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EVALUATION OF POLYPHENOL BIO-AVAILABILITY THROUGH INTESTINAL ABSORBTION WITH SPECIAL REFERENCE TO MADHU SAMYOGA AND SAMSKARA- AN EX-VIVO EXPERIMENTATION

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ABSTRACT

Utility of Honey is based on its unique quality called *Yogavahi* (one that takes the properties of other substances along with its own), by which it potentiates the efficacy of drugs used along with it either in Mixed or Processed form. Honey has been tested experimentally for its anti-obesity and anti-hyperglycemic properties which show different effects with respect to Mixed and Processed forms. Honey Processed with *Triphala (*polyherbal combination of *Terminalia chebula, T. bellerica, Emblica officinalis)* decoction has significant anti-obesity activity, whereas that Mixed with *Triphala* Decoction *is* more effective in hyperglycemia. Study was aimed at evaluation of Polyphenol bio-availability with special reference to Mixed and Processed form of Honey by ex-vivo experimentation. Kevala Madhu, Jala samskarita Madhu, Trhiphala Kashaya mishrita Madhu and Triphala Kashaya samskarita madhu were subjected to Ex-vivo experimentation and later subjected to total phenol estimation. Honey processed with Triphala decoction increases the intestinal absorption of phenolic compounds and thus enhances the bio-availability. Heating process with Honey has a direct effect on phenolic absorption through intestine due to increase in total phenolics.

KEYWORDS: Poly phenols, Samyoga, Samskara, Triphala, Madhu, Bio-availability

INTRODUCTION

Life style diseases are the most discussed health risks due to causation of more number of deaths worldwide, involving conditions like diabetes, obesity and varieties of metabolic disorders[1]. Obesity increases risk of Type2 Diabetes mellitus[2]. Therapeutic implications for both obesity and diabetens remain similar involving diet and physical exercise [3]. Therapeutic modalities in Ayurveda invariably indicate *Madhu* and *Triphala* in both *Prameha* and *Sthoulya*, where *Madhu* with *Triphala kashaya* is termed as *Sarva pramehahara*[4]. Though *Purana Madhu* alone has been attributed with *Lekhana karma*[5], processing (*Samskara)* with *dravyas* having similar activities may further potentiate desirable effects[6] and this has been attributed to

*Yogavahi*twa[7]. While explaining Ama & *Pakwa Madhu Lakshana*, *Acharya Kaiyadeva* attributes different modalities of *Madhupaka* to achieve specific outcomes[8].

Honey is a complex material having as much as 181 different constituents[9] consisting maximum amount of oligosaccharides exerting anti-diabetic effect [10, 11]. Number of substances like flavonoids, phenolic acids and invert sugar associated with protein enzymes characterize honey constituents have established antioxidant / anti-hyperglycaemic and cyto-protective potential, collectively or individually. Phenolic acids such as Gallic acid have been found in honey and even in *Triphala* have established cardio protective activity[12]. Dark coloured honey samples have exhibited more noteworthy anti-oxidant activitiy[13].

Studies confirmed that, *Triphala* possesses high amount of phenols/polyphenols accounting for its activity towards several free radicals. The glycosylated forms have long been considered not to be absorbed from the gut unless hydrolyzed by the micro flora in the large intestine or in the distal ileum[14]. Only a few studies have been performed so far on the consequence of binding to proteins on both activity and bio-availability of the phenol compounds[15,16]. Other reliable methods for the estimation of intestinal uptake of food components, such as *in-vitro* or *in-vivo* perfusion techniques, are also available. Polyphenol bioavailability can be evaluated by using segments of the small intestine of rats. Two polyphenolic compounds, tannic acid and catechin, can be taken as representatives of high and low molecular weight Polyphenol with Gallic acid and flavonoid structures, respectively [17]. Role of Polyphenol has been emphasized in bringing about such pharmacological activities through anti-oxidant potential[11], which is said to be influenced by heating process[18]. There is a need to emphasize an understanding of *Ayurvedic* epistemology and the principles of *Dravyaguna vijnana*, while embarking on modern drug research [19] Present study was aimed at evaluation of Polyphenol bio-availability with special reference to *Samyoga* and *Samskara* of *Madhu* by ex-vivo experimentation. Since Triphala is not a good source for Flavonoid, only Gallic acid (with or without bound form) will be considered for the study in comparison with standard Gallic acid and Tannic acid.

MATERIALS AND METHODS

Deseeded fruits of *Haritaki* (*Terminalia chebula* Retz), *Vibhitaki* (*Terminalia bellerica* Retz) and *Amalaki (Emblica officinalis Geartn*) were procured from Local

market of Mysore city, and samples were authenticated by the subject expert by comparing with the voucher specimen maintained at *Dravyaguna* Museum, Department of *Dravyaguna*, JSSAMC, Mysuru. Honey was procured from Honey national society of India. Both Triphala and Honey were subjected for purity tests. Equal proportion of *Haritaki* (*Terminalia chebula* Retz), *Vibhitaki* (*Terminalia bellerica* Retz) and *Amalaki* (*Emblica officinalis* Geartn) were mixed and pounded into coarse powder. *Triphala Kashaya* was prepared as per general *Kashaya paka vidhi* of *Sharanghadhara Samhita*[20]. One part of coarse powder was boiled with 16 parts of clean potable water over *Mandagni* and reduced to 1/8th of the mixture. *Kashaya* was filtered using 4 folded clean and dry cotton cloth. *Madhu* and *Triphala Kashaya* were taken in equal proportion and was mixed homogeneously and *Paka* was carried out till the original quantity of *Madhu* was retained and *Paka Lakshanas* was observed [21]. Eight parts of Madhu was mixed with 1 part of water and was heated over Mandagni till volume was reduced to original volume of Madhu[22] (Photo 1 & 2) Samples were further subjected for physico-chemical evaluation. At the time of analysis, unprocessed Madhu was homogeneously mixed with freshly prepared *Triphala Kashaya* and further diluted using distilled water as per the requirement of the test protocol. *Triphala Kashaya Samskarita Madhu* & *Jala Samskarita Madhu* and Kevala Madhu samples were diluted using only distilled water.

PHARMACOGNOSTICAL AND PHYSICO-CHEMICAL STUDY

Pharmacognostical and physico-chemical tests were performed as per the test protocol[23].

Foreign matter evaluation of Triphala, Total ash content, acid insoluble ash, Water soluble ash, Extractive values, Moisture Content, Specific gravity, Total Phenolic Content and pH of honey were estimated.

EX-VIVO EXPERIMENTAL STUDY

Healthy wistar albino rats were procured from animal breeding facility, Department of pharmacology, JSS University were maintained at JSS COLLEGE OF PHARMACY Animal house. Animal ethical clearance was obtained from JSS JSS pharmacy college, Mysuru prior to commencement of the experimental study. All the 12 animals which were selected for experiment were maintained in 4 different cages separately, each cage/ group consisting 3 rats. Animals were acclimatized for the

period of 30 days before commencement of experiment (Photo – 3).

Twelve healthy Wistar albino rats were considered for this experiment and were divided into four groups constituting three rats in each group. The solutions of *KM (Kevala Madhu), JSM (Jala samskarita Madhu), SYM (Triphala Kashaya mishrita Madhu) and TSM (Tripahal Kashaya samskarita Madhu)* were taken respectively.

Ex- vivo study was performed as per established protocol with minor changes[24] These rats were weaned for 20days with regular diet. Before each experiment, the rats were fasted overnight, and following day rats were sacrificed using anesthetic halothane overdose. Immediately the rats were taken for analysis. Under aseptic conditions with the help of animal dissection procedures, the rats were cut open (photo – 4), and the whole intestine was taken out from the rat. The fecal matter present in the intestine was flushed out, The segment was washed with phosphate buffered saline pH 7.5, tied at one end and loosely ligated at the other end and the cleaned intestine now was taken for the experiment. 2ml of solutions containing *KM, JSM, SYM, TSM* are introduced into the intestines and the other end is tightly ligated and kept in ringer lactate solution for 30min. The content was now taken out from the gut and was collected in vials. Contents were centrifuged to separate the mucosal and epithelial cells and other debris. After the end of the experiment, the vials containing the solutions were subjected to Total phenolic content estimation as per standard protocol[25]. Values obtained before, during and after experimentation were tabulated and expressed in terms of mean.

OBSERVATION AND RESLUTS

Observations made during pharmacognostic and physico-chemical studies have been enumerated in Table 1, 2, 3 and 4. Total phenolic estimation was done at the end of ex-vivo experiment is depicted in Table 5. During Madhupaka process with both water and Triphala Kashaya, following observations were made.

Madhupaka Lakshanas:

a) Tantumatam (formation of thread between fingers)

b) Kshipte jale nignam (Sinks to the bottom of water when droped)

c) vastre kshavalekshikshata darvya (Easily separable from cloth without leaving stain)

Table No. 1 Organoleptic tests of *Triphala*

Drugs	Color	Shape	Odour	Taste
Haritaki	Yellowish brown in colour with shiny	Ovate and wrinkled longitudinally	**Odourlless**	Astringent, slightly bitter and sweetish in the

	and smooth texture.			end.
Vibhitaki	Dark brown to black with hairy texture	Globular and obscurely 5 angled	**Odourless**	Astringent.
Amalaki	Black	Globose, curled pieces of pericarp of dried fruit	**Odourless**	Sour & Astringent.

Table No. 2 Organoleptic tests of *Madhu:*

Drug and Tests	Colour	Odour	Taste
Purana Madhu	Light Brown	Characteristic odour	Sweet
Kashaya Samskarita Madhu	Dark Brown	Characteristic odour	Sweet & Astringent
Jala Samskarita Madhu	Light Brown	Characteristic odour	Sweet

Table No 3 Physico-chemical study of *Triphala*

Sl.No	Test	Results	Sl.No	Test	Results
1.	Foreign matter	Absent	4.	Water soluble ash	2.04 %
2.	Total ash	2.96 %	5.	Water extract	39.2 %
3.	Acid insoluble ash	0.14 %	6.	Moisture content	7.1%

Table No 4 Physico-chemical study of *Madhu*:

Sl. No	Test	KM	JSM	TSM	SYM
1.	Specific Gravity	1.391.6 g/ml	1.3751g /ml	1.3757g /ml	
2.	Moisture content	21.5%	24.7%	20.7%	

Table No 5 Total phenolic content estimated in samples during ex-vivo study:

SL. NO.	GROUP	Before experiment mg/kg	After Experiment mg/kg	Mean difference	% Absorption
1	*Triphala Kashaya samskaritha madhu*	25058	8060.4	16997.6	67.8%
2	*Triphala kashaya Mishrita madhu*	21942	20544	1398	6.8%
3	*Jala samskarita madhu*	588.39	441.7	146.69	24.9%
4	*kevala madhu*	724	641.8	82.2	11.35%

DISCUSSION

Previous studies conducted on *Purana Madhu* revealed potent anti-hyperlipidemic and anti-hyperglycemic activities. Usually *Madhu* is utilized as a food product but the same can be used as *Anupana* along with *Aushadhi Dravya* to potentiate the activity which is related to *Yogavahitwa*[26]. Such *Aushadhi Dravyas* may be either used in *Samyoga* or after *Samskara* to obtain specific outcomes. One such combination is *Triphala* & *Madhu*, having common Pharmacological activities such as *Sthoulya hara* (*Medhodhatu vruddhi*) & *Pramehahara* where *Madhu* in the form of *Samskara* & *Samyoga* has been indicated respectively. Studies conducted to validate such concepts were found true through experimental & clinical protocol. *Triphala Kashaya Samskarita Madhu* showed significant efficacy in obesity as well as hyperlipidaemia[27] but had very little effect on serum glucose levels whereas *Madhu* mixed with *Triphala kashaya* exerted potent anti-hyperglycemic[28] potential among streptozotocin induced hyperglycemic rats. The difference in their efficacies is attributed to Phyto-chemical, Physico-chemical & nutritional aspects of *Madhu* and hence detailed analysis become essential. Observations made during previous studies are suggestive of altered physical & chemical profile after ageing of honey which is mainly related to enzymatic & interactive reactions over sugars, proteins & phenolic compounds [29].

Colour of *Purana Madhu* was comparitively darker than the one which was freshly collected. Darker honey samples are often said to have health benefits compared to lighter ones & this justifies utility of *Purana Madhu* in *Chikitsa*[30]. Dark brown colour & characteristic organoleptic nature of *Kashaya Samskarita Madhu* can be attributed to added Phyto-chemicals through *Triphala Kashaya* & heat effect which brings specific changes in the sugar components[31]

The Ex-vivo method is used to assess the rate and the degree to which polyphenols are digested and absorbed by taking small intestines together with microflora colon fermentation. In vivo studies of the pathways for polyphenol absorption are seriously hindered by the lack of access to the human intestinal epithelium. The alternative is to use animal model or cell culture experiments or the common techniques of ex-vivo experimentation. Rat is regarded as best experimental model being considered to be most suitable model for nutrition studies due to high degree of similarity between rat and human to digestion, anatomy, physiology of GI tract, the composition of intestinal

microbiota being very similar. Due to these similarity the results of Polyphenol absorbtion and their activity in rats might also be applied to human gut it represents a good platform and for the simulation of various human intestinal application.

Oral bio-availability is the product of fraction absorbed, fraction escaping gut wall elimination and fraction escaping hepatic elimination. Factors that influence bio-availability can be divided into physiological, physico-chemical and bio-pharmaceutical. It has been well established that physico-chemical properties determine oral absorption and drug metabolism. Some Polyphenol with different structures cannot be hydrolysed by intestinal enzymes and therefore, cannot be digested and absorbed until they reach the colon. After the intake of Polyphenol, Aglycones can be absorbed from the small intestine and the maximal plasma concentrations often reach 1-2 hr after ingestion[32, 33, 34]. However most Polyphenol in food are in the form of esters glycosides or polymers that cannot be absorbed in the native form. These substances must be hydrolysed by intestinal enzymes before substances can be absorbed. Hence whole intestine was considered for the study rather taking only segments of intestine [35]. In general, low molecular weight polyphenols are partially absorbed into the body directly or after phase II enzyme-dependent metabolic conversion in small intestinal cells. High molecular weight tannins and even low molecular weight polyphenols are transported into the large intestine in their original form and are excreted unaltered or are broken down by enterobacteria[36,37]. Hence, the stomach is the minor location of absorption of Polyphenol and therefore excluded in the present study.

If the phenolics contain a sugar molecule, such as glucose, galactose or xylose, they will be absorbed through the small intestine by the cytosolic beta–glucosidase/lactase phlorizin hydrolase. The absorption is also specific to carrier molecules[33].

Total phenolic content was significantly increased after heating at 80˚C, 100˚C, and 120˚C for 30 minutes each. Maillard reaction products are responsible for the increase in total phenolics after heating[38]. Among the four samples taken, *Triphala Kashaya samskaritha Madhu* is seen to be absorbed by [67%] followed by *Triphala Kashaya Samyukta Madhu* (6.8%) when injected into the intestinal lumen. The heat treatment is assumed to release bound phenolic compounds[39] and form complexes with sugar molecules aiding their easy absorption

from the intestine, as compared to the unheated samples thus making phenolic compounds absorbed more. Honey when heated showed significant increase in Polyphenol content. Change in sugar content of honey is also reported in previous works. Glucose and fructose ratio changes considerably after heating of honey. Permeability of different sugars varies in intestine and hence may be responsible of enhanced absorption of Polyphenol after processing. Role of specific sugar in phenolic absorption needs to be studied in detail.

CONCLUSION

Madhu samskara with *triphala kashaya* increases the intestinal absorption of Polyphenol and thus facilitates the bio-availability. Heating process with *madhu* has a direct effect on phenolic absorption through intestine. Merely, using *triphala kashaya* along with *madhu* without processing will not improve the intestinal absorption of phenolic compounds.

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Preparation of *Madhu Samskara*

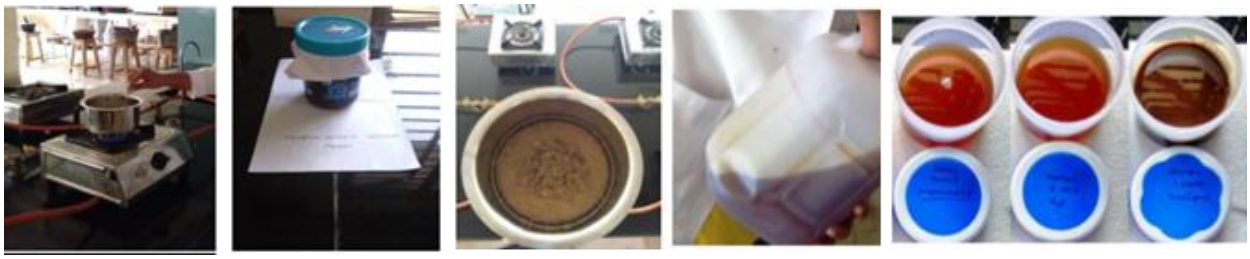
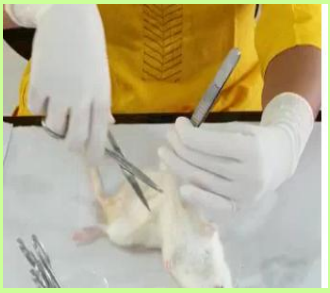


Photo - 1

		
KEVALA MADHU	***JALA SAMSKARITA MADHU***	***Triphala Kashaya Samskarita Madhu***
Photo - 2		

Experimental animals- wistar albino rats	
	
Wistar Albino rats	Experimental study
Photo 3 & 4	