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Comparative Evaluation of Anti-Inflammatory Activity of Shodhana Processed Guggul

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***Abstract*---**Shodhana is a technique that has been around since the Samhita period. Shodhana is an Ayurvedic purification treatment that involves soaking, rubbing, and washing hazardous medicinal herbs (upavishadravyas) with specialised media such as gomutra (cow's urine), Godugdha (cow's milk), Guduchi Kwath, Triphala Kwath, Pancha Tikta Kwath, Dash Moola Kwath, Nimba Patra Kwatha with Haridra Churna, Nirgundi Patra and other pharmaceutical procedures to remove the doshas. Shodhana is a significant approach for removing Doshas from practically all types of medications (impurities or toxic contents) and substances are then processed further. In the present study guggul is used and guggulu shodhana is done by using different liquid media such as distilled water, Gomutra (cow urine), Triphala Kwath. The present study aimed to determine the anti-inflammatory activity of shodhit guggul by using carrageenan-induced

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paw edema and Freund's adjuvant-induced arthritis in in Wistar Albino rats. By the pharmacological study it is concluded that the Triphala shodhit Guggul showed enhanced anti-inflammatory activity as compared with Distilled water and Gomutra shodhit Guggul. Thus, the purpose of Shodhana process of Guggul is proved experimentally showing enhancement of pharmacological activity.

***Keywords*---**Shodhana, guggul, Carrageenan, Freund's Adjuvant, Triphala kwath.

Introduction

Ayurveda - The knowledge of life has been known to mankind from the beginning of time. It increases life expectancy, promotes good health, and cures ailments. Different sorts of medications, discovered in nature from natural resources, whether herbal, animal, or mineral, have been used to attain these goals and objectives. (Dr. Chaube anjana et al,1994) Shodhana is an Ayurvedic purification treatment that involves soaking, rubbing, and washing hazardous medicinal herbs (upavishadravyas) with specialised media such as gomutra (cow's urine), Godugdha (cow's milk), Guduchi Kwath, Triphala Kwath, Pancha Tikta Kwath, Dash Moola Kwath, Nimba Patra Kwatha with Haridra Churna, Nirgundi Patra and other pharmaceutical procedures to remove the doshas. (Bhava prakash Nighantu 2008). Poisonous plants are subjected to shodhana sanskara (purification process), before their therapeutic use. This process use to remove doshas and to reduce toxicity of poisonous plant considerably and keeps it at required optimum level. (Ayurvedic formulary of India 2003)

Types of shodhana

Shodhana technique has been broadly classified into Samanya shodhana and Vishesha Shodhana. Samanya shodhana also known as General/Common purification and Vishesha shodhana known as Specific purification. (Charaka Samhita 1984)

1. Samanya shodhana (General/Common purification).

This medthod is applicable for particular group of drugs. (Venkateshwarlu G., et. al 2010)

Method 1: Cut the drug into small pieces. Then Soak in media (Gumutra, Cow Milk, Kwatha etc.) for 3 days. Change the media every day. On 4th day wash it with warm water, dry under sun & store.

Method 2: The drug should be tied in a pottali and boiled (Swedana) with media in Dolayantra for 5 prahara (15hrs). Milk must be above the level of Pottali and maintain the level of media throughout the Swedana process with repeated addition of Media.

2. Vishesha shodhana (Specific purification)

Specific Purification of Guggula increases the medicinal properties and helps in modifying the pharmacological actions. (Ilanchezhian R., et. al. 2011)

Need For Purificationl of Guggul

Purification of a natural herb has two goals. The first step is to get rid of any external or internal contaminants (Taru et.al. 2013). The second reason is to boost the therapeutic value of the product. As Guggul is unorganized drug dust, dry leaves, and other foreign components found in Guggul are examples of external contaminants (Warrier P. K et.al., 1995). Purification makes the herb safer and more effective to use. In some cases, extra therapeutic properties are included in the formulation. (Chaudhary A., et al., (2010)

Material and Methods

Collection and Authentication of Plant Material:
Guggul *(Commiphora mukul*) is procured from a local market and authenticated from Agharkar Research Institute in Pune.
Pharmacological Evaluation:
Experimental Animals:
Wistar albino rats of either sex, weighing 150-250 gms, were used in this study. The experimental protocol was approved by the Institutional Animal Ethical Committee. Animals were kept in standard conditions in an animal house approved by the Committee for the purpose of controlling and supervising Animal Experiments (CPCSEA). (Asad et.al. 2008) Albino rats were housed in Polypropylene cages and kept at 24°C ± 2°C with a 12 h light/dark cycle. They were fed ad libitum with a standard pallet diet and had free access to water. (Arora., et al., (1971)

Anti-Inflammatory activity was carried out by Following Models
Carrageenan induced paw edema:
One of the most common techniques for screening anti-inflammatory drugs is based on the ability of such agents to inhibit the edoema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) are used, including formaldehyde, carrageenan, and others. The impact can be measured in a variety of ways. The hind limb can be dissected and weighed at the talocrural joint. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared to the controls. Many methods for measuring paw volume have been described, including simple and less accurate methods as well as more sophisticated electronically devised methods. The assessment's value is determined less by the apparatus and much more by the irritant used. Some irritants cause only short-term inflammations, whereas others cause paw edoema to last for more than 24 hours. (Verma S et. al 2010)

Experimental Design:

Table. 1
Grouping of animal for Carrageenan induced paw edema

Group	Traetment and Dose/Day
I Control	Control (Distilled water, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1%(w/v)
II Diclofenac	Diclofenac Soduim (10mg/kg, p.o.) + 0.5ml subcutaneous injection of

Soduim	carrageenan; 1%(w/v)
III Crude 100	Crude(100mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1%(w/v)
IV Crude 150	Crude (150mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1%(w/v)
V WSG 100	WSG (100mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1%(w/v)
VI WSG 150	WSG (150mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1%(w/v)
VII TSG 100	TSG (100mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1% (w/v)
VIII TSG 150	TSG (150mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1% (w/v)
IX GSG 100	GSG (100mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1% (w/v)
X GSG 150	GSG (150mg/kg, p.o.) + 0.5ml subcutaneous injection of carrageenan; 1% (w/v)

(WSG- Water Shodhit Guggul, TSG- Triphala shodhit Guggul, GSG- Gomutra Shodhit Guggul)

Preparation of drug solution

Preparation of standard drug solution:
25mg tablet of Diclofenac Soduim was added in 10ml distilled water, and added 1% Gum acacia. Shake the resulting mixture to form a uniform suspension.
Preparation of test drug solution:
Add appropriate quantity of drug as per body weight into Water, and added 1% Gum acacia as suspending agent. Shake the resulting mixture to form a uniform suspension.
Preparation of 1% Carrageenan solution:
To 10ml of normal saline (0.9% w/v) add slowly 0.1 gm of accurately weighed Carrageenan. Stir slowly and continuously the resulting solution using a magnetic bead until gets a uniform solution.
Preparation of wetting solution: To 2-3ml of wetting compound add 1liter of distilled water. To it add 0.5gm of NaCl and stir well and slowly. The wetting solution must be freshly prepared.

Procedure

Paw edoema was induced in the hind paws of mice in this model by subcutaneous injection of 50 microliters of carrageenan, 1 percent (w/v). The contralateral paw served as a control, receiving the same volume of vehicle injection (PBS). The progression of the edoema was tracked by measuring the thickness of footpad swelling with a Plethysmometer at 0, 1, 2, 3, and 6 hours after carrageenan injection. 30 minutes before carrageenan administration, animals were given crude guggul, water treated guggul, Triphala treated guggul, and Gomutra treated guggul (100mg/kg, 150mg/kg), or water (control animals). As a positive control, Diclofenac Soduim (10mg/kg) was used.

Evaluation

The increase in paw volume after 3 or 6 hours is calculated as a percentage of the volume measured immediately after the irritant injection for each animal. Animals that have been effectively treated have much less edoema. For each time interval, the difference in average values between treated animals and control groups is calculated and statistically evaluated. The difference at different time intervals provides some insight into the duration of the anti-inflammatory effect. For active drugs, a dose-response curve is generated, and ED50 values are calculated. (vogel 2002)

Freund's Adjuvant Induced Paw Edema:

Arthritis was induced in the left hind paw by injecting a 0.1 ml (0.1% w/v) suspension of killed Mycobacterium tuberculosis bacteria homogenized in liquid paraffin. Drug treatment began on the first day, i.e. the day of adjuvant injection (0 day), 30 minutes before adjuvant injection, and was continued until the 21st day. The volume of the paws was measured using a plethismometer on the fourth, eighth, fourteenth, and twenty-first days (Ugo Basile, India). On each day, the mean changes in injected paw edoema with respect to initial paw volume were calculated, and the percent inhibition of paw edoema with respect to the untreated group was calculated using the formula below. The changes in body weight were tracked on a daily basis.

Measurement of paw edema:

Before the experiment, plethysmometry (model 7140 plethysmometer; Ugo Basile, Comerio, Italy) was used to measure paw volume, and plethysmometer (dynamic plantar esthesiometer 37400; Ugo Basile) was used to measure mechanical touch sensitivity of the paws at 4, 8, 14, and 21 days after FA administration. The plethysmometer is made up of two vertically connected water-filled Perspex cells, the larger of which is used to measure the volume displacement caused by dipping the rat paw in. The water level in the smaller tube, which houses the transducer, is proportional to the volume of water into which the paw is dipped. The transducer measures the conductance between 2 vertical Pt-Ir wire electrodes and compensates for changes in conductivity brought about by salinity and temperature alterations and operates the digital screen, which shows the exact volume of the paw in cm.

Experimental Design:

Table 2
Grouping of animal for Freund 's adjuvant induced paw edema:

Group	Treatment and Dose/Day
I Control	Control (Distilled water, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1%(w/v)
II Diclofenac Soduim	Diclofenac Soduim (10mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1%(w/v)

III Crude 100	Crude(100mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1%(w/v)
IV Crude 150	Crude (150mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1%(w/v)
V WSG 100	WSG (100mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1%(w/v)
VI WSG 150	WSG (150mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1%(w/v)
VII TSG 100	TSG (100mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1% (w/v)
VIII TSG 150	TSG (150mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1% (w/v)
IX GSG 100	GSG (100mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1% (w/v)
X GSG 150	GSG (150mg/kg, p.o.) + 0.1ml subcutaneous injection of freund's adjuvant; 1% (w/v)

Preparation of drug solution:

Preparation of standard drug solution:

25mg tablet of Diclofenac Sodium was added in 10ml distilled water, and added 1% Gum acacia. Shake the resulting mixture to form a uniform suspension.

Preparation of test drug solution:

Add appropriate quantity of drug as per body weight into Water, and added 1% Gum acacia as suspending agent. Shake the resulting mixture to form a uniform suspension.

Preparation of wetting solution:

To 2-3ml of wetting compound add 1liter of distilled water. To it add 0.5gm of NaCl and stir well and slowly. The wetting solution must be freshly prepared.

Procedure:

In this model, 0.1 ml of lambda freund's adjuvant 1 % (w/v) was subcutaneously injected into the hind paw of mice to induce edoema. The contralateral paw served as a control, receiving the same volume of vehicle injection (PBS). The course of the edema was monitored by measuring the thickness of footpad swelling after 0, 4, 8, 14, and 21 days after freund's adjuvant injection by using a Plethysmometer. Animals received crude guggul, water treated guggul, Triphala treated guggul and Gomutra treated guggul (100mg/kg, 150mg/kg) or water (control animals). Diclofenac Soduim (10mg/kg) was used as positive control. (Vogel 2002).

Result and Discussion

This study deals with pharmacological findings of crude guggul and purified guggul.

a) Anti-Inflammatory Activity:

Carrageenan was injected in hind paw of rat to produce paw edema.

Table. 3
Carrageenan induced paw edema

Groups	Dose mg/kg	Change in paw volume (Mean ± SEM)				
		0hr	1st hr	2nd hr	3rd hrs	6th hrs
Carrageenan	100	0.97 ±0.04	2.32 ±0.041	2.38 ±0.017	2.48 ±0.025	2.54 ±0.030
Diclofenac Sodium	5	0.9 ±0.018	1.12 ±0.046	1.36 ±0.024	1.48 ±0.039	1.54 ±0.022
Crude	100	0.98 ±0.038	1.35 ±0.026	1.56 ±0.028	2.11 ±0.037	2.26 ±0.036
Crude	150	0.94 ±0.022	1.45 ±0.028	1.75 ±0.024	2.3 ±0.018	2.42 ±0.034
DSG	100	0.95 ±0.024	1.42 ±0.040	1.69 ±0.034	2.1 ±0.053	2.25 ±0.035
DSG	150	0.96 ±0.015	1.47 ±0.031	1.75 ±0.021	1.86 ±0.038	2 ±0.041
TSG	100	1.01 ±0.02	1.29 ±0.026	1.56 ±0.05	1.5 ±0.037	1.47 ±0.021
TSG	150	0.95 ±0.027	1.5 ±0.039	1.31 ±0.040	1.21 ±0.048	1.08 ±0.025
GSG	100	0.96 ±0.021	1.59 ±0.034	1.49 ±0.024	1.42 ±0.041	1.41 ±0.037
GSG	150	1 ±0.018	1.23 ±0.023	1.12 ±0.039	1.04 ±0.03	1.02 ±0.041

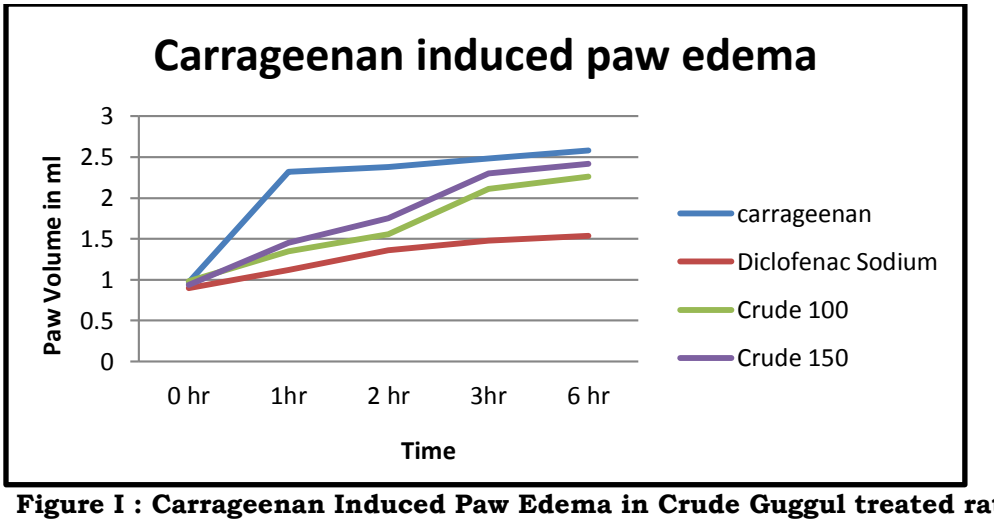

Figure I : Carrageenan Induced Paw Edema in Crude Guggul treated rat

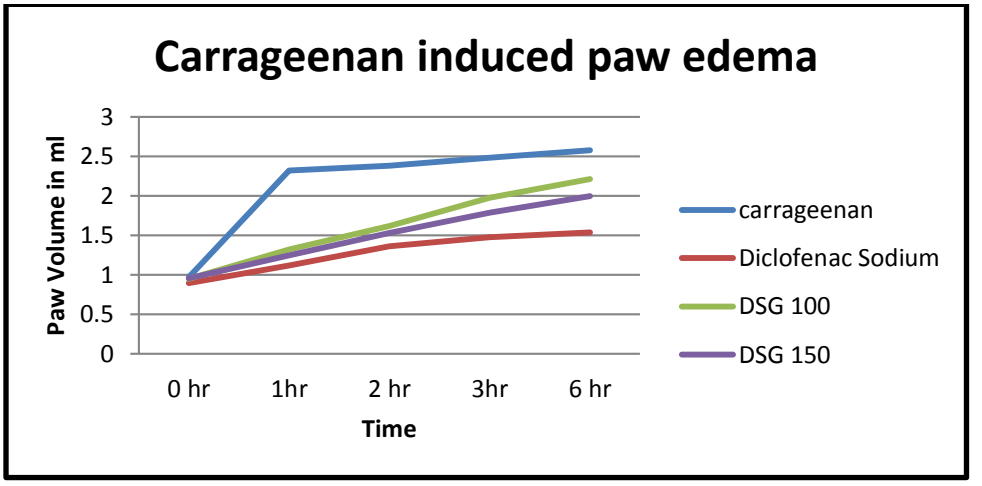

Figure II : Carrageenan Induced Paw Edema in Distilled water Shodhit Guggul treated rat

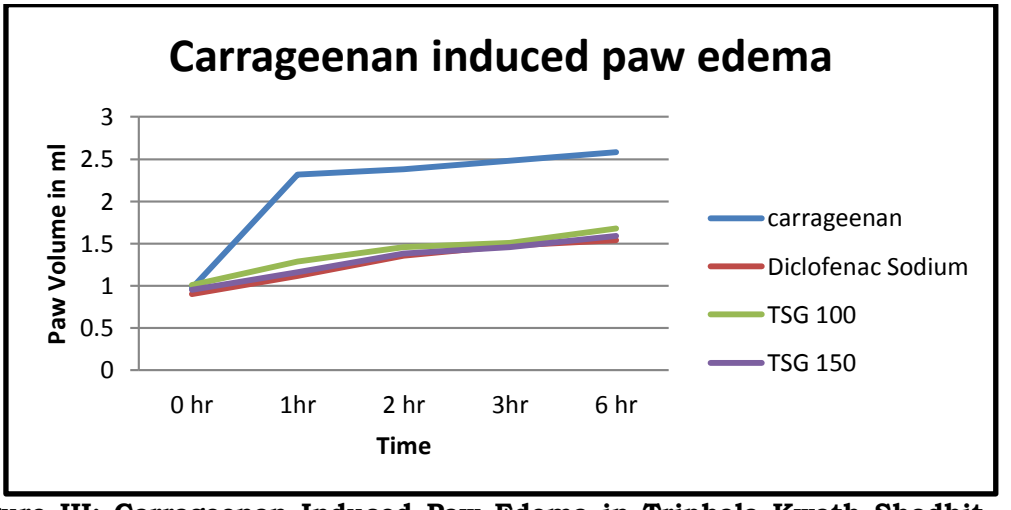

Figure III: Carrageenan Induced Paw Edema in Triphala Kwath Shodhit Guggul treated rat

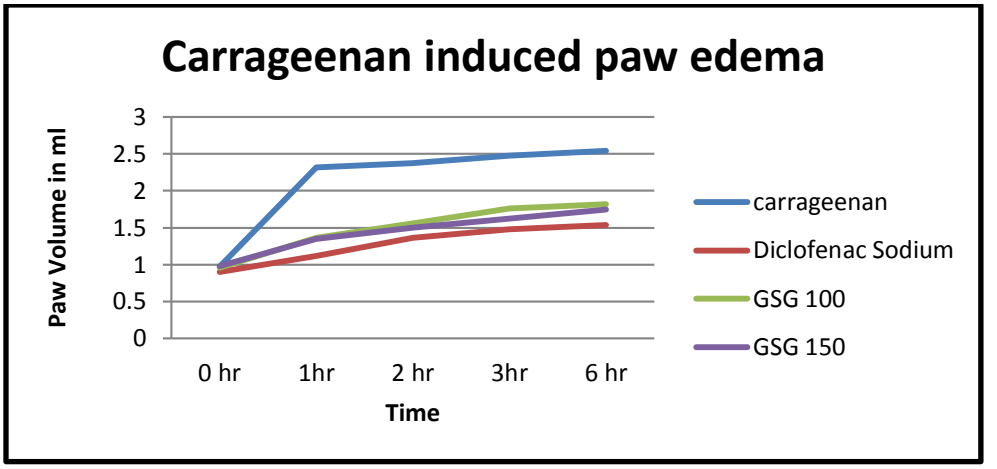

Figure IV: Carrageenan Induced Paw Edema in Gomutra Shodhit Guggul treated rat

Time-dependent increases in paw volume were observed in the vehicle-treated groups at 1/2, 1, 2, 3, and 6 hr after administration of carrageenan injection. Carrageenan-induced inflammation was significantly reduced in all purified samples, and Diclofenac at a dose of 10mg/kg inhibited paw edoema significantly. Among the purified guggul Triphala shodhit guggul (TSG) shows significant inhibition of paw edema as compare to distilled water shodhit guggul and Gomutra shodhit guggul.

b) freund's adjuvant induced paw edema in rat:

Table 4
Freund's Adjuvant Induced Paw Edema

Groups	**Dose mg/kg**	**Daily change in paw volume (Mean ± SEM)**				
		Day 0	**4th day**	**8th day**	**14th day**	**21st day**
Control	Vehicle	0.95 ±0.037	2.32 ±0.041	2.38 ±0.017	2.48 ±0.025	2.54 ±0.030
Diclofenac Sodium	5	0.89 ±0.018	1.12 ±0.046	1.36 ±0.024	1.48 ±0.039	1.54 ±0.022
Crude	100	0.98 ±0.038	1.35 ±0.026	1.56 ±0.028	2.11 ±0.037	2.3 ±0.036
Crude	150	0.94 ±0.022	2.3 ±0.028	1.75 ±0.024	2.3 ±0.018	2.42 ±0.034

DSG	100	0.95 ±0.024	1.42 ±0.040	1.69 ±0.034	2.1 ±0.053	2.27 ±0.035
DSG	150	0.96 ±0.015	1.47 ±0.031	1.75 ±0.021	1.86 ±0.038	2 ±0.041
TSG	100	1.01 ±0.02	1.32 ±0.026	1.48 ±0.05	1.51 ±0.037	1.68 ±0.021
TSG	150	0.95 ±0.027	1.16 ±0.039	1.38 ±0.040	1.46 ±0.048	1.59 ±0.025
GSG	100	0.96 ±0.021	1.38 ±0.034	1.56 ±0.024	1.76 ±0.041	1.82 ±0.037
GSG	150	0.98 ±0.018	1.55 ±0.023	1.5 ±0.039	1.62 ±0.03	1.75 ±0.041

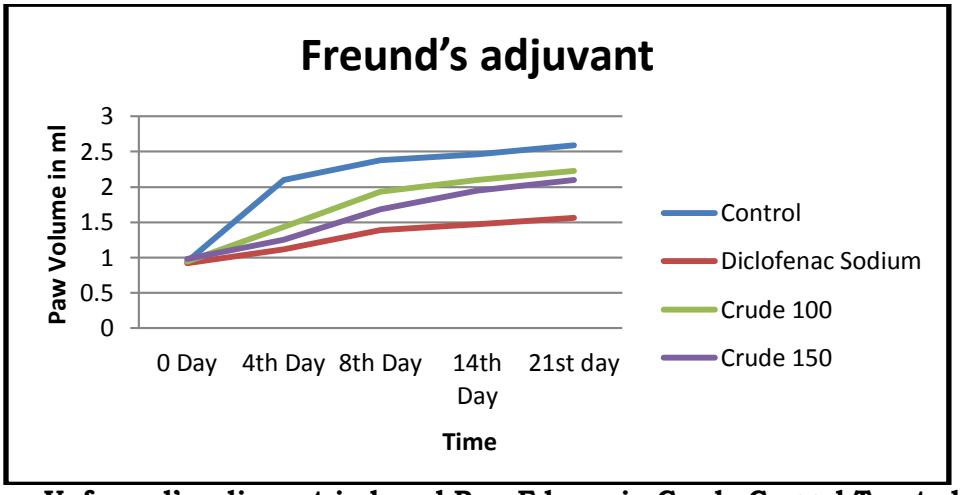

Figure V: freund's adjuvant induced Paw Edema in Crude Guggul Treated rat

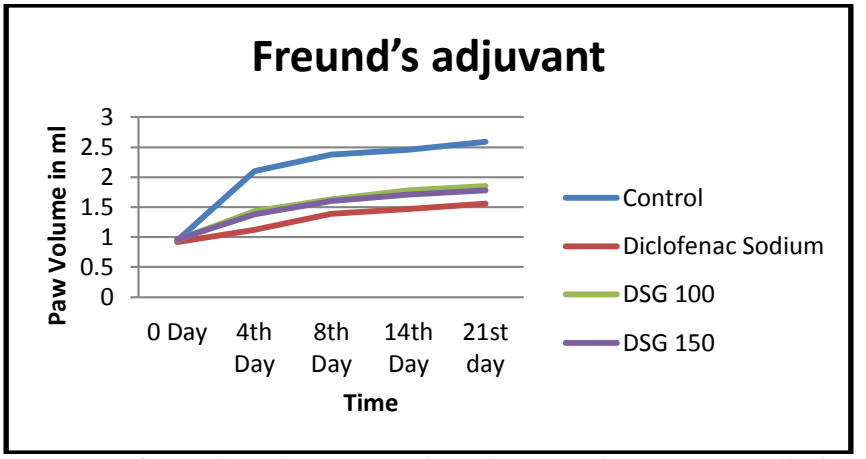

Figure VI: freund's adjuvant induced Paw Edema in Distilled Water shodhit Guggul treated rat

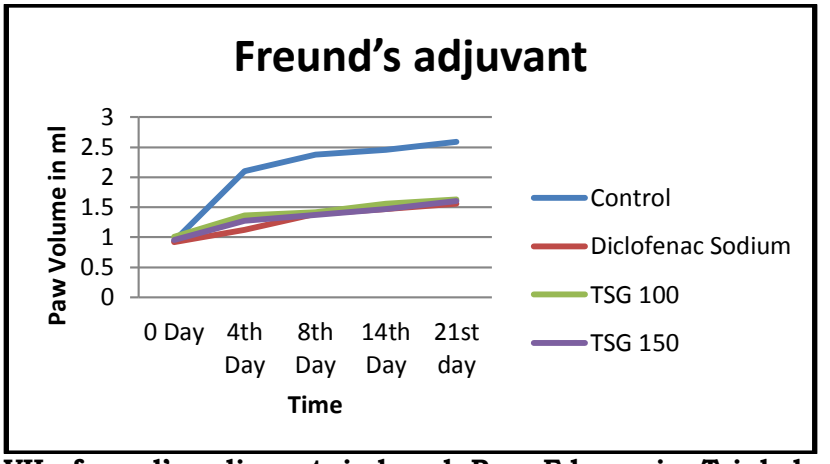

Figure VII: freund's adjuvant induced Paw Edema in Triphala Kwath Shodhit Guggul treated rat

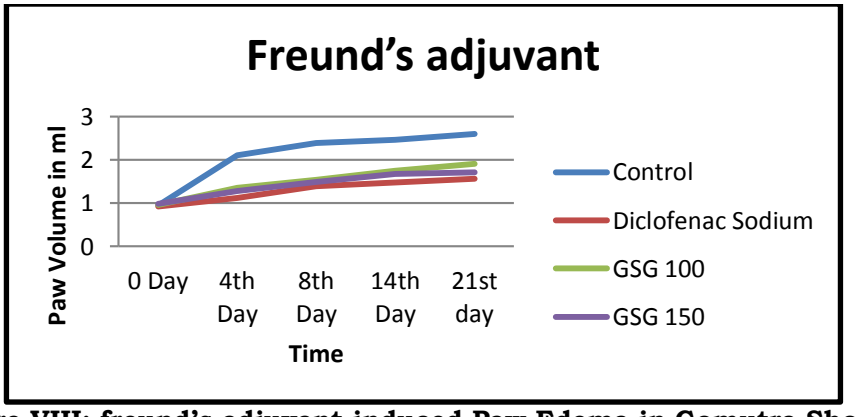

Figure VIII: freund's adjuvant induced Paw Edema in Gomutra Shodhit Guggul treated rat

Time dependent increased in paw volume was observed at 0, 4, 8, 14, 21 days after administration of freund's adjuvant injection in vehicle treated groups were increased. freund's adjuvant induced inflammation was significantly reduced in all the purified samples, and Diclofenac at the dose of 10mg/kg caused significant inhibition of paw edema. Among the purified guggul Triphala shodhit guggul (TSG) shows significant inhibition of paw edema as compare to distilled water shodhit guggul and Gomutra shodhit guggul.

Conclusion

Guggul was purified using various purification media such as distilled water, Triphala Kwath decoccation, and Gomutra. The study of Shodhana process revealed pharmacological changes. By the pharmacological study it is concluded that the Triphala shodhit Guggul showed enhanced anti-inflammatory activity as compared with Distilled water and Gomutra shodhit Guggul. Thus, the purpose of Shodhana process of Guggul is proved experimentally showing enhancement of pharmacological activity.

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