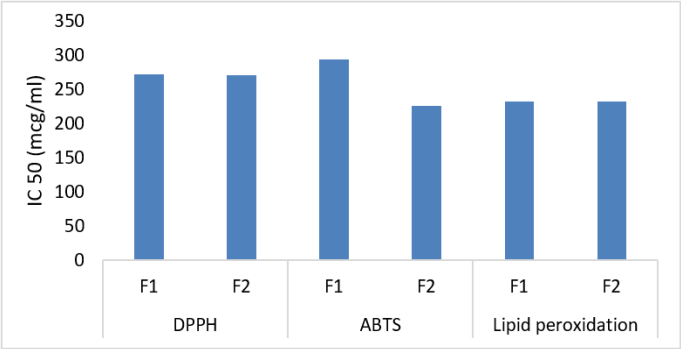

Figure 1: Determination of IC_{50}.

RESULTS AND DISCUSSION

In-vitro antioxidant study

The *in vitro* antioxidant study was carried out to evaluate the antioxidant potential of *Amruthotharam Kashayam* (F1) and Kashaya mixture (F2). The overall antioxidant activity was found to be good in both formulations. The formulation F2 showed better antioxidant action shown in Table 1 and Figure 1.

Pharmacological Activity

Obesity and its accompanying diseases, such as diabetes, provide significant problems for fundamental science and clinical research.

The various studies were discovered that the disorders caused by high-fat feeding were similar to the human metabolic syndrome, which is indicated by a rise in body weight (obesity), moderate hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and compensatory hyperinsulinemiaas well as a decreased glucose disappearance rate, and this may extend to cardiovascular complications.[25,26] A rat model of high fat density (HFD) diet-induced obesity is well-controlled and has many characteristics with human obesity. Obesity-related cardiovascular problems can be studied using a mouse model of obesity based on HFD diet.[27]

The present work investigates the antiobesity action of Amruthotharam Kashaya formulations prepared by classical method and modern method on high fat diet induced obesity in Wistar rats. This study will help us to understand whether changing the formulation method, will there be any change in pharmacological effect.

The animals were grouped in the eight groups containing 6 rats in each group. The obesity was induced using high fat diet. The animals were fed with high fat diet for four week of period. Furthermore the animals were treated with alloxan to induced diabetes. The process was carried out for four week of time period for determination of fasting glucose level and postprandial glucose level in blood. The treatment was carried out to evaluate the change in body weight and glucose level in blood after 4 week of treatment with formulation F1 and F2.

Body weight changes

The body weights (g) of all the animals were measured at 4 weeks of the study. After the induction of high fat diet to the rats, weight was increased in the weight was observed in all rats selected in each group. After the treatment with formulations F1 and F2 on animals with HFD and HFD-DIA, there was decreased in the body weight observed from 254.52- 230.7 g (group F1.1), 184.48- 252.25 (group F1.2), 254.03- 223.72 gm (group F2.1), 252.87-220.05 g (group F2.2), 254.10- 221.55 g in standard group. The results concluded that both formulations F1 and F2 reduced increased body weight of rats. However, the formulation F2

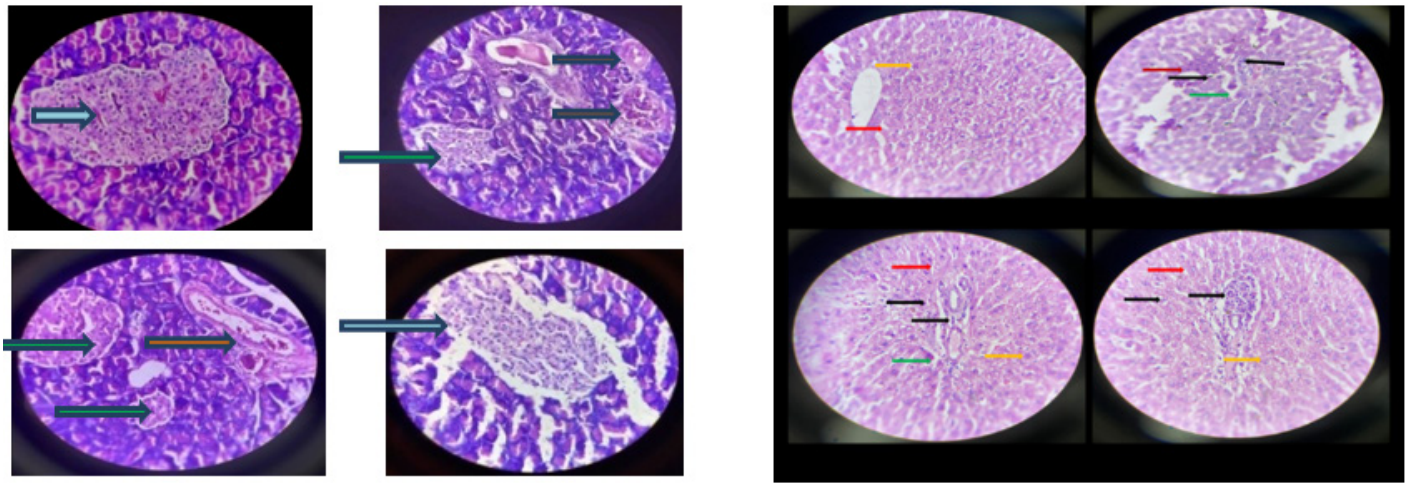

Figure 2: Histopathology of Liver and Pancreas.

showed better action in decreasing body weight compared to the formulation F1 prepared by classical method, shown in Table 2.

Change in blood glucose level

The fasting glucose level and postprandial glucose level in blood was measured and given in Table 3 and 4. After the induction of diabetes in groups normal, HFD- DIA, F2.1, F2.2 and standard there was increased in fasting glucose level and postprandial sugar level in blood observed. After the treatment with formulations F1 and F2 on animals with HFD- DIA, there was decreased in the fasting glucose level observed from 177.00- 161.33 mg/dL (group F2.1), 173.00- 156.83mg/dL (group F2.2), 171.50- 155.33mg/dL in standard group. Also decreased in the postprandial sugar level in the blood was observed ranges from 171.33- 137.33 mg/dL (group F2.1), 169.33- 132.33 mg/dL (group F2.2), 164.33- 130.17 mg/dL in standard group. Both the formulation successfully reduced increase fasting and postprandial sugar level in blood. It was concluded that formulation F2 reduced increase fasting and postprandial sugar level in blood more efficiently shown in Table 3 and Table 4.

Effect on Serum Biochemical Parameters

The rats with high fat diet and alloxan induced diabetes solution (i.e. HFD + DIA) when treated with formulation F1 (i.e., F2.1 group VI) and formulation F2 (i.e., F2.2 group VII), there was a significant decreased in the total cholesterol level observed in group VII (155.42 mg/dL) when compared with group VI (175.5 mg/mL) on the 9th week after treatment shown in Table 5 All the studied on comparison with standard group treated with standard drug (Metformin group VIII) concluded that Amruthotharam Kashaya prepared by modern method (F2) was found to be effective in reducing total cholesterol level in serum.

Effect on serum triglycerides levels (mg/dL)

The rats with high fat diet and alloxan induced diabetes solution (i.e. HFD + DIA) when treated with formulation F1 (i.e. F2.1 group VI) and formulation F2 (i.e. F2.2 group VII), there was a greater reduction in the triglyceride level observed in group VII (35.17 mg/dL) when compared with group VI (43.72 mg/dL) on the 9th week after treatment. All the studies on comparison with standard group treated with standard drug (Metformin group VIII) in Table 6 concluded that *Amruthotharam kashaya* prepared by modern method (F2) was found to be effective in reducing triglyceride level in serum.

Effect on serum HDL levels (mg/dL)

The rats with high fat diet and alloxan induced diabetes solution (i.e. HFD + DIA) when treated with formulation F1 (i.e. F2.1 group VI) and formulation F2 (i.e. F2.2 group VII), the increased in HDL level was observed in group VII (38.78 mg/dL) when compared with group VI (33.83 mg/dL) on the 9th week after treatment. All the studies on comparison with standard group treated with standard drug (metformin group VIII) in Table 7, concluded that *Amruthotharam kashaya* prepared by modern method (F2) was found to be effective in significantly improving HDL level in serum.

Effect on serum LDL levels (mg/dL)

The rats with high fat diet and alloxan induced diabetes solution (i.e. HFD + DIA) when treated with formulation F1 (i.e. F2.1 group VI) and formulation F2 (i.e. F2.2 group VII), there was a greater reduction in the LDL level observed in group VII (55.68 mg/dL) when compared with group VI (66.26 mg/dL) on the 9th week after treatment. All the studies on comparison with standard group treated with standard drug (metformin group VIII) in Table 8 concluded that Amruthotharam kashaya prepared by modern method (F2) was found to be effective in reducing LDL level in serum.

Effect on serum VLDL levels (mg/dL)

the rats with high fat diet and alloxan induced diabetes solution (i.e. HFD + DIA) when treated with formulation F1 (i.e. F2.1

Table 3: Change in the fasting glucose level in blood after treatment with formulations.

Groups	Change in fasting glucose level in blood (mg/dL)			
	1st Week	2nd Week	3rd Week	4th Week
Normal	91.33±0.82	92.33±1.03	93.33±1.37	94.17±1.33
HFD-DIA	194.67±3.33	201.00±3.03	207.50±3.73	214.83±2.86
F2.1	177.00±2.37	173.50±2.43	165.50±1.97	161.33±2.07
F2.2	173.00±1.55	167.33±1.63	162.50±1.87	156.83±1.17

Table 4: Change in the postprandial glucose level in blood after treatment with formulations.

Groups	Change in postprandial glucose level in blood (mg/dL)			
	1st Week	2nd Week	3rd Week	4th Week
Normal	117.67±0.52	118.83±1.17	120.00±0.89	120.33±0.52
HFD-DIA	193.67±3.20	202.17±1.60	212.50±1.38	222.17±2.14
F2.1	171.33±2.50	161.33±1.51	150.00±1.41	137.33±1.97
F2.2	169.33±2.16	159.00±1.26	145.67±2.42	132.33±2.07
Standard	164.33±2.42	155.17±1.94	143.50±2.26	130.17±1.17

Table 5: Effect of F1 and F2 formulations on serum total cholesterol level.

Group	Serum total cholesterol level (mg/dL)		
	0 week	4th week	9th week
F1.1	104.53± 1.30	243.87± 1.54	190.1± 1.28
F1.2	103.95± 0.94	243.95± 0.94	185.8± 1.96
F2.1	105.52± 0.70	244.28± 1.39	175.58± 1.70
F2.2	104.68± 1.09	243.85± 1.72	155.42± 1.70
Standard	104.78± 1.12	244.95± 1.37	173.5± 1.60

Table 6: Effect of F1 and F2 formulations on serum triglycerides level.

Group	Serum triglycerides level (mg/dL)		
	0 week	4th week	9th week
F1.1	23.27±0.69	89.17±0.67	65.02±1.34
F1.2	23±1.04	87.6±0.56	54.75±1.90
F2.1	23.15±0.66	87.82±1.39	43.72±1.48
F2.2	23.07±0.46	88.02±0.96	35.17±1.88
Standard	23.08±0.64	88.3±1.01	44.93±1.31

Table 7: Effect of F1 and F2 formulations on serum HDL level.

Group	Serum HDL level (mg/dL)		
	0 week	4th week	9th week
Normal	42.88±1.22	46.92±0.75	47.53±0.85
HFD	42.53±1.37	20.17±0.90	18.55±0.80
HFD-DIA	43.42±0.35	20.4±0.74	19.62±0.52
F1.1	42.98±1.10	20.72±1.05	25.62±1.12
F1.2	42.78±0.77	20.77±1.23	30.98±1.47
F2.1	42.18±1.55	20.05±0.55	33.83±1.00
F2.2	42.33±1.00	20.7±0.75	38.78±0.74
Standard	42.67±1.35	20.85±1.10	32.67±1.04

Table 8: Effect of F1 and F2 formulations on serum LDL levels (mg/dL).

Group	Serum LDL level (mg/dL)		
	0 week	4th week	9th week
F1.1	50.18±1.03	95±0.99	94.78±1.89
F1.2	51.58±1.14	95.2±0.95	85.57±1.59
F2.1	51.1±1.09	94.57±1.52	66.26±1.62
F2.2	51.38±1.32	95.25±1.88	55.68±2.05
Standard	51.28±0.77	95.72±0.78	65.25±2.10

Table 9: Effect of F1 and F2 formulations on serum VLDL level.

Group	Serum VLDL level (mg/dL)		
	0 week	4th week	9th week
F1.1	14.41±0.75	50.13±0.85	45.58±1.07
F1.2	14.75±0.89	50.53±1.46	37.43±1.16
F2.1	14.58±0.66	50.97±1.97	32.65±1.76
F2.2	14.7±0.92	50±1.24	26.13±1.49
Standard	14.58±0.70	50.22±1.68	33.02±0.97

group VI) and formulation F2 (i.e. F2.2 group VII), there was a significant reduction in the VLDL level observed in group VII (26.13 mg/dL) when compared with group VI (32.65 mg/dL) on the 9th week after treatment. All the studies on comparison with standard group treated with standard drug (metformin group VIII) in Table 9 concluded that Amruthotharam kashaya prepared by modern method (F2) was found to be effective in reducing VLDL level in serum.

Histopathological Evaluation

Histopathology of liver

Histopathology of liver tissue of rats treated with high fat diet and HFD- alloxan induced diabetes showed infiltered cells (Figure 2B, 2C) when compared to normal healthy control rats liver tissue which showed normal hepatocyte and normal sinusoids (Figure 2A). The rats with high fat diet and HFD-alloxan induced diabetes, when treated with formulation F1 and F2 showed decreased number of infiltered cells (Figure 2D, E, F, G). While comparing the formulation F1 and F2, F2 showed better action with no infiltered cells as compared to the rats treated with metformin (Figure 2).

Pancreatic slice of HFD-alloxan-induced diabetic rats treated with Formulation -1 (Kasaya) at 1.5gm/kg body weight demonstrating a modest number of islets of langerhans (orange arrows) and a normal islet of langerhans with many beta cells (green arrows) (Figure 2D). PF2: Pancreatic slice of HFD-alloxan-induced diabetic rats treated with Formulation -2 (extract combination) at 1.5gm/kg body weight revealing a limited number of islets of langerhans (orange arrows) and normal islets of langerhans with abundant beta cells (green arrows) (Figure 2E). PMET: pancreatic slice of diabetic rats treated with Metformin demonstrating normal pancreatic islet of langerhans with increased beta cell population (blue arrows), shown in Figure 2.

The formulation contains guduchi *(Tinospora cordifolia)*, haritaki (*Terminalia chebula*), and shunthi (*Zingiber officinale*). Because natural medicines are derived from wild sources, maintaining consistent product quality is challenging owing to external variables such as well as geographic location, nutrient availability, temperature variations, light and water availability, and soil conditions. The two well-known and widely used medical systems in India are called Ayurveda and Unani Medicine respectively. In India's AYUSH systems, about 8,000 different herbal treatments have been given official status. Tabatabaei M *et al*., 2016[28] One of these medicines is called *Amruthotharam/Amrutottaram Kashayam*, and it treats metabolic issues by treating inflammation as its primary mechanism of action. You LB *et al*., 2019 *Tinospora cordifolia, Terminalia chebula*, and *Zingiber officinale* are used in the composition these natural medicines are derived from wild sources, Singh MP *et al*., 2009 it can be difficult to maintain a consistent product quality due to external factors and climatic conditions. Mukherjee PK *et al*., 2002,[20] Both of the formulations were found to possess an amount of antioxidant activity that was sufficient after being examined as a whole. The F2 formulation has a stronger antioxidant activity than the F1 formulation. Bahmani M, *et al*., 2012[21] The purpose of the pharmacological study was to ascertain whether or not the formulation was successful in combating diabetes and obesity. The evaluation took into account changes in both the fasting glucose level and the postprandial sugar level in the blood. Body weight change was also taken into account. It has been demonstrated that the contemporary method formulation F2 is successful in lowering both excess sugar levels and additional body weight. The total cholesterol level, triglyceride level, HDL level, LDL level, and VLDL level are some of the blood

biochemical indicators that are investigated in this part of the research. It has been demonstrated that consumption of F2 can assist in the lowering of total cholesterol, triglyceride levels, LDL levels, and VLDL levels in the blood, as well as the elevation of HDL levels**.** Ighodaro OM *et al*., 2017[23] Histopathology of liver tissue treated with high fat diet and HFD-alloxan induced diabetes demonstrated infiltered cells as compared to normal healthy control rats liver tissue, which displayed normal hepatocytes and sinusoids. The number of infiltered cells was decreased in rats with high fat diet and HFD-alloxan induced diabetes who were treated with formulations F1 and F2.

CONCLUSION

The populace of developing nations uses medicinal herbs as an alternate form of treatment. Recent years have seen an increase in the scientific foundation of herbal treatment. Studies leading to the scientific explanation of plant medicinal qualities are enabling this approach to achieve increased credibility and acceptability within the medical community in response to requests from both the public and medical authorities. Antioxidant activity of *Amruthotharam kashayam* (F1) and the Kashaya mixture (F2) was examined. It was revealed that both formulations had high antioxidant activity. The F2 formulation was shown to be more effective in protecting against oxidative stress. The pharmacological study was carried out to evaluate the action of formulation against diabetes and obesity. The evaluation was carried out based on parameters such as change in body weight and change in fasting glucose level and postprandial sugar level in blood. The formulation F2 prepared by modern technique was found to be effective in reducing increased body weight and sugar level. The study also evaluates the effect of formulations (F1 and F2) on serum biochemical parameters such as total cholesterol level, triglyceride level, HDL level, LDL level, VLDL level. The formulation F2 was found to be effective in reducing total cholesterol level, triglyceride level, LDL level, VLDL level and improving HDL level in the serum.

Histopathology of liver tissue of rats treated with high fat diet and HFD- alloxan induced diabetes showed infiltered cells when compared to normal healthy control rats liver tissue which showed normal hepatocyte and normal sinusoids. The rats with high fat diet and HFD- alloxan induced diabetes, when treated with formulation F1 and F2 showed decreased number of infiltered cells. The *Amruthotharam* is indeed a very potent medicine which treats overall metabolic disorder as it alters the cellular mishaps.

ACKNOWLEDGEMENT

I express my deep sense of gratitude towards, Urjayu Research Center, Panaji, Goa, India for providing Histopathological analysis reports. I express my gratitude to Amsar Veda, Goa for providing samples of all herbal extracts

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Dhane K, Gupta MK, Hyam S, Patil A. Preparation and *in-vitro* and *in-vivo* Evaluation of Ayurvedic Formulation "Amruthotharam" Formulated by Classical and Modern Technique. Indian J of Pharmaceutical Education and Research. 2023;57(1s):s126-s134.