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THE EFFECT OF SHODHANA (PURIFICATION PROCESSES) ON ANTI-ARTHRITIC ACTIVITY OF GUGGUL IN THE FREUND'S COMPLETE ADJUVANT MODELS OF ARTHRITIS IN RATS

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ABSTRACT

It has been reported that crude guggul may induce liver toxicity. It has been proved that suddha guggul can be given safely in the treatment of various disorders. Several reports that shodana process can purify the guggul and can overcome the side effects of crude guggul. The objective of the present study is to elucidate the effect of shodhana process on antiarthritic activity of guggul in rats. The unpurified guggul (180 and 360 mg/kg) and guggul purified either with cow urine (180 and 360 mg/kg) or with water (180 and 360 mg/kg) or cow milk (180 and 360 mg/kg) by shodhana process were administered orally in rats. There was an evaluation of paw volume, arthritic score, mobility score, body weight, haematological parameters and histopathology of tibiotarsal joint in Freund's complete adjuvant (FCA) induced arthritic model. There was an increase in arthritis score, paw volume, WBC, ESR, CRP in cow milk purified and water purified guggul as compared to unpurified guggul with significant decrease in liver toxicity. The same parameters were found unaltered in cow urine purified guggul with significant decrease in liver toxicity as compared to unpurified guggul. There was a significant decrease in necrosis and lymphocytic infiltration in tibiotarsal joint in cow urine purified treated animals as compared to FCA treated animals but not significant change as compared to unpurified guggul treated animals. Cow urine purified guggul may significantly decreases the adverse effect profile of guggul without affecting therapeutic efficacy.

Key Words: Arthritis, Cow urine, Guggul, Rats, Shodhana process

INTRODUCTION

Guggul, an oleo gum resin, obtained from *Commiphora wightii* [1] (Syn. *Commiphora mukul*) is belonging to family Burseraceae. The therapeutic significance of the same has been reported in various traditional literature of ayurveda. The usage of suddha guggul has been indicated in obesity, tumours, malignant sores, ulcers, intestinal worms, leucoderma, sinus, edema and sudden disabled seizures [1-3]. Further, it has been well reported that guggul can significantly lower the lipid levels [4, 5]. The effect of guggul has been reported to be effective in blood coagulation disorders [6-8], myocardial infarction [9], coronary heart disease [10 - 12] and inflammatory joint disorders [13].

Guggulosterone (E and Z) [14], the active chemical constituents of guggul, have been reported to influence numerous natural processes like tumour cell proliferation and apoptosis [15]. Despite its well documented therapeutic benefits, it has been reported that unpurified guggul exhibits toxic effects like liver toxicity, skin rashes, irregular menstruation, diarrhoea [16] and its usage warrants medical supervision especially in patients of hepatic disorders, inflammatory bowel syndrome and diarrhoea. Further, it has been reported that such adverse effects of crude guggul can be reduced by shodhana process, a one of the purification methods. The process include cleansing, detoxification and transformation of crude guggul using fluid media such as Guduchi Kwath, Triphala Kwath, Milk (Godugdha), Panchtikta Kwath, Dashmoola Kwath, Nimba patra, Kwath with Haridra churna, Cow urine (Gomutra) and Nirgundi patra [17-19].

In light of such reports, it has been proposed to study the influence of shodhana process using cow urine, cow milk and water on unpurified guggul in mediating its anti-arthritic activity.

MATERIALS AND METHODS

Procurement of the crude materials: Crude guggul was procured from Sanjivani Ayushdhalay, Bhavnagar; Gujarat, India and authentified as guggul (*Commiphora mukul* Linn) at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi.

Preparation of drug sample: Fine powder of unpurified and three purified guggul either with process by cow urine or cow milk or water was used. Unpurified and purified guggul (2 gm) was triturated with honey.

Experimental Animals: Total sixty-six wistar albino rats of either sex (120-200 gm) were used. Animals were acclimatized for one week 12 hr/12hr light/dark cycle. Animals had free access to standard pellet diet and purified drinking water *ad libitum*. Protocol for the present study was approved by IAEC of Babaria Institute of Pharmacy, Vadodara with permission from CPCSEA with approval number: Ph.D/12-13/04.

Shodhana process of crude guggul

Crude guggul was placed over a piece of cotton cloth which was later tied to loosely wrap the guggul to form a pouch. A pouch was hung into an earthen vessel containing one of the different shodhana dravyas or the boiling fluids *viz*., cow's urine, cow's milk and water ensuring that the pouch was completely dipped in to the extraction fluids. The earthen vessel was gently heated to just boil the liquid. The heating was continued with occasional shaking of the pouch until all the soluble fraction passes into the fluid. The pouch was taken out after ensuring

that all solubilized matter of the guggul has been digested in the fluid. The solubilized guggul was filtered and concentrated to a syrupy mass. 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 plastic containers.

Evaluation of antiarthritic activity using FCA model

Groups of animals: Animal were divided into total eleven groups of six animals in each as follows: Group 1 received normal saline (0.9% NaCl), Group 2 received FCA (0.1 ml) only, Group 3 to Group 11 received FCA (0.1 ml) along with either Indomethacin (10mg/kg, p.o) or unpurified guggul (180mg/kg, p.o) or unpurified guggul (360 mg/kg, p.o) or cow urine purified guggul (180mg/kg, p.o) or cow urine purified guggul (360 mg/kg, p.o) or water purified guggul (180mg/kg, p.o) or water purified guggul (360 mg/kg, p.o) or cow milk purified guggul (180mg/kg, p.o) or cow milk purified guggul (360 mg/kg, p.o) respectively.
Animals were injected 0.1 ml of FCA into sub planter area of left hind paw on day 1 under light ether anaesthesia. There was a measurement of joint diameter, paw volume arthritic score, mobility and body weight at every alternative day till the day 28.

Measurement of paw volume: The left rear paw volumes of all animals were measured at the time of FCA injection on day 1 and at distinctive time interims till day 28 with the help of plethysmometer [20, 21]. The change in paw volume was measured as the difference between initial and final paw volume.

Measurement of arthritic score: The morphological peculiarity of the arthritis like redness, swelling and erythema [22, 23] was checked by situational visual criteria like normal paw – 0, mild swelling and erythema of digit – 1, swelling and erythema of the digit – 2, extreme swelling and erythema – 3, total deformation and inability to utilize the appendage – 4 on different days.

Measurement of mobility score: Chronic inflammation and/or arthritis reduce the use of the untreated paw by the adjuvant-diseased rats. On the day 28th, each rat was kept on a table top and permitted to move freely. The impaired function of the adjuvant-treated paw as dismissed in light of the fact that in a few rats this paw was swollen as well as filled with puss and few of the rats had the capacity utilize this appendage. The mobility evaluations were carried out by two independent observers, blind to the treatment modules. The mobility score was assigned as follows – 6: no impairment in movement, 5: rats touch the ipsilateral rear paw completely on the floor, 4: rats touch just the toe of the ipsilateral rear paw on the floor, 3: rats touch the contralateral rear paw completely on the floor, 2: rats touch just the toe of the contralateral rear paw on the floor, 1: rats utilizes only the fore paws, and score 0 indicate absence of movement [22, 23].

Rat Body Weight: The body weight of all rats was measured using a calibrated weighing balance till the day of sacrifice [24, 25].

Determination of haematological parameters
On the day 28, blood collection was done by retro-orbital plexus. Haematological parameters such as white blood cells (WBC), red blood cells (RBC) and haemoglobin (Hb) were measured as per the standard protocol. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured. After collection of blood, animals were sacrificed and hind paw were isolated in each animal and stored at -20° C. Histopathological analysis was done on hind paw on day 28.

Statistical Analysis

Results are presented as Mean ± SEM. Statistical difference between the means of the various groups were evaluated using one-way ANOVA followed by Tuckey's test using sigma stat software ($p<0.5$ was considered statistically significant).

RESULTS

Effect of unpurified and purified guggul on paw volume
There was a significant ($p<0.001$) increase in the paw volume in FCA treated rats. Initially, from day 4 to 10, there was no significant reduction in any of the guggul treated animals. As compared to FCA treated animals, there was a significant ($p<0.001$) and dose-dependent decrease in paw volume from day 10 onwards in animals treated with either unpurified guggul or cow urine purified guggul or water purified guggul or cow milk purified guggul (180 and 360 mg/kg, p.o with each treatment) or Indomethacin (10 mg/kg) (Table 1).

Effect of unpurified and purified guggul on arthritic score
There was a significant increase ($p<0.001$) in arthritic score in FCA treated animals as compared to normal animals. There was a significant reduction in arthritis score in unpurified guggul treated animals. Moreover, animals treated with unpurified guggul and cow urine purified guggul (180 and 360 mg/kg) showed dose dependent reduction of arthritic score as compared to normal animals. (Table 2)

Effect of unpurified and purified guggul on mobility score
There was a significant reduction in mobility in FCA treated animals as compared to normal animals. Animals treated with either unpurified guggul or cow urine purified guggul or water purified guggul or cow milk purified guggul (180 and 360 mg/kg, p.o with each treatment) showed significant ($p<0.001$) and dose dependent recovery in mobility score as compared to control group (Table 3).

Effect of unpurified and purified guggul on body weight
There was a significant decrease ($p<0.001$) in body weight in FCA treated animals as compared to normal animals. Treatment with either unpurified guggul or cow urine purified guggul or water purified guggul or cow milk purified guggul (180 and 360 mg/kg, p.o with each treatment) showed significant ($p<0.001$) and dose dependent increase in body weight when compared with FCA treated animals (Table 4)

Effect of unpurified and purified guggul on RBC, WBC and Hb levels
There was a significant reduction ($p<0.001$) in the level of RBCs and Hb with significant elevation in WBCs level in FCA treated animals as compared to normal animals. Animals treated with either unpurified guggul or cow urine purified guggul showed significant increase in the RBCs and Hb levels with decrease in WBCs level as compared to FCA treated animals (Table 5).

Effect of unpurified and purified guggul on ESR level
There was a significant ($p<0.001$) increase in ESR in FCA treated animals as compared to normal animals. There was a significant and dose-dependent decrease in ESR level in animals treated with unpurified and cow urine purified guggul as compared to FCA treated animals. A significant reduction was seen in ESR level in animals treated with Indomethacin (10 mg/kg) as compared to control animals (Table 6).

Table 1: Effect of unpurified and purified guggul on paw volume

Treatment	Paw volume (mL) (Mean ± SEM)		Volume Change
	Before FCA induction	After 28th day	
Normal	-	-	-
Disease Control	0.384 ± 0.021	0.314 ± 0.010	0.07
Standard drug	0.364 ± 0.025	0.082 ± 0.001 **	0.282
GUG – 1 (180 mg/kg)	0.304 ± 0.029	0.135 ± 0.013 **	0.169
GUG – 1 (360 mg/kg)	0.270 ± 0.038	0.099 ± 0.003 **	0.171
GUG – 2 (180 mg/kg)	0.254 ± 0.036	0.105 ± 0.006 **	0.149
GUG – 2 (360 mg/kg)	0.304 ± 0.026	0.118 ± 0.003 **	0.186
GUG – 3 (180 mg/kg)	0.354 ± 0.018	0.224 ± 0.015 **	0.130
GUG – 3 (360 mg/kg)	0.345 ± 0.023	0.204 ± 0.010 **	0.141
GUG – 4 (180 mg/kg)	0.299 ± 0.028	0.173 ± 0.008 **	0.126
GUG – 4 (360 mg/kg)	0.329 ± 0.025	0.191 ± 0.009 **	0.138

Each value expressed as Mean ± SEM of 6 animals after respective treatment. Data were analyzed by one-way analysis of variance (ANNOVA) followed by Turkey's test. +++ p <0.001 when compared with normal and *** p<0.001 when compared with control.

Table 2: Effect of unpurified and purified guggul on arthritic score

Treatment	Arthritic Score
Normal	0 ± 0.00
Disease Control	3.83 ± 0.17 +++
Standard drug	1.5 ± 0.22 **
GUG – I (180 mg/kg)	3 ± 0.42
GUG – I (360 mg/kg)	2.5 ± 0.22 *
GUG – II (180 mg/kg)	3.5 ± 0.22
GUG – II (360 mg/kg)	3 ± 0.45
GUG – III (180 mg/kg)	3.83 ± 0.17
GUG – III (360 mg/kg)	3.5 ± 0.22
GUG – IV (180 mg/kg)	3.83 ± 0.17
GUG – IV (360 mg/kg)	3.83 ± 0.17

Each value expressed as Mean ± SEM of 6 animals after respective treatment. Data were analyzed by one-way analysis of variance (ANNOVA) followed by Turkey's test. +++ p <0.001 when compared with normal and *** p<0.001 when compared with control.

Table 3: Effect of unpurified and purified guggul on mobility score

Treatment	Mobility Score
Normal	6 ± 0.00
Disease Control	2.5 ± 0.22 +++
Standard drug	6 ± 0.00 ***
GUG – I (180 mg/kg)	4.5 ± 0.22 ***
GUG – I (360 mg/kg)	5.17 ± 0.16***
GUG – II (180 mg/kg)	4.5 ± 0.22 ***
GUG – II (360 mg/kg)	5.17 ± 0.16 ***
GUG – III (180 mg/kg)	3.5 ± 0.22 ***
GUG – III (360 mg/kg)	4.25 ± 0.21 *
GUG – IV (180 mg/kg)	3 ± 0.00
GUG – IV (360 mg/kg)	3.5 ± 0.22 *

Each value expressed as Mean ± SEM of 6 animals after respective treatment. Data were analyzed by one-way analysis of variance (ANNOVA) followed by Turkey's test. +++ p <0.001 when compared with normal and *** p<0.001 when compared with control.

Table 4: Effect of unpurified and purified guggul on body weight

Treatment	Body weight (gm)		
	0th day	28th day	Mean changes in body weight
Normal	153.12	213.87	55.0 ± 5.38
Disease Control	175.16	182.83	8.66 ± 0.92+++
Standard drug	166.83	210.33	42.16 ± 1.01***
GUG – I (180 mg/kg)	164.00	183.45	20.1± 1.15 *
GUG – I (360 mg/kg)	166.33	185.33	15.66 ± 1.71
GUG – II (180 mg/kg)	165.33	192.33	27.83 ± 1.47 ***
GUG – II (360 mg/kg)	166.33	187.83	21.50 ± 1.56 **
GUG – III (180 mg/kg)	167.33	197.33	29.83 ± 1.99 ***
GUG – III (360 mg/kg)	148.59	174.60	24.67 ± 2.12 ***
GUG – IV (180 mg/kg)	158.84	190.91	33.59 ± 1.12 ***
GUG – IV (360 mg/kg)	165.60	194.00	29.66 ± 2.16 ***

Each value expressed as Mean ± SEM of 6 animals after respective treatment. Data were analyzed by one-way analysis of variance (ANNOVA) followed by Turkey's test. +++ p <0.001 when compared with normal and *** p<0.001 when compared with control.

Table 5: Effect of unpurified and purified guggul on WBC, RBC, Hb levels

Treatment	WBC (Cu.mm)	RBC (Million/cu.mm)	Hb (g/dL)
Normal	6.93 ±0.055	9.70 ± 0.30	16.23 ± 0.59
Disease Control	10.10 ±0.090+++	5.80 ± 0.11+++	6.93 ± 0.66+++
Standard drug	7.17 ±0.084***	9.34 ± 0.21***	15.37 ± 0.32***
GUG – I (180 mg/kg)	8.09 ±0.056***	8.146 ± 0.17 ***	11.65 ± 0.32***
GUG – I (360 mg/kg)	7.60 ±0.079***	8.94 ± 0.10***	13.53 ±0.40***
GUG – II (180 mg/kg)	8.29 ±0.047***	7.93 ± 0.23***	11.01 ±0.24***
GUG – II (360 mg/kg)	7.88.±0.055***	8.67 ± 0.1***	12.78±0.20***
GUG – III (180 mg/kg)	9.06 ± 0.066***	7.76 ± 0.46***	10.81 ± 0.16***
GUG – III (360 mg/kg)	8.43±0.048***	8.54 ± 0.20***	12.55 ± 0.28***
GUG – IV (180 mg/kg)	9.39 ± 0.060	7.48 ± 0.18***	10.61 ±0.19***
GUG – IV (360 mg/kg)	8.90±0.088***	8.17 ± 0.10***	12.33±0.32***

Each value expressed as Mean ± SEM of 6 animals after respective treatment. Data were analyzed by one-way analysis of variance (ANNOVA) followed by Turkey's test. +++ p <0.001 when compared with normal and *** p<0.001 when compared with control.

Table 6: Effect of unpurified and purified guggul on ESR level

Treatment	ESR (mm/h)
Normal	3.08 ± 0.84
Disease Control	13.10 ± 0.29 +++
Standard drug	3.25 ± 0.29 ***
GUG – I (180 mg/kg)	6.425±0.46***
GUG – I (360 mg/kg)	4.74 ± 0.51 ***
GUG – II (180 mg/kg)	7.448±0.59***
GUG – II (360 mg/kg)	5.46 ± 0.37 ***
GUG – III (180 mg/kg)	8.502 ±0.81***
GUG – III (360 mg/kg)	5.64 ± 0.64 ***
GUG – IV (180 mg/kg)	9.68±0.26**
GUG – IV (360 mg/kg)	6.56 ± 0.41 ***

Each value expressed as Mean ± SEM of 6 animals after respective treatment. Data were analyzed by one-way analysis of variance (ANNOVA) followed by Turkey's test. +++ p <0.001 when compared with normal and *** p<0.001 when compared with control.

Table 7: Effect of unpurified and purified guggul on CRP level

Treatment	CRP
Normal	1.68 ± 0.64
Disease Control	11.63 ± 0.74 +++
Standard drug	1.80 ± 0.48 ***
GUG – I (180 mg/kg)	4.11 ± 0.20 ***
GUG – I (360 mg/kg)	4.08 ± 0.28 ***
GUG – II (180 mg/kg)	4.82 ± 0.19 ***
GUG – II (360 mg/kg)	4.30 ± 0.26 ***
GUG – III (180 mg/kg)	5.53 ± 0.14 ***
GUG – III (360 mg/kg)	5.13 ± 0.30 ***
GUG – IV (180 mg/kg)	9.99 ± 0.34
GUG – IV (360 mg/kg)	6.15 ± 0.27 ***

Each value expressed as Mean ± SEM of 6 animals after respective treatment. Data were analyzed by one-way analysis of variance (ANNOVA) followed by Turkey's test. +++ p <0.001 when compared with normal and *** p<0.001 when compared with control.

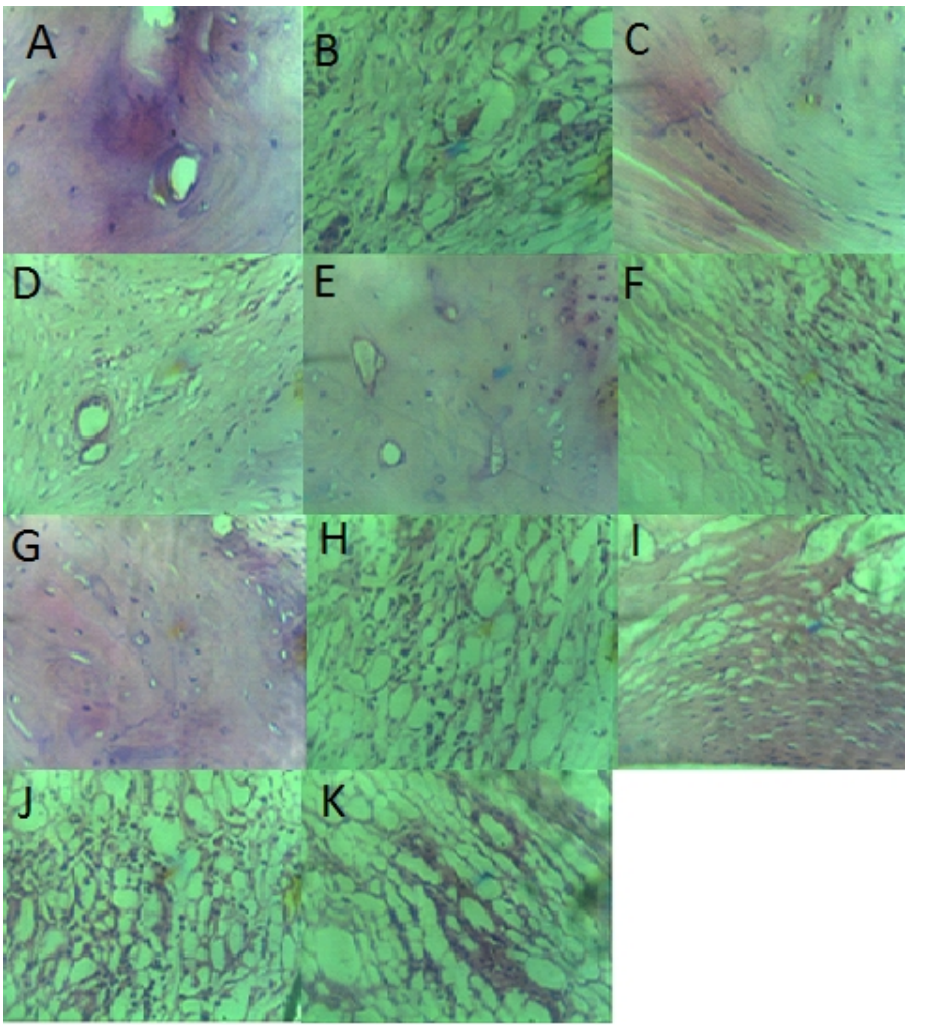

Figure 1: Histopathological representation of tibiotarsal joints

A-Normal control animal, **B-** FCA treated animals, **C-**Indomethacin treated animals, **D-** Unpurified guggul (180 mg/kg) treated animals, **E-** Unpurified guggul (360 mg/kg) treated animal, **F-** Cow urine purified guggul (180 mg/kg) treated animals, **G-** Cow urine purified guggul (360 mg/kg) treated animals, **H-** Water purified guggul (180 mg/kg) treated animals, **I-** Water purified guggul (360 mg/kg) treated animals, **J-** Cow milk purified guggul (180 mg/kg) treated animals, K- Cow milk purified guggul (360 mg/kg) treated animals

Effect of unpurified and purified guggul on CRP levels

There was a significant ($p<0.001$) increase in CRP level in animals treated with FCA as compared to normal animals. Animals treated with either unpurified guggul or cow urine purified guggul or water purified guggul or cow milk purified guggul in doses of 180 mg/kg and 360 mg/kg in each treatment showed significant ($p<0.001$) decrease in CRP levels as compared to FCA treated animals (Table 7).

Effect of unpurified and purified guggul on inflamed joint

There was no evidence of necrosis and lymphocytic infiltration in results of histopathology of tibiotarsal joints in normal animals (Figure I A). There was significant increase in inflammation in connective tissue along with significant necrosis and lymphocytic infiltration in FCA treated animals (Figure I B). The same inflammation was found to be decreased in animals treated with Indomethacin. The results of histopathology of tibiotarsal joint indicated that there was an inflamed connective tissue, mild necrosis and lymphocytic infiltration in the tibiotarsal joint in animals treated with either unpurified guggul or cow urine purified guggul or water purified guggul or cow milk purified guggul in dose of 180 mg/kg in each treatment. There was no evidence of inflamed connective tissue, necrosis and lymphocytic infiltration in cow urine purified guggul (360 mg/kg) while the other treatments as mentioned above with dose of 360 mg/kg indicated the presence of mild necrosis and lymphocytic infiltration (Figure I C-K).

DISCUSSION

It has been reported in the ayurvedic literature that shodhana process– an ancient method may be utilized for purification of guggul. Further, there are various evidences of liver toxicity with high dose of unpurified guggul. In light of these evidences, we have evaluated the influence of purification of shodhana process on either anti-arthritic action or adverse effect profiles of crude guggul. The results from our study indicated that all three purified guggul showed anti-arthritic activity among which cow urine purified guggul has shown more prominent antiarthritic activity as compare to other purified guggul. Though, such anti-arthritic activity of cow urine purified guggul has similar action as compared to unpurified guggul, there was no adverse effects observed with cow urine purified guggul which may lead to increase its usage with respect to safety.

Freund's complete adjuvant induced arthritis has been reported as a model for primary and secondary chronic arthritis, involving the role of prostaglandins and immune reaction respectively. There was a report for the release of different inflammatory mediators in the initiation of pain along with swelling of the limbs and joints, bone deformations, disability of joint function [26]. Arthritic score is index of the joint inflammation after immunization [27] and a selective reduction in the arthritic score in the present study indicated the immunosuppressive effects of guggul by virtue of its anti-inflammatory properties [27].

Moreover, health status and diseases recovery is indirectly concerned with body weight [28, 29]. It has been reported that there was a significant reduction in body weight in joint inflammation. Likewise, there was a significant reduction in body weight in FCA treated animals with treatment of either unpurified guggul or cow urine purified guggul or water purified guggul or cow milk purified guggul, showing its therapeutic potential in arthritis. It has been reported that there was a decreased response of the bone marrow to erythropoietin and there was a destruction of premature RBCs in FCA induced arthritis, resulting into the decrease in levels of Hb and RBCs. The restoration in such parameters with cow urine purified guggul treatment may indicate that such guggul treatment may affect the function of bone marrow, raising its potential in the treatment of arthritis. Further, reduction in ESR level in animals treated with various guggul treatment indicated the anti-arthritic activity of guggul, extending it potential in the arthritis induced by either inflammation or stress or cell necrosis [30].

It has been reported that elevated level of the IL-1β inflammatory response in FCA induced arthritis result in increase in granulocyte and macrophage colony stimulating factors which is associated with elevated level of WBC [31, 32]. The significant decrease in WBC level was seen in cow urine purified guggul treated animals as compared to FCA treated animals, indicating its proposed role of in the inhibition of the release of inflammatory mediators.

CRP, a critical biomarker for different inflammatory and degenerative condition [33] may be increased in diseases associated with inflammation or tissue destruction and may also indicate the diseases progression especially in arthritis condition. Such levels of CRP were found to be decreased more significantly by guggul with various purification method, indicating better role of purified guggul in arthritis condition.

In conclusion It has been suggested that cow urine purified guggul by shodhana process may be the most effective purified form which can reduce the adverse effects of raw guggul without affecting its anti-arthritic activity.

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