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ACUTE AND SUBACUTE TOXICITY STUDIES ON SHODHANA PROCESSED GUGGUL

Poonam Taru, Mukta Abhyankar*, Vaishali Undale and Ashok Bhosale

Seth Govind Raghunath College of Pharmacy, Saswad, Pune, Mahrashtra, India

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Correspondence to Author:

Mukta Abhyankar

Assistant Professor & H.O.D, Department of Pharmacognosy, Seth Govind Raghunath College of Pharmacy, Saswad, Pune, India

E-mail: abhyankarmukta@rediffmail.com

ABSTRACT

Ayurveda– The science of life is known to the mankind since time immemorial. It prolongs life span, maintains positive health and cures diseases. Due to its high effectiveness, popularity and the socio-economic status it was recognized as an independent branch of learning and has been known as “Rasa Shastra” and “Rasa chikitsa” in the field of Ayurveda. After the development of rasa Shastra it was made possible for the minerals and metals, precious and semiprecious stones to pass through various pharmaceutical processes like shodhana, Marana etc. for several times so as to convert these in to a form or compound which may suit to the human body and could be observed and assimilated easily into the system without exhibiting any toxic symptom. There are two objectives to purify a natural herb. There are two objectives of purification. First is to remove the external and internal impurities. The second reason is to increase the medicinal value. The shodhana processes of guggul can be done by general purification and specific purification. Present work involves study of hematological, biochemical, Histopathological changes in mice treated with Guggul before and after shodhana process.

INTRODUCTION:

Concept of Shodhana Process (Purification): Shodhana technique is in existence since samhita period. In charaka samhita it has been indicated by “Shuddha” and “Shaucha” words. Shodhana is an important technique necessary for almost all kinds of drugs to remove their Doshas (impurities or toxic contents). *Shodhana* is a process of purification and detoxification by which physical and chemical blemishes and toxic materials are eliminated and substances are subjected for further processing [1, 2].

Different Liquid Media Prescribed For Shodhana: As the different techniques are described for the Shodhana of guggulu, Likewise various liquid media are also prescribed for the purification of guggulu. They are as follows;

Guduchi Kwath, Triphala Kwath, Milk (Godugdha), Pancha Tikta Kwath, Dash Moola Kwath, Nimba Patra Kwatha with Haridra Churna, Cow Urine (Gomutra), Nirgundi Patra.

Types of Shodhana: Shodhana technique has been broadly subdivided into two major types as following;

1. Samanya shodhana (General purification).
2. Vishesha shodhana (Specific purification)

The Need For Purificationl of Guggul: There are two objectives to purify a natural herb. First is to remove

the external and internal impurities. The second reason is to increase the medicinal value.

As Guggul is unorganized drug, external impurities are in the form of dust, dry leaves and other foreign materials present in Guggul. After purification, the herb becomes safer and more effective for use. Sometimes additional medicinal values are also instilled in the formulation [2, 3, 4].

MATERIALS AND METHODS: Guggul, an oleo gum-resin, is a plant exudates of Family Burseraceae. In India it is mainly produced by four species. They are namely *Commiphora mukul* Engl, *Commiphora wightii* (Arnott.) Bhand, *Commiphora berry, Commiphora agallocha* etc.These species can be differentiated from each other by the following points; namely stem, color of flower, calyx, corolla, fruits etc. Of the above mentioned species, *Commiphora wightii* (Arnott.) was selected for the further process.

Collection And Authentication of Plant Material: Guggul is collected from local market and authenticated from Agharkar research institute pune.

Animal Stock: Swiss albino mice aged 5-6 weeks, weighing about 24-28gm, were used in the present study. The animals are maintained under controlled temperature, humidity and automated light cycles (12 h light, 12 h dark).

Toxicity Studies were carried out by following Methods:

Acute Toxicity Studies [5]: A total of 30 mice were randomly allotted to one control and 4 treated groups. The different shodhit guggul in each case was administered orally in three doses, namely 1.0, 2.0 and 3g/kg. The animals were observed for 24 h for signs of toxicity and mortality.

Subacute Toxicity [6]: Five mice were randomly selected and marked for individual identification. Animals were fasted 24hrs prior to dosing. In the present study, the doses of crude as well as purified guggul 300 mg/kg/day were selected, The drugs were suspended in distilled water by adding suspending agent Gum Acacia and orally given to each group of mice daily for 14 days, while the control group received the water vehicle.

Toxic manifestations such as signs of toxicity, mortality and the body weight changes were monitored daily. At the end of the study, all animals were fasted for 16-18 h and then anesthetized on day 15th. Blood samples for hematological and biochemical analysis was taken from common carotid artery. All mice were sacrificed after the blood collection. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

Estimation of Hematological & Biochemical Parameters: After 14th Days of drug administration the blood were collected from mice by retro-orbital method, and serum was separated by centrifugation at 3500 rpm below 30^{o}C for 20 mins. The collected serum was used for estimation of SGOT, SGPT, serum creatinine, Total protein etc.

Estimation of Histopathological Parameters: After 14th Days of drug administration, on 15th day all animals were fasted for 16-18 h and then anesthetized by using anesthetic ether. All mice were sacrificed after the blood collection. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

RESULT AND DISCUSSION:

Haematological Parameters: Sub-acute toxicity study of crude and purified guggul were studied in mice. Haematological evaluation of blood plasma is as follows:

TABLE 1: HAEMATOLOGICAL STUDIES ON MICE AFTER SUBACUTE TREATMENT WITH CRUDE AND PURIFIED GUGGUL

Sr No.	Parameters	Control	Crude	D/W	TSG	GSG
1.	Hemoglobin	10.2	5.1	6.9	7.2	7.8
2.	RBC	4.8	3.90	4.10	4.30	4.49
3.	WBC	5,500	4,300	6,800	7,800	8,200

From the above results it is concluded that the Haemoglobin count, Red blood cell count(RBC), white blood cell count in the purified guggul is increased as compare to crude guggul.

Biochemical Parameters: Sub-acute toxicity study of crude and purified guggul were studied in mice. Biochemical evaluation of blood serum is as follows:

TABLE. 2 : EFFECT OF CRUDE AND PURIFIED GUGGUL ON BIOCHEMICAL PARAMETERS

Sr No.	Parameters	Control	Crude	D/W	TSG	GSG
1.	Total Protein(gm/dl)	5.8	5.24	6.49	6.90	6.88
2.	Total Bilirubin(mg/dl)	0.91	1.2	0.9	0.5	0.7
3.	Direct Bilirubin(mg/dl)	0.15	0.32	0.3	0.1	0.3
4.	Indirect Bilirubin(mg/dl)	0.34	1	0.6	0.4	0.4
5.	SGPT(U/L)	17	66.9	20.4	13.7	31.1
6.	SGOT(U/L)	30	71.3	25.2	20.4	26.2
7.	Serum creatinine(mg/dl)	0.9	0.4	0.3	0.4	0.5

a) **Total Protein**: Total Protein count in the purified guggul is increased as compare to crude guggul.

b) **Bilirubin**: Total Bilirubin, Direct Bilirubin and Indirect Bilirubin values in the purified guggul were decreased as compare to crude guggul. Increase in this values may leads to liver disease.

c) **SGPT (Serum Glutamate Pyruvate Transaminase) and SGOT (Serum Glutamate Oxaloacetate Transaminase)**: SGPT and SGOT values in the purified guggul were decreased as compare to crude guggul. Increase in this values indicate signs of liver diseases.

d) **Serum creatinine**: Serum creatinine value in the purified guggul, no significant change could be observed as compare to purified guggul.

Histopathological Parameters:

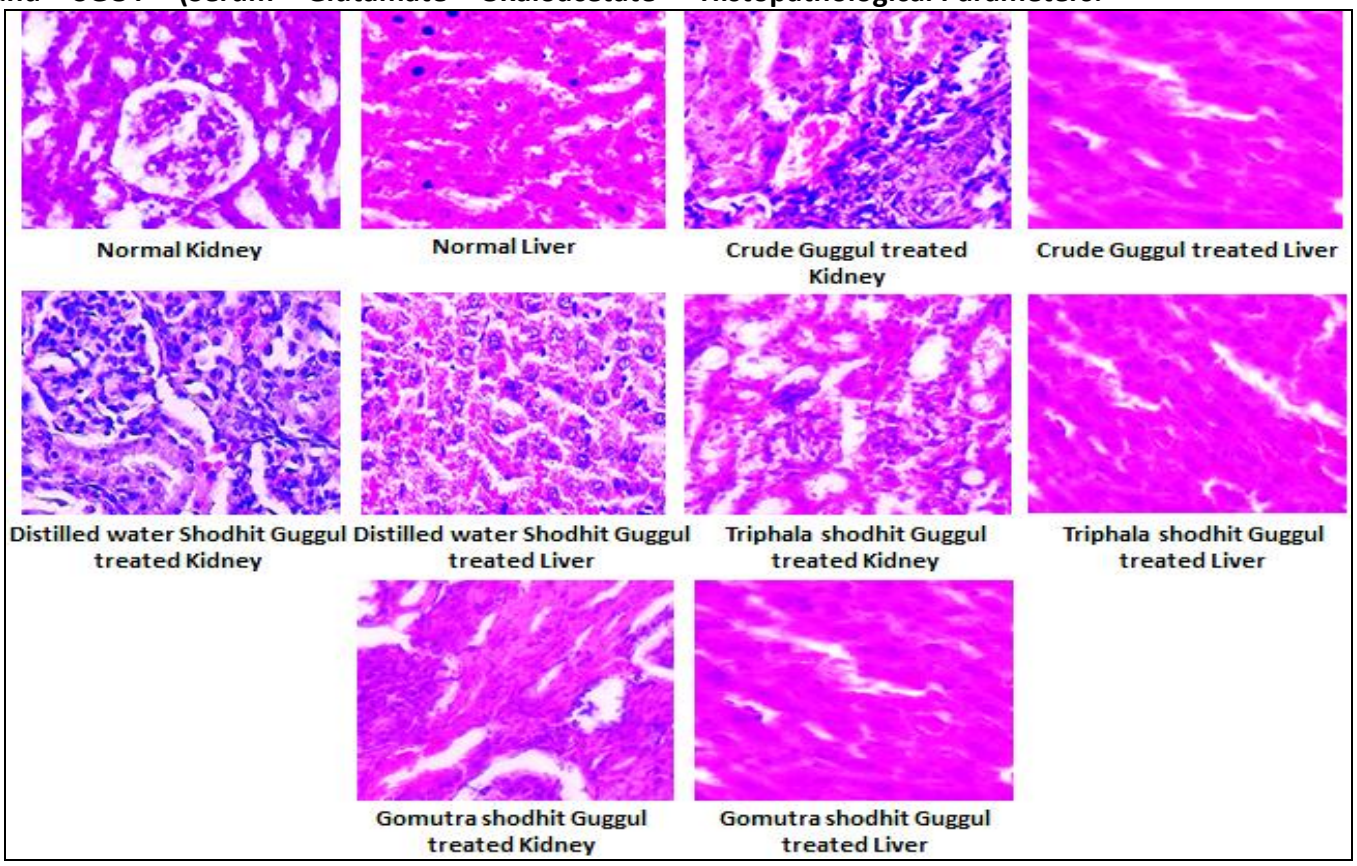

FIGURE 1: HISTOPATHOLOGICAL EVALUATION

Photomicrograph of mice Liver and Kidney showing normal histological appearance with no evidence of necrosis. (H & E 400 X). In the present study histopathological and biochemical parameter analysis did not reveal any marked liver injury. From toxicity assessment point of view the three changes can be considered as important that is elevation in blood urea, blood sugar and blood protein. In TSG treated group all these three changes were observed in addition moderate elevation in alkaline phosphatase activity was observed. In contrast to this in GSG administered group only two parameters i.e. increase in serum protein and blood urea level was observed. On the basis of this summing up it can be suggested that analysis of biochemical parameters indicate GSG to be less toxic in comparison to TSG.

CONCLUSION: Guggul was subjected to purification process by using various purification media Distilled water, Triphala Kwath decoccation and Gomutra. Shodhana process is used to remove the external and internal impurities and to increase the medicinal value. It was subjected to various studies like Hematological and Histopathological. By toxicity study, it was concluded that after Shodhana process the toxicity of purified guggul was reduced as compare to crude guggul. Thus, the purpose of Shodhana process is practically proved. No toxicity was observed in purified guggul as compare to crude guggul which shows some symptoms of toxicity.

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