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Original Article

An Analytical & *In-Vivo* Study to Evaluate the Effect of Triphala Kaṣāya Vāsita Godhūma (Triticum aestivum grains soaked in Triphala Kaṣāya) on its Glycemic Index compared to Raw Godhūma (Raw Triticum aestivum grains)

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ABSTRACT

Diabetes is generally referred to as a Metabolic Disorder or a Lifestyle Disorder, that is mainly characterized by the symptom of Hyperglycemia caused by the defective insulin secretion, congenital or acquired. Diabetes requires management of diet and lifestyle with or without medications to keep the blood sugar under control. Foods with medium Glycemic Index (GI) is best preferred for Diabetics, because high GI foods will rapidly increase the blood sugar level, and low GI will cause grave health issues. In practice, wheat is the highly recommended diet for diabetics as the GI is medium in comparison to rice that belongs to a high GI group. But wheat causes digestive problems, and flatulence in addition to the Gluten allergy that many people suffer from. However in Āyurveda, the Classical Text Aṣṭāṅga Hṛdaya *(AH)* mentions in Prameha Cikitsa Adhyāya (Chapter on Treatment of Diabetes) that Godhūma (grains of Wheat - Triticum aestivum L) has to be kept soaked in Triphala Kaṣāya (Decoction prepared with the fruits of Terminalia chebula, T. bellerica and Phyllanthus emblica) and then dried and powdered before being used as a diet for a diabetic. The grains of Godhūma were kept soaked in Triphala Kaṣāya and then dried and powdered. A comparative analytical study was done for the two samples- treated wheat grains (Triphala Kaṣāya Vāsita Godhūma) and untreated/raw wheat grains. An *In vivo* study was also done to assess the GI value of both the samples of wheat. The analytical and *In vivo* study results prove that while the GI is still maintained within medium range, the allergen gluten is completely eliminated, total fat and total protein have reduced while still maintaining the nutritive value, in the treated sample when compared to the raw sample. Based on the results obtained from the analytical and in-vivo study, it can be concluded that Triphala Kaṣāya Vāsita Godhūma becomes even more compatible and healthy for a diabetic, in gluten allergy and discomforts caused by wheat consumption, in comparison to the untreated wheat grains.

Key words: *diabetes, āyurveda, prameha, godhūma, triphala kaṣāya, glycemic index, gluten.*

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INTRODUCTION

Diabetes mellitus is a chronic multisystemic disease that is categorized as a Lifestyle disorder. It is a non-communicable disease, but the proportion of people affected with this is rapidly increasing. Long term Hyperglycemia affects the micro vessels thus leading to Diabetic Retinopathy and Neuropathy. This has a pronounced impact on the quality of life and the overall life expectancy of a Diabetic. Along with medications, diet and exercise are equally important to maintain control over blood sugar levels in a diabetic. In fact, borderline elevated blood sugar levels can be controlled with diet itself. However, Āyurveda advocates treating Godhūma with Triphala Kaṣāya and be used as food by a diabetic and not the raw godhūma. The classical text mentions that this becomes more compatible and healthier for the patient. Hence the Analytical and *In vivo* study were done to understand the variations in the treated sample of Godhūma and how it affects the GI.

AIMS & OBJECTIVES

To analyze and compare variations in Phytochemical constituents of Godhūma before and after being subjected to Vāsana with Triphala Kaṣāyam.

To experimentally evaluate the effect of Triphala Kaṣāya Vāsita Godhūma on the Glycemic Index in comparison to raw Godhūma through *In vivo* Study.

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MATERIALS & METHODS

The study was conducted in two phases.

A) Analytical phase

The steps involved in the Analytical Phase are as follows.

1) Preparation of Triphala Kaṣāya

The three Myrobalans- Fruits of Harītaki (Terminalia chebula L)[1], Vibhītaki (Terminalia bellerica Roxb)[2] and Āmalaki (Phyllanthus emblica Gaertn)[3] were taken in the ratio of 1:1:1 (60 gms each) and 16 times water (2880 ml) was added. This was placed on mild fire and boiled until the quantity of the decoction was reduced to 1/8th (360 ml). The decoction is allowed to cool and then filtered. The method of preparing the decoction is as mentioned in Āyurvedic Pharmaceutical textbook Śārṅgadhara Samhita.[4]

2) Vāsana (Soaking) of Godhūma

Raw Godhūma grains (350 gms) collected from the authentic organic cultivators is now added slowly to the prepared Triphala Kaṣāya and kept soaked in such a way that the level of Triphala Kaṣāya is just above the immersed grains. This is left undisturbed for 12 hrs from sunset (6.19pm as on 31st August 2018) until sunrise (6.15 am as on September 1st 2018). The Kaṣāya is drained off completely and the soaked Godhūma grains are collected.

3) Drying of Godhūma grains

The treated Godhūma grains are placed in a clean tray. The tray containing the grains is now placed inside the Hot Air Oven and the temperature is set at 30-degree C. Occasionally the sample was checked to see the extent of dryness. After 21 hours the Godhūma was completely dry and was removed from the Hot Air Oven and then powdered.

4) Comparative Physico- Phytochemical analysis

The Raw and the treated sample of Godhūma were subjected to Physicochemical, Phytochemical and Nutritional profiling to understand the difference in the sample after the procedure was done.

B) *In vivo* Study Phase

Ethical Statement

The study Number was CKL/TOX/IAEC/2018-1/113.

Study Sample : Raw and Treated Grains of Triticum aestivum (Godhūma) / (Triphala Kashaya treated wheat grains and raw wheat

Place of Study

Department of Toxicology, CARE Keralam Ltd, KINFRA park, Koratty, Thrissur, District, Kerala, India. Reg no-1620/PO/RcBi/S/12/CPCSEA dated09/09/2014.

Name, address and registration number of breeders from which animals acquired

CARE Keralam Ltd, Koratty, Thrissur, Reg no-1620/PO/RcBi/S/12/CPCSEA dated09/09/2014.

Study compliance

The study was performed as per the Animal Ethics Committee Guidelines.

The study plan as laid and approved by Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was followed exactly. This was agreed upon by the Sponsor and the Animal Committee.

STUDY DESIGN

Number of Groups

The objective of this study in Wistar albino rats was to experimentally evaluate the effect of Triphala Kaṣāya treated wheat grains on its GI compared to raw wheat grains.

24 Albino rats (205-245g) were utilized in this study. The Animals were maintained under standard laboratory conditions. They were provided with food and water. The rats were acclimatized to laboratory set up for 7 days.

After adaptation period, the weight of the animals was rechecked. They were made to fast for 12 hours (overnight fasting). They were divided into four groups (six animals per group), their fasting blood glucose were determined and were treated in the following manner.

Test System and Management

Husbandry

Conditions: Animals had supply of adequate fresh air with IVC system (Air changes 15 per hour), and the room temperature 21.0 to 24.0°C, relative humidity was 57-65%, exposure to light was 12 hours light followed by a dark cycle of 12 hours. The temperature and relative humidity were recorded every day.

Housing: Each animal was housed in a standard polysulphonate cage (Size: L 300 x B 170 x H 140 mm). It had a stainless-steel top grill mesh with the facilities for containing food in the form of pellets and water for drinking was placed in a water bottle with a sipper tube made of stainless steel.

Acclimatization: The animals were acclimatized for a period of seven days that is the minimum period in laboratory setup. They were regularly monitored for the clinical signs.

Diet: Laboratory animal feed (Manufactured by Feed mill of School of Animal Nutrition and Feed Technology, Kerala Veterinary & Animal Sciences University) was given to the animals.

Water: Water was provided ad libitum until the end of the study. Charcoal filtered bore-well water was provided to the animals. This was also activated and then put through ultraviolet rays' exposure, in Aqua guard water filter cum purifier.

Safety precautions

All the necessary accessories required for the conduction of the study like gloves, mask etc were used.

Sample size

Total number of rats is 24, 4 groups with 6 rats each. Male Wistar Albino rats were used because, they are small rodents that are commonly selected for initial studies on GI, for its availability, low cost and compatibility. 24 rats were decided to be used in this study, as this is the minimum number to arrive at a meaningful statistical conclusion of acceptable standards.

Method of assessment

The glucose level in the blood of the animals were taken at zero time. The blood draw was from the tail vein. The first reading is before they are administered 2g of the samples. The blood glucose levels were regularly measured at 30, 60, 90 and 120th min. The glycemic response was calculated as The Incremental Area under the Blood Glucose Curve (IAUC). The. Graph was drawn from the concentration-time graph of blood glucose rejecting the area below the fasting level.[5]

Blood glucose curves were drawn from blood glucose values of animals at time 0, after 30, 60, 90 and 120th minute interval after consuming

the glucose (control) and formulated food samples of each group. The Incremental Area Under the Curve (IAUC) was thus calculated for all the animals. After the values were obtained, the following formula was applied to calculate the Glycemic Index.

Glycemic Index (GI) = IAUC for food samples (2g) X 100/ IAUC for Glucose (2g)

STATISTICAL DATA

Blood glucose (mg/dl) for each animal at different time intervals and Incremental Area Under the Curve (IAUC) (Tables 3 & 4) are presented. The graph is drawn representing time along the X axis and the blood sugar along the Y Axis and the IAUC is calculated using Trapezoid Rule. The graphical representation is given below.

DATA COMPILATION

The raw data was compared with the computer results of the data (in the form of appendix) and were verified. The data on blood glucose levels were subjected to computer Statistical processing. Each data was analyzed and presented appropriately in tabular columns. The findings of animal experiment were presented according to the standard reporting procedure.

INTERPRETATION OF RESULTS

The Results were thus interpreted using statistical analysis.

The data on blood glucose readings, generated from the present study, were expressed as mean ± SEM and subjected to computer statistical analysis with the help of Graph Pad Prism software, Version 5.00, USA. 2007. One-way ANOVA with Dunnett's post-test was done for all the treatment groups comparing with the control group data. All analysis and comparisons were evaluated at 5% significance level.

AMENDMENTS AND DEVIATIONS

There were no amendments and deviations.

RESULTS AND DISCUSSION

Analytical study (Tables 5-7)

Comparative qualitative analysis of the constituents present in untreated and treated wheat

The Qualitative assessment done in the two samples of Raw and Treated Godhūma, revealed that there is no variation in the constituents. It can be understood from the Table that Alkaloids, Flavonoids, Phenol, and Tannins were present in Godhūma Pre and Post treatment with Triphala Kaṣāya. Whereas constituents like Saponins, Sterols, Triterpenoids, Glycosides and Steroids were absent Pre and Post treatment with Triphala Kaṣāya. There was no change in both the samples.

Comparative wet analysis of untreated and treated wheat

When parameters like Acid insoluble ash, Alcohol soluble extract, Gluten and Water-soluble extract were checked, the significant change was found in Gluten. 9.01% of Gluten that was detected in raw wheat became completely absent in the treated wheat sample. This is a very significant change because, though Gluten is considered to be a highly nutritious protein, it causes a lot of health problems to individuals. Gluten is the main causative factor for Celiac Disease.[6] In small amounts, Gluten ingested may not cause problems, but in the long run, it can even cause neurodegenerative diseases. The effect of treating raw wheat with Triphala Kaṣāya is that while the values of Acid insoluble ash, Alcohol soluble extract, and Water-soluble extract remain almost unchanged, Gluten is completely eliminated in treated sample.

The value of carbohydrate in treated sample was 88.16% , comparatively more than untreated wheat that was 75.91%. The Energy value in untreated wheat was higher (373.83%) in comparison to treated wheat (368.39). In a similar manner, Total fat was also high in untreated wheat (2.39%) compared to treated wheat (1.75%). The most significant value obtained was the Total protein. In untreated wheat, the value of Total protein was 12.17% in comparison to the almost negligible value obtained in treated sample (.63%). There was no significant variation in the values obtained in Crude fibre, Total ash, Moisture, Sodium, Potassium and Calcium levels.

In a Diabetic, low protein and low-fat diet is the most recommended. By low protein diet, the focus is on deriving the required calories from complex carbohydrates and not proteins. Depending on the pathology the person has, the protein to be ingested has to be decided. In case of a diabetic, the primary target of the diet followed is to keep the blood sugar levels in control.

Protein is mandatory for growth, and hence cannot be totally avoided in the diet. The quantity of protein required in the diet varies depending upon the extent to which the kidney or liver is damaged and the amount of protein required by the individual in maintaining his health. The suggested low-protein diet especially in Diabetics or any kidney pathology is 0.6g/kg[7] per day. If large quantities of protein are consumed, it cannot be digested and so gets excreted in the urine. On the contrary a low protein diet will avoid the stress on the kidneys and the liver.

In a similar way, for a diabetic, a low fat diet[8] is recommended. The rest of the parameters like Fiber, Total ash, Moisture, Potassium, Calcium, and Sodium are almost of the same value in the untreated and treated sample.

In vivo study

The GI is defined as the Incremental blood glucose area under the curve following a test food. This is expressed as the percentage of the corresponding area following a carbohydrate equivalent load of a reference food. The food is characterized on the basis of the postprandial glycemic response and not on the chemical composition. Recent researches have laid stress on the fact that foods with high glycemic index bear the risk of coronary heart disease.

Triphala is one of the most important Āyurvedic formulation. This is the collective term given to the fruits of Harītaki, Vibhītaki and Amalaki respectively. It has a wide range of application for the management of different kinds of Prameha (diabetes)[9] in the Ayurvedic literature either individually or in combination with other formulations.[10] Triphala contains about 194 bio actives,[11] that makes it an extremely efficient therapeutic agent. In ancient Ayurvedic literature, Aṣṭāṅga Hṛdaya, Prameha Chikitsa, wheat is recommended as a diet for diabetics after being soaked in Triphala Kaṣāya.[12] Hence the present study evaluated the variations in GI when wheat is subjected to the procedure as mentioned in the Classical Ayurvedic Text.

In this study, the glycemic index of the foods ranged from 20.5% in normal lab rat diet, 49.4% in raw wheat sample, to 58.7% in Triphala Kaṣāya treated wheat sample. There was no distinct difference between the GI of the raw and Triphala Kaṣāya treated wheat grains, but the values were significantly (<0.001) lower when compared with the GI of reference sample, Glucose (GI=100).

Blood glucose levels at different time intervals, IAUC and glycemic index of Wistar rats are shown in Tables and Figures.

Dietary carbohydrates could raise the blood glucose levels especially during the postprandial period and scientific studies have established that there is a very strong relationship between the diet and diabetes.[13]

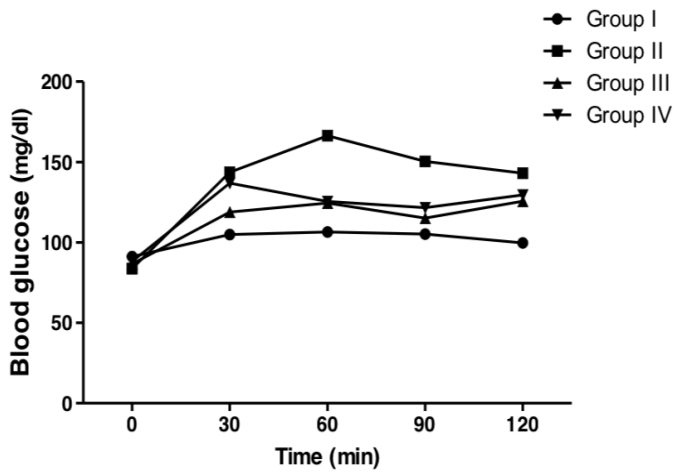

Figure 1: Blood glucose (mg/dl) at different time intervals.

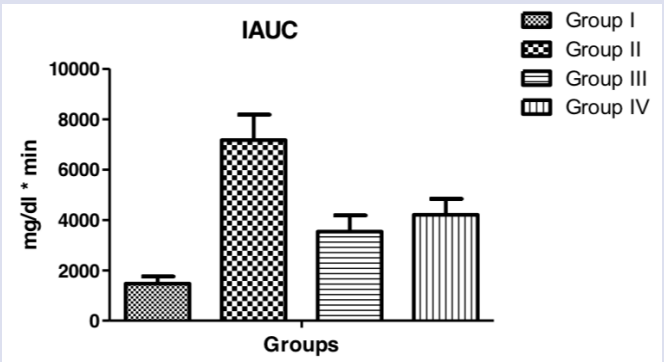

Figure 2: Incremental area under the curve (IAUC) of different groups.

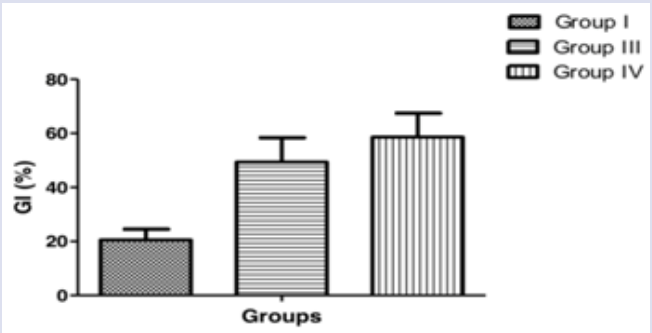

Figure 3: Glycemic index (GI) of different groups.

Table 1: The groups of animals and the administration of sample.

Groups	No. of animals	Treatment
I- Normal control	6	Normal diet
II - Glucose control	6	Glucose solution (2g in distilled water, per oral)
III- Raw Wheat grain	6	Raw Wheat grain- not soaked in Triphala Kaṣāya. 2g, per oral
IV- Treated Wheat grain	6	Wheat grain kept soaked in Triphala Kaṣāya. 2g, per oral

Table 2: Specifies the details of the animals.

Animal Model	: Rats
Strain	: Wistar albino rats
Reason for selection of species	Rat is one of the recommended species by : regulatory agencies for conducting toxicological studies among rodents.
Source	CARe KERALAM Ltd., KINFRA Small Industries Park, Koratty-– 680 309, Thrissur
No. of animals & Sex	: 9 Male Rats
Body weight range Receipt	: 205-245 g
Age at treatment	: 8 to 12 Weeks
Identification	: Cage cards

Table 3: Blood Glucose observations for each animal at different time intervals and incremental area under the curve (IAUC).

Groups	Blood Glucose Levels (mg/dl)					Incremental Area under the Curve (IAUC)
	0 Min	30 Min	60 Min	90 Min	120 Min	
I	87	119	147	113	137	863.4
	119	185	174	148	155	870
	71	157	192	177	135	2130
	64	136	158	170	161	1013
	82	147	134	140	134	2505
	79	118	193	154	136	1455
II	87	119	147	113	137	4290
	119	185	174	148	155	5040
	71	157	192	177	135	10350
	64	136	158	170	161	9615
	82	147	134	140	134	6030
	79	118	193	154	136	7695
III	88	101	118	96	120	2010
	92	143	146	145	141	5475
	76	107	117	109	125	3885
	79	143	116	123	135	5190
	100	110	136	99	130	1801
IV	85	109	114	119	103	2880
	86	129	141	139	162	5670
	84	197	127	111	111	5895
	88	139	120	148	143	5115
	85	125	117	110	117	3390
	87	104	124	115	117	2910
	99	127	124	107	127	2250

Table 4: The blood glucose levels at different intervals were calculated.

Groups	Blood glucose level (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
I	91.167 ± 3.497	104.833 ± 4.143*	106.500 ± 5.032***	105.167 ± 5.382***	99.667 ± 4.047***
II	83.667 ± 7.813	143.667 ± 10.366	166.333 ± 9.861	150.333 ± 9.340	143.000 ± 4.824
III	86.667 ± 3.575	118.833 ± 7.748	124.500 ± 5.402***	115.167 ± 7.378**	125.667 ± 5.439
IV	88.167 ± 2.242	136.833 ± 12.911	125.500 ± 3.413***	121.667 ± 7.079*	129.500 ± 7.957

Table 4a: In vivo details.

Groups	Incremental Area Under the Curve (IAUC) mg /dl*min	Glycemic Index (GI) %
I	1472.733 ± 285.413***	20.54 ± 3.981***
II	7170.000 ± 1008.248	100 ± 0.000
III	3540.167 ± 642.557**	49.38 ± 8.962***
IV	4205.000 ± 632.289*	58.65 ± 8.819***

Incremental Area Under the Curve (IAUC) and Glycemic Index (GI) for different diets
Values are expressed as mean ± SEM
*P<0.05, **P<0.01 and ***P<0.001 when compared with Group II

Table 5: Physico chemical evaluation.

Constituents	Untreated	Treated
Alkaloids	Present	Present
Flavonoids	Present	Present
Phenol	Present	Present
Saponins	Absent	Absent
Sterols	Absent	Absent
Tannins	Present	Present
Triterpenoids	Absent	Absent
Glycosides	Absent	Absent
Steroids	Absent	Absent

Table 6: Wet analysis.

Parameters	Untreated	Treated
Acid Insoluble Ash	0.02%	0.02%
Alcohol Soluble Extract	5.02%	5.79%
Gluten	9.01%	Not Detected
Water Soluble Extract	13.53%	10.74%

Table 7: Nutritive value assessment.

Nutritive Values	Untreated	Treated
Carbohydrate	75.91%	88.16%
Crude Fibre	1.75%	1.61%
Energy Value	373.83%	368.39%
Total Fat	2.39%	1.75%
Total Protein	12.17%	0.63%
Total Ash	1.49%	1.54%
Moisture	8.04%	8.55%
Sodium	0.28%	0.16%
Potassium	0.81%	0.77%
Calcium	0.01%	0.01%

The GI of foods (%) can be classified into three, that is, high (>70%), medium (56-69%) and low GI (<55). In high GI foods, the sugar is rapidly released into the blood stream, while low-GI foods is characterized by the gradual release of glucose into the blood stream.

In this study, even though there was no marked difference between values of GI of both grain samples, GI of Triphala Kaṣāya treated wheat grains were found to be on a slightly higher side, 58.7% (medium GI) compared to raw wheat grains, 49.4% (low GI) but P value obtained was not significant. There are a number of factors like starch composition/ properties, dietary fiber content, food processing, storage etc that influences the GI. In the present study, Kaṣāya treated wheat grains were prepared by keeping raw wheat soaked in Triphala Kaṣāya followed by drying. From the values obtained in the analytical study it is evident that there has been changes in the treated sample.

Not only in a diabetic, but also in glucose intolerance and hyperglycemia, the GI of the foods consumed are very important. In the *In vivo* study, healthy rats were chosen as animal models, because the target was basically to understand the effect on GI value. Now that the effect on GI and the variations in phytochemical and nutritional parameters are clear, a clinical study can be designed to standardize the dose and dosage form of wheat for consumption.

CONCLUSION

Thus, the Comparative analytical study, depicts that though the level of carbohydrate is moderately elevated, the Total energy value is less in the treated sample when compared to the untreated sample. Total protein has become almost negligible, while Total fat has reduced in a minimal way in the treated sample. In a diabetic, the diet recommended is a low protein and low fat, so as to avoid the stress on the system. When Godhūma is kept soaked in Triphala Kaṣāya, the Guru[14] (difficult to digest) and Santarpaṇa Guṇa[15] (nourishing aspect) is maintained as well as the Kleda guṇa (pathology stimulating factor) present in the body is dried up by Triphala Kaṣāya. Triphala is an excellent remedy in alleviating the Kleda and Medas (fat content) in the aggravated form. This is directly indicated in Prameha,[16] as Prameha is caused by the Vṛddhi (aggravation) of Kleda and Medas.[9] The predominant symptom in Prameha, ie- Dhātu śaithilya (weakening of the tissues) is prevented due to the Guru, Snigdha Guṇa (oily nature) of Godhūma. Thus Godhūma and Triphala Kaṣāya make a perfect balance to counteract Prameha. The most significant finding was the complete elimination of Gluten in the treated sample. Gluten is a known allergen for a number of people worldwide and is the major causative factor in Celiac Disease. Though there are a lot of food materials available in the market that are Gluten Free, it does not ensure the nutritive value. Food is meant primarily for maintaining the nutrition apart from making it suitable for the pathological condition. As far as a diabetic is considered, diet and activity are equally important as that of the medications that he is consuming. Hence Godhūma kept soaked in Triphala Kaṣāya is nutritive at the same time suitable for the Samprāpti Vighaṭāna of Prameha (counteracting the diabetes pathology).

From the *In vivo* study, it can be concluded that Triphala Kaṣāya Vāsita Godhūma is definitely more compatible and healthy in comparison to raw wheat grain. The P-Value obtained from the statistical analysis is not significant, indicating that even when wheat is kept soaked in Triphala Kaṣāya, there is no significant change in the GI.

The nutritive value is enhanced and the allergen gluten is eliminated, in Triphala Kaṣāya Vāsita Godhūma. The data from this analytical and

In vivo study should be used to conduct Clinical Studies and check the variation in the Blood glucose Levels and can be standardized as a recommended diet for a Diabetic.

SUGGESTIONS

The Analytical and *In vivo* studies have helped us in determining the pharmacodynamics of the sample.

In future the study can be extended into a clinical study, now that the basic data is available.

This data can be used in deciding the way in which Godhūma has to be processed for consuming, because GI is affected by a number of factors including the method of cooking.

The study can be done by trying out different procedures of levigation mentioned in the classical texts. The soaking can be done for 3 days or 5 days or even 7 days.

Different proportion of the three fruits can be taken to prepare Triphala Kaṣāya and used in the study.

This can be tried in definite types of Prameha- Vataja, pittaja or kaphaja and the response can be studied.

FUTURE SCOPE OF THE STUDY

Wheat is already an accepted diet for a Diabetic. Triphala is also a widely used formulation. But regular consumption of wheat produces a lot of unwanted effects even in a healthy person. Regular use of Triphala is not recommended as it is extremely dry in nature. But in conditions of aggravated Kleda and Medas, as seen in Prameha, the combination of Godhūma and Triphala helps achieve the target of maintaining health and breaking the pathology simultaneously. The next step is to conduct a Clinical Trial standardizing the method of processing, administration and dose in which treated Godhūma can be prescribed to Diabetics.

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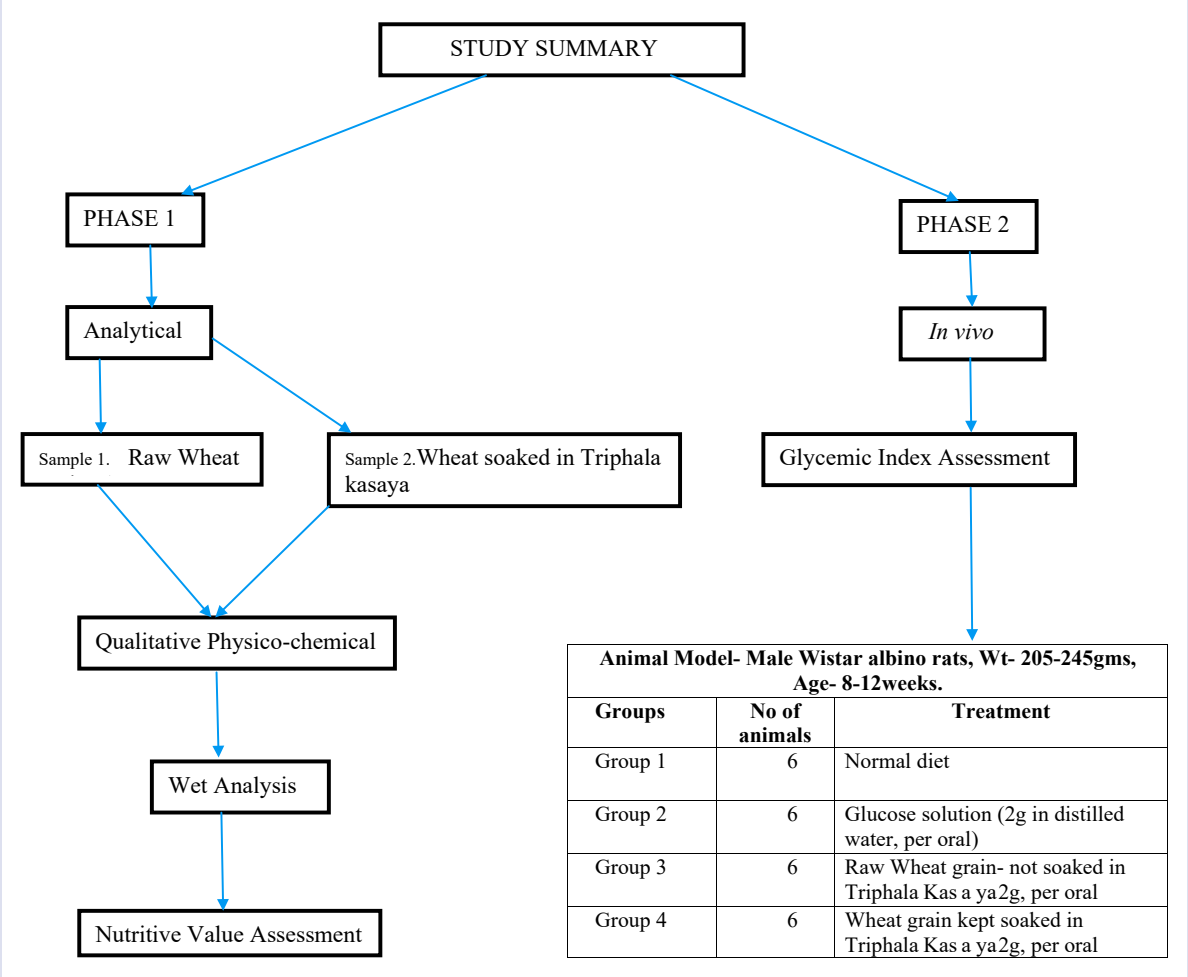
GRAPHICAL ABSTRACT



Animal Model- Male Wistar albino rats, Wt- 205-245gms, Age- 8-12weeks.		
Groups	**No of animals**	**Treatment**
Group 1	6	Normal diet
Group 2	6	Glucose solution (2g in distilled water, per oral)
Group 3	6	Raw Wheat grain- not soaked in Triphala Kas a ya2g, per oral
Group 4	6	Wheat grain kept soaked in Triphala Kas a ya2g, per oral

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