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THE EFFECT OF SHODHANA (PURIFICATION AND POTENTIATION PROCESSES) OF *COMMIPHORA MUKUL* BURG. (GUGGUL) ON ANTI-INFLAMMATORY ACTIVITY IN ALBINO RATS

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ABSTRACT

Objective: The study was designed to evaluate the effect of purification (shodhana) on phytochemicals and anti-inflammatory properties of *Commiphora mukul* Burg. (Guggul) against Carrageenan induced rat hind paw oedema in albino rats.

Introduction: Ancient literature classifies *Commiphora mukul* as mild toxic drug. Ayurvedic texts clearly state that it must be used only after subjecting them to various Shodhana processes to eliminate toxicity and enhancing the efficacy. Several experiments report that Shodana process can purify the Guggul and can overcome the side effects of Crude Guggul.

Methodology: In the present study the Guggul an oleo-gum resin was purified by Shodhana process called Swedhana. The unprocessed and the products after each shodhana processed were studied to assess the influence of shodhana on phytochemicals and anti-inflammatory properties.

Results and Discussions: The Shodhana process on Guggul significantly enhanced the Guggulsterone Z and showed enhanced anti-inflammatory properties. The Guggul processed with triphala Kashaya (three fruits aqueous extract) significantly enhanced the anti-inflammatory properties. The Triphala anti-oxidant ingredients viz. galloyl glucose and other polyphenols might be included in triphala processed guggul and it may be attributed to the enhanced anti-inflammatory activity. The Shodhana processed products of guggul showed more therapeutic efficacy when compared with unprocessed guggul and the results were comparable with standard drug treatments.

Conclusion: It may be concluded that the traditional system of purification (Shodhana) can influence the phytochemicals and pharmacological profile of the plant drugs and useful in adopting these Shodhana processes in enhancing the safety, efficacy and potentiation the drugs.

INTRODUCTION

Initially the researchers worldwide concentrated on phytochemicals and pharmacological analysis of medicinal plants and their products. This was further continued to isolate, characterize the lead molecule. Attempts were made to structurally modify them with the intention of reducing the toxicity and enhancing the efficacy of them. However, these attempts were gone in vain. Therefore, many of them are being withdrawn. Since several centuries Ayurveda is utilizing these highly toxic to less toxic herbs after subjecting them to shodhana processes for treating human ailments. The Traditional medicines are gaining increasing popularity worldwide for the treatment of various diseases in recent times. Even there is a growing interest in research on Ayurveda science. As a first phase, analysis of components in the herbs and the development of new drugs from natural origin, as well as quality assurance and toxicological investigations of them is carried out worldwide. But there are claims that most of medicinal herbs have demonstrated none or mild side effects. But it is established behind doubt that certain herbs are highly toxic. Even Ayurveda, classified such herbs into Visha (Toxic) eg. Aconite and upavisha (moderately toxic) eg. Nux-vomica. Despite this, such toxic herbs are being adopted by Ayurvedic practioners for treating various diseases. The traditional shodhana procedures have been claimed to reduce the toxicity and enhance the efficacy of various toxic herbs. These shodhana processes are so well explained in the literature and evolved logically into an ancient science of detoxification. It is also clear from the literature that a single detoxification or purification process is not sufficient to detoxify all the herbs. Therefore, many shodhana processes have been evolved ranging from simple washing to boiling the herbs in dola-yantra (drug packed in cloth & hanged in the specified liquid and boiled for specified time) in various media (e.g. extracts of herbs, cow milk, cow urine, lime etc.). There is a more than one Shodhana process is applied for detoxification/potentiating of certain herbs. The process include from simple cleaning to detoxification and transformation of crude guggul using fluid media such as Guduchi (*Tinospora cordifolia*) Kwath-Kashaya (herb or herbs aqueous extract), Triphala (Three fruits) Kwath or Kashaya (dried riped fruits powders of Amla (*Emblica officinalis)* Phylanthaceae family, Chebulic-Myrobalan (*Terminalia chebula*) & Beleric-myrobalan *(Terminalia belerica*) Combretácea-Family), Milk (Godugdha-Cow milk), Panchtikta (five bitter herbs) Kwath, Dashmoola (ten roots) Kwath, Nimba patra (*Azadirachta indica*-leaf) Kwath with Haridra (Indian saffron) churna (powder), Cow urine (Gomutra) and Nirgundi patra (*Vitex nigundo*) leaf [1-4]. The herbs Kashayam/Kwath freshly prepared by boiling an herb or group of herbs (showing same chosen medicinal properties) in water. The each type of Kashayam has different therapeutic properties depending on its ingredients [4].

The guggul, an oleo-gum-resin is obtained from *Commiphora mukul* (Syn. *Commiphora wightii*), family Burseraceae. The therapeutic applications of the guggul has been reported in various traditional literature of Ayurveda. The usage of suddha (pure) guggul has been indicated in the treatment of obesity, tumours, malignant sores, ulcers, intestinal worms, leucoderma, sinus, and oedema [4-7]· The guggul has been reported to be effective in the management of blood coagulation disorders [6-8], myocardial infarction [9], Coronary heart disease [10-12] and in the treatment of joint inflammatory disorder [13].

Guggulosterone (E and Z), the active chemical constituents of guggul is reported to influence numerous natural processes like tumour cell apoptosis [14-15]. The literature indicates that unprocessed/unpurified guggul or partially purified guggul is used in the treatment of hyperlipidemia, anti-cholesterol, platelet aggregation inhibitory and fibrinolytic action, ischemic heart diseases, melatonin induced hypothyroidism, obesity and immunomodulatory properties. It is reported that the Z-guggulsterone showed good thyroid stimulating activity in rats which leads to reduction of cholesterol and serum lipids [16-19]. The purified Guggul lipid (a mixture of Guggulsteroids), completely inhibited ADP-adrenaline-induced platelet aggregation. It is reported to reduce serum triglycerides and cholesterol as well as LDL and VLDL cholesterols but elevates levels of HDL cholesterol significantly [20-23]. It is reported in the treatment of rheumatoid arthritis and in the controlling of various inflammations [24-25] and anti-cancer properties [26]. However, there is no relevant study on Shodhana processed guggul by using cow milk and triphala aqueous extract is carried out for its efficacy in the treatment of inflammation. Hence, in the present study it was hypothesized that these Shodhana processes must have some qualitative and quantitative influence on the phytochemical profiles of toxic to mild toxic or poisonous herbs; thereby they alter the efficacy and safety of them. Keeping this hypothesis in view, the present study is planned to scientifically validate the Shodhana processes prescribed in Indian system of medicine for *Commiphora mukul* (Guggul) for its anti-inflammatory properties**.**

MATERIALS & METHODS

PLANT MATERIALS

The oleo-gum-resin of guggul was collected from Yucca enterprises, Mumbai, India and identified by its diagnostic characters. The authenticity of the plant product was confirmed by Prof K. Prabhu, Dept. of Pharmacognosy, SCS College of Pharmacy, Harapanahalli, Davanagere Dist., Karnataka. A voucher specimen (SCS/P.COG/13/04-05) has been placed in the herbarium of the Department of Pharmacognosy.

Purification of Guggul [1-4]

The Guggul subjected to Swedana (another type of Shodhana) process was performed by placing the crude guggul in the cotton cloth tied loosely and wrap the guggul to form a pouch. The guggul packed pouch was hung into an earthen vessel containing one of the different shodhana dravyas (liquids) or boiling fluids viz., cow's milk or triphala aqueous extract ensuring that the pouch was completely dipped into the extraction liquids till end of the process. The earthen vessel was gently heated to just boil the liquid then the heating was continued with occasional shaking of the pouch until all the soluble fraction dissolved into the fluids. The pouch was taken out after ensuring that all solubilized matter of the guggul has been digested in the fluid taken. The solubilized guggul was filtered and concentrated to a syrupy mass and it was poured in to shallow tray smeared with cow ghee and allowed to dry. The dried mass called 'suddha guggul' or 'purified guggul' was cut in to small rectangular pieces and stored in air tight glass container. The products obtained from the above processes are designated as below:

GUG1: Unprocessed guggul
GUG2: guggul processed with cow milk
GUG3: guggul processed with triphala Kashaya

PHYTOCHEMICAL ANALYSIS [27-31]
STANDARDIZATION

All the Guggul products were analyzed for extractive values, ash values, loss on drying and foreign organic matter.

PHYTOCHEMICAL ANALYSIS

The ethyl acetate extracts of Shodhana processed and unprocessed Guggul were analyzed for qualitative changes in phytochemicals.

ANALYSIS OF SHODHAN MATERIALS

The cow milk and thriphala aqueous extracts were analyzed for phytochemicals after shodhana.

TLC ANALYSIS

The Guggulsterone-E and Guggulsterone Z are therapeutically active compounds present in Guggul. Therefore, these two compounds were analyzed in all the shodhana processed and unprocessed products by TLC method.

Standard sample: 1% Guggulsterone-E in methanol, 1% Guggulsterone-Z in methanol.

Test sample:The processed and unprocessed Guggul products (2G) (i.e. GUG1, GUG2 and GUG3) were extracted with 25 ml of ethyl acetate by refluxing separately. The ethyl acetate extracts were concentrated and diluted with methanol.
Solvent system: Toluene-Ethylacetate(93:7)
Developing time: 30 minutes
Detection: Vanillin-sulphuric acid

ANTI-INFLAMMATORY STUDY [32-33]

Treatment protocol
Group –I: Control 0.5 ml of saline p.o, 0.1ml 1% carrageenan
Group –II: Diclofenac sodium 25mg/kg, p.o, 0.1ml 1% carrageenan
Group –III: GUG-1 250 mg/kg p.o, 0.1ml 1% carrageenan
Group –IV: GUG-2 250 mg/kg p.o, 0.1ml 1% carrageenan
Group –V: GUG-3 250mg/kg p.o, 0.1ml 1% carrageenan

The Albino rats of either sex were divided into 5 groups of 6 animals each and they were fasted for 12h and water *ad libitum*. Animals of group I were treated with 0.5 ml of saline p.o and groups-II, III, IV and V were treated with diclofenac sodium 25mg/kg p.o, GUG1, GUG2 and GUG3 250 mg/kg respectively. In all the groups of animals, acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline in right hind paw of the rats after 45 min of respective treatments. All the data were expressed as mean±S.E.M. Statistical significance was calculated using one-way ANOVA with Dunnett's test. P values <0.05 were considered statistically significant. The results were summarized in Table no.5 and Fig.2

Fig 1: Guggul-Plant & Oleo-gum resin

Table 1: Effect of Shodhana on Pharmacognostic standards of Guggul

S.no.	Method (G% w/w)	GUG 1	GUG 2	GUG 3
1.	Loss on drying	4.8±0.05	6.85±0.04	5.8±0.20
2.	Water soluble extractive	14.56±0.60	7.2±0.04	18.4±0.07
3.	Alcohol (70%) extractive	18.42±0.09	2.43±0.06	4.2±0.067
4.	Petroleum ether (60-80ºC) extractive	10.12±0.07	7.81±0.06	8.56±0.12
5.	Total ash	8.42±0.075	7.92±0.076	11.4±0.72
6.	Acid insoluble ash	4.91±0.025	4.12±0.057	4.69±0.061
7.	Water soluble ash	3.13±0.022	2.6±0.021	4.85±0.045
8.	Foreign organ matter	5.11±0.1	1.1±0.5	0.8±0.12

Table 2: Effect of Shodhana on Phytochemicals of Guggul

Phytochemical constituents	**GUG1**	**GUG2**	**GUG3**
Steroids	++++	+++	+++
Carbohydrates	+++	++	++
Flavanoids	+	+	+
Tannins	-	-	+++
Proteins	-	+++	-

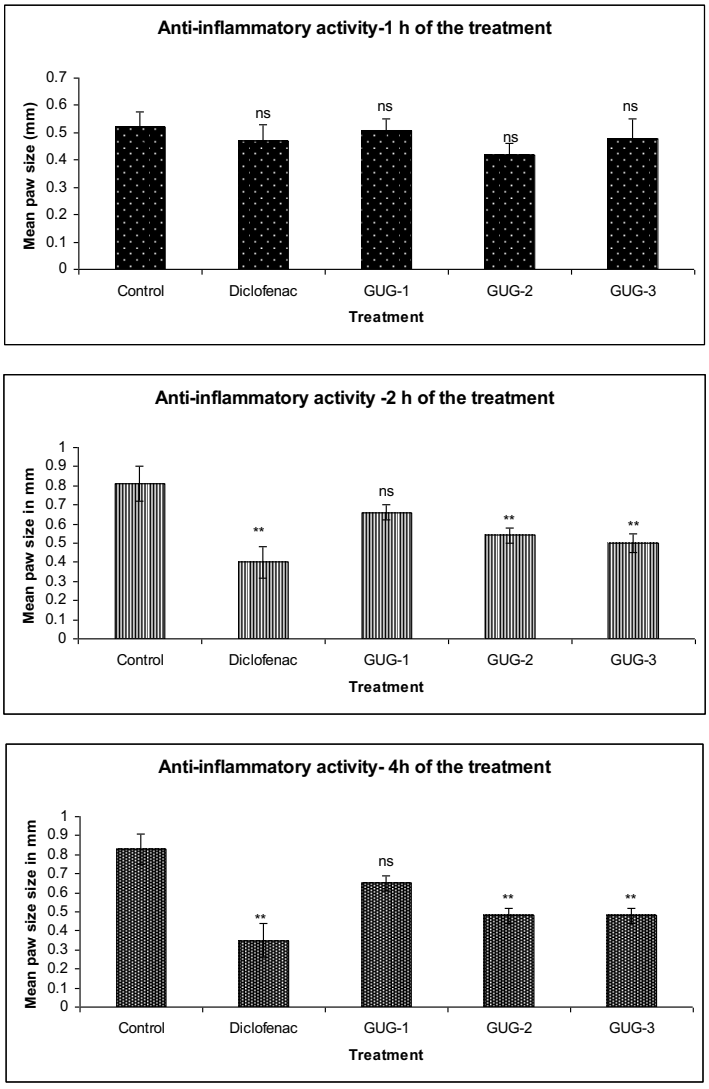
Table 3: Phytochemical analysis of Shodhana processed materials after Shodhana

Phytochemicals	**Cow milk**	**Triphala Aqueous Extract**
Steroids	+	+
Carbohydrates	+	+
Flavanoids	+	+
Tannins	-	+++

Table 4: TLC analysis of Guggul before and after Shodhana

S.no.	Samples	Guggulsterone E (Rf)	Guggulsterone Z (Rf)
1.	Guggulsterone E	0.38	-
2.	Guggulsterone Z	-	0.46
3.	GUG1	0.38	0.46
4.	GUG2	0.39	0.46
5.	GUG3	0.38	0.46

Fig:2 Effect of Shodhana on Anti-inflammatory activity of Guggul in carrageenan induced paw edema in Albino Rats



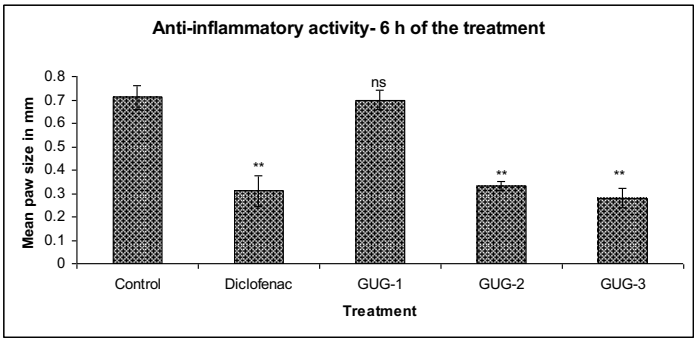


Table 5: Effect of Shodhana on Anti-inflammatory Activity of Guggul in Carrageenan induced paw edema in Albino Rats

Treatment	Dose (mg/kg)	Mean paw-size in mm				% Inhibition			
		1h	2h	4h	6h	1h	2h	4h	6h
Control	-	0.52±0.056	0.81±0.09	0.83±0.077	0.71±0.05	-	-	-	-
Diclofenac Sodium	25	0.47±0.06 ns	0.40±0.083**	0.35±0.090**	0.31±0.064**	5.0	50.98	57.98	55.86
GUG 1	250	0.51±0.04ns	0.66±0.04ns	0.65±0.039ns	0.70±0.04ns	1.9	18.38	21.96	1.1
GUG 2	250	0.42±0.04 ns	0.54±0.04**	0.48±0.04**	0.33±0.02**	19.2	37.7	42.37	53.5
GUG 3	250	0.48±0.07 ns	0.50±0.05**	0.48±0.04**	0.28±0.04**	3.8	33.7	42.37	60.47

Values are mean ± SEM for six rats
ns = non-significant, **P* <0.05, ***P*<0.01 V/s Control

The paw volume was measured by plythesmometer at 1, 2, 4 and 6h after carrageenan injection. All the data analyzed for the statistical significance. Mean increase/decrease in the paw volume was measured and the percentage reduction in paw inflammation was calculated with reference to negative control by using following formula.

% inhibition = Test – Control X100/Control

C = Average volume of edema in control animals
T = Average volume of edema in treated animals

RESULTS AND DISCUSSIONS

PHARMACOGNOSTIC ANALYSIS

It is evident from the results that, loss on drying after shodhana by both the processes was increased from 4.8 %G w/w ± 0.05 to 6.85 %G w/w ± 0.04 and 5.8 %G w/w ± 0.20 respectively. This increase in the loss on drying is understandable as the water content during Swedhana treatment with cow milk and Triphala kashaya might have been diffused in to the Guggul.

Water soluble extractive value was reduced from 14.56 %G w/w ± 0.60 to 7.2 %G w/w ± 0.04 after swedhana with cow milk and increased to 18.4 %G w/w ± 0.07 after processing with triphala kashaya. It may be inferred that some portion of the water-soluble substances might dissolved in cow milk and hence, water soluble extractive value after swedhana treatment with cow milk was reduced. Surprisingly the water soluble extractive values after swedhana with triphala kashaya was increased from 14.56 %G w/w ± 0.60 to 18.4 %G w/w ± 0.07. The exact reason for this could not be explained. Probably some of the water-soluble substances from the triphala kashaya might be added up into the Guggul during processing with triphala kashaya.

Alcohol soluble extractive value after both the methods of shodhana was drastically reduced from 18.42 %G w/w ± 0.09 to 2.43 %G w/w ± 0.06 and 4.2 %G w/w ± 0.067. The exact reason for such reduction in the values could not be explained. Similar results were obtained in case of petroleum ether extractive values.

The total Ash value, acid insoluble ash and water-soluble ash values were altered to a very lesser extent. Further there was a marked reduction in the foreign organic matter. All these parameters can be used for standardization of the guggul before and after Swedhana. The results were summarized in table 1.

PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical analysis showed the presence of steroids, carbohydrates, flavanoids, in the sample before swedhana. It was observed that proteins were present in the product after swedhana with cow milk in addition to other guggul constituents. Similarly, tannins were present in the product after shodhana with triphala kashaya along with other guggul constituents. The cow milk protein might be absorbed in to the guggul during the swedhana with cow milk. As a result of this, product after processing with cow milk showed the presence of proteins content more. It is reported that antioxidant polyphenolic compounds like galloyl glucose, gallotannins, ellagitannins and other an is present in *Terminalia chebula* and *Terminalia belerica* [34], of triphala Ayurvedic medicine. The galloyl glucose from these plants is added into the GUG-3 during shodhana with triphala kashaya. The results were summarized in table 2.

Phytochemical analysis of shodhana processed materials

It was observed from the phytochemical analysis of Cow milk after Swedhana with Guggul showed the presence of steroids, carbohydrates and flavanoids. It is due to the diffusion of some of phytochemicals from guggul to processing maters. The results were summarized in table 3.

TLC Analysis

All the three products GUG-1, GUG-2 and GUG-3 were subjected to TLC study to identify chemical markers. Two distinct spots were observed in all the three samples at Rf values 0.38 and 0.46 in toluene: ethyl acetate (7:3). These two spots were

identified as guggulsterone-E and guggulsterone-Z. The intensity of guggulsterone-Z spot was more than the guggulsterone E in GUG2. The results were summarized in table 4.

ANTI-INFLAMMATORY ACTIVITY

The Guggul is the most popularly used anti-inflammatory and anti-arthritis drug in the Indian system of medicine. However, it is used only after subjecting it to simple shodhana processes to enhance the absorption and reduce the mild side effects. In our study, the Shodhana processing methods by applying Swedhana processing on Guggul with cow milk and triphala extract is applied. Therefore in the present study an attempt is made to analyse the influence of both the methods of Swedhana on the anti-inflammatory activity of it. All the three products of Guggul were tested for anti-inflammatory property against acute inflammation model i.e. carrageenan induced rat hind paw edema. The GUG2 and GUG3 not showed anti-inflammatory activity at 1sth but showed significant anti-inflammatory activity at 2nd, 4th and 6thh after carrageenan challenge. The Diclofenac sodium (25 mg/kg) was used as standard in the present study. The shodhana processed samples showed significant anti-inflammatory activity which is comparable to that of standard drug treatment. The GUG3 showed maximum anti-inflammatory activity (60.47% inhibition) at 6th h. The results were summarized in table 5 and fig.2.

Since both isomers of guggulsterones are known to possess potent anti-inflammatory property and even the antioxidant principles and anti-inflammatory compounds of triphala Kashaya that are added into GUG-3 may also contribute to the enhanced anti-inflammatory property of it [35].

CONCLUSION

It is reported in several experiments that Shodana process can purify and can overcome the side effects of Crude Guggul. The Shodhana processes Swedhana methods, enhanced the softness of Guggul, reduced brittleness and altered the physico-chemical profile. In our study it showed the changes in physico-chemical properties and reflect in pharmacognostic standards and phytochemicals profile of Guggul before and after Shodhna. These standards are utilized in the standardization of the plant materials before and after shodhna processes in Indian system of medicine. It is reported that anti-inflammatory phytochemicals of triphala was included in triphala kashaya processed Guggul [20]. There is an addition of some unidentified phytochemicals and or shodhana processing materials in processed Guggul products (GUG2 & GUG3) and may be responsible for the enhanced therapeutic efficacy. The both processed Guggul has significant anti-inflammatory effect on experimentally induced inflammation in rats. The mechanism of anti-inflammatory action is relevant to oxygen free radical scavenging and inhibition of the formation of inflammatory cytokines [36]. The anti-inflammatory activity of both the processed Guggul may be due to oxygen free radical scavenging and inhibition of the formation of inflammatory cytokines in the rats.

Ancient method of shodhana possesses significantly detoxified and potentiated the Guggul. Since significant activity is seen with both Shodhana processed Guggul, it may be suggested that any one of ancient methods of shodhana processes may be adopted when it is intended to be used as anti-inflammatory agent. It is worthwhile to adopt Shodhana processes as per Ayurveda in the development of herbal formulations with applications of modern technology to assess its safety and efficacy.

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