

Supporting Information

Bergenin loaded gum xanthan stabilized silver nanoparticles suppresses synovial inflammation through modulation of immune response and oxidative stress in adjuvant induced arthritic rats

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Supplementary Methods:

S1. GX-AgNPs-BG stability study

Drug loaded GX-AgNPs-BG containing known amount of drug were incubated with diluted human blood plasma and stored at room temperature. Samples (2 mL) were withdrawn at pre-determined time intervals and centrifuged 12000 rpm for 30 min. Supernatant containing free drug was carefully isolated and read spectrophotometrically. Drug released during incubation period with plasma was quantified accordingly and percent drug retained by nanoparticles was determined.

S2. Toxicity Parameters:

Acute oral toxicity study was performed according to the Organization of Economic Co-Operation and Development (OECD) guideline 420 for testing of chemicals. Toxicity was evaluated following various parameters along with behavioral changes and mortality that includes changes in body weight, organ weight and histology of vital organs. Here are the results of different parameters focused for this toxicity studies.

(a) Body Weight:

In acute toxicity testing rats (n=6/group) treated consecutively for a period of 7 days with BG (10-500 mg/kg; *p.o.*), GX-AgNPs-BG (0.5-1 mg/kg; *p.o.*) or vehicle controls received 0.9% saline. During the 7 days of observation period, treated rats did not show any signs of behavioral distress or any alteration in the sleeping pattern, food and water intake and metabolism. Additionally mortality was also not recorded throughout the study.

The body weight of each rat was carefully monitored and gradual increase was observed in vehicle control and treated groups while changes between the groups was not significantly different as depicted in **table S1**.

Table S1. Effect of BG and GX-AgNPs-BG on body weight

Group	Doses (mg/kg; <i>P.O.</i>)	Body Weight (g)			
		Day 1	Day 3	Day 5	Day 7
Vehicle Control	-	150±4.01	152±5.21	154±4.61	156±4.08
BG	10	154±3.41	158±4.36	161±4.11	164±3.11
BG	50	150±6.51	153±4.01	154±2.61	158±5.31
BG	100	156±4.81	158±3.68	160±5.36	162±4.56
BG	250	152±5.61	154±4.31	156±3.11	159±5.31
BG	500	158±4.95	160±5.31	164±5.81	167±3.51
GX-AgNPs-BG	0.5	159±6.03	161±2.51	163±5.01	166±4.81
GX-AgNPs-BG	1	155±2.01	158±5.21	162±3.11	165±5.41

Values are expressed as the mean ± SD (n = 6; rats); p > 0.05 using one-way ANOVA followed by Tukey's multiple comparison test.

(b) Organ weight:

The organs including liver, spleen and kidney were carefully excised in the present study and weighed after 7 days of oral treatment. The weight of each organ in the treatment groups did not show any significant difference (p > 0.05) than that of vehicle control as mentioned in **table S2**.

Table S2. Effect of BG and GX-AgNPs-BG on weight of vital organs of rats

Group	Doses (mg/kg; <i>P.O.</i>)	Weight (g)		
		Liver	Kidney	Spleen
Vehicle Control	0.9 % saline	6.0±4.01	0.54±4.01	0.4±5.21
BG	10	6.3±3.41	0.49±3.01	0.48±6.31
BG	50	6.0±4.01	0.53±2.33	0.4±5.21
BG	100	6.3±3.41	0.50±3.95	0.48±6.31
BG	250	6.0±4.71	0.48±5.64	0.46±8.01
BG	500	6.0±4.01	0.56±5.65	0.42±5.21
GX-AgNPs-BG	0.5	6.3±3.41	0.45±7.33	0.48±6.31
GX-AgNPs-BG	1	6.0±4.71	0.53±2.68	0.46±8.01

Values are expressed as the mean ± SD (n = 6; rats); p > 0.05 using one-way ANOVA followed by Tukey's multiple comparison test.

(c) Histology:

Each selected organs include (liver, kidney and spleen) from all groups i.e: BG and GX-AgNPs-BG or vehicle control were processed for histological analysis using hematoxylin and eosin (H&E) stain. Histological studies revealed no abnormalities in liver, kidney and spleen tissue of treated rats as found to be similar as that of vehicle control.

- (i) The liver tissue displayed normal hepatocytes without any enlargement in sinusoidal vein, central vein and portal triad in all treated groups compared to control.
- (ii) Kidney micrograph has shown normal architecture of glomerulus and Bowman's capsules with no degeneration, necrosis, or inflammation.
- (iii) Histological features of spleen showed normal splenocytes with prominent nucleus in both treated and control groups.

Thus, histopathological evaluation indicated that the treatment did not have any adverse effect on morphology of the tissues. Therefore, **(Fig. S1)** concluded that BG (10-500 mg/kg) and GX-AgNPs-BG (0.5-1 mg/kg) treatment did not produce any toxic effect in rats.

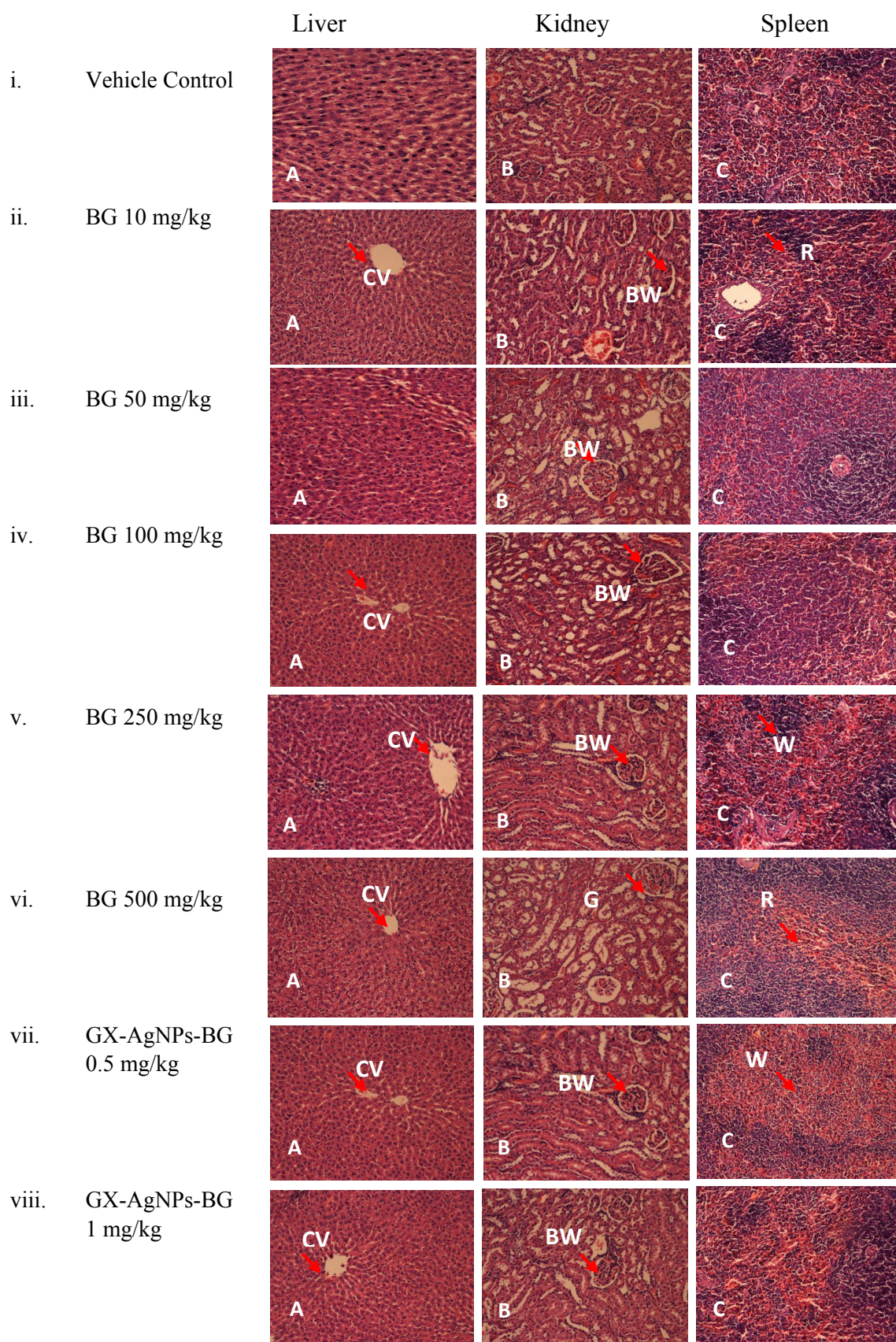


Figure S1: Microphotograph of H&E stained of liver, kidney and spleen tissues at magnification x40, i-viii (A) shows liver tissue of Vehicle control, BG (10-500 mg/kg) and GX-AgNPs-BG (0.5-1 mg/kg); i-viii (B) shows kidney tissue of Vehicle control, BG (10-500 mg/kg) and GX-AgNPs-BG (0.5-1 mg/kg) and i-viii (C) shows spleen tissue of Vehicle control, BG (10-500 mg/kg) and GX-AgNPs-BG (0.5-1 mg/kg) indicating no significant damage was observed in any tissue of the treatment groups as compared with the control group.

Indicators: Portal Triad (pt); Central Vein (CV), Bowman's capsule (BW), glomerulus (G), Red pulp (R) and White pulp (W).

In the light of these aforementioned findings, we may conclude that BG and GX-AgNPs-BG is non-toxic molecules and did not produce any evidence of toxicity. The body weight changes are

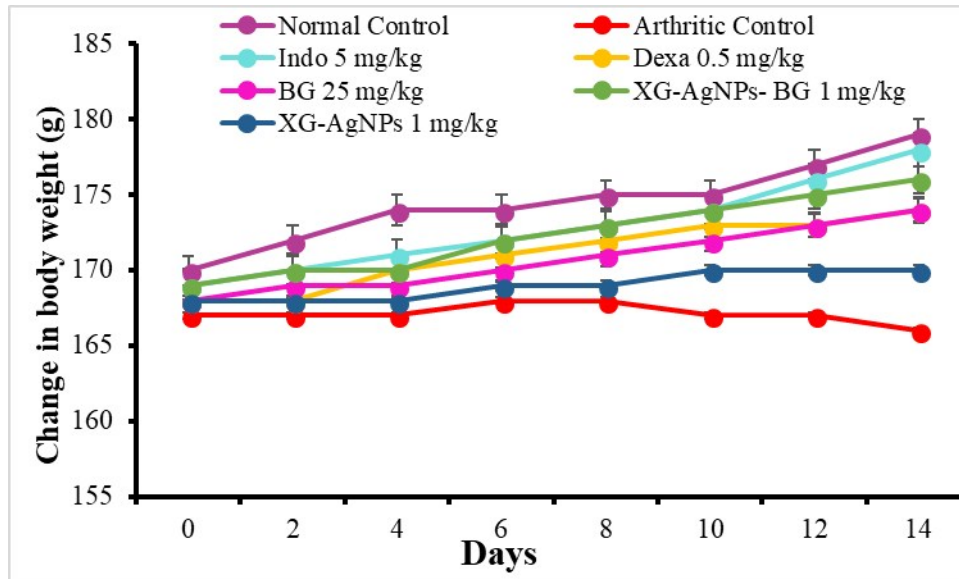
sensitive indicator of general health status of animals [1], we observed normal increment in body weight in all treated groups suggesting that BG and its nano particles did not interfere with the normal metabolism and growth of animals, similarly, no significant changes were observed in vital organs. The histological examination revealed no remarkable changes in the internal organs. Overall the structure and morphology of liver, kidney and spleen were remain intact and no damage were observed in all treated and vehicle control groups.

S3. Body and organs weight during arthritis treatment

a) During the course of experiment the body weight of arthritic rats in treated and untreated groups (arthritic control) were recorded after the induction of CFA on alternate days [2].

In the arthritic control group, the mean body weight was gradually decline compared to normal control at every time point. However, the normal control animals and the treated arthritic groups including; BG (25 mg/kg) or GX-AgNPs-BG (1 mg/kg) or dexamethasone (0.5 mg/kg) and/or indomethacin (5 mg/kg) demonstrated gradual rise in their mean body weights which was significantly different with the arthritic control group as mentioned in

Fig. S2. However, arthritic animals treated with nano-cargo (GX-AgNPs; 1 mg/kg) did not cause any



significant change in weight.

Figure S2: Effect of BG (25 mg/kg), GX-AgNPs-BG (1 mg/kg) and GX-AgNPs (1 mg/kg), Indo (5 mg/kg) and Dexa (0.5 mg/kg) on body weight of CFA induced arthritic rats. Each group is represented by the Mean \pm SD of (n = 5).

b) At the end of experiment, the arthritic rats of all the experimental groups were euthanized and the thymus/spleen were carefully excised and weighed. The spleen and thymus Index were calculated as ratio of organ weight to rat body weight (mg/g) [3].

Thymus and spleen indexes have been significantly associated with diverse immunological functions. In the present study, thymus and spleen indexes were significantly increased in arthritic control rats than that of normal control which implied indirectly that B cells and T cells in peripheral lymphoid organ proliferated severely. However, BG (25 mg/kg), GX-AgNPs-BG (1 mg/kg), dexamethasone (0.5 mg/kg) and indomethacin (5 mg/kg) treatments decreased spleen index significantly compared to CFA-induced arthritic control group. Thymus weight was not significantly altered in treatment groups and found to be almost similar to normal control as showed in **Fig.S3**. Moreover, nano-carrier GX-AgNPs (1 mg/kg) treatment exhibited no significance influence on thymus and spleen index.

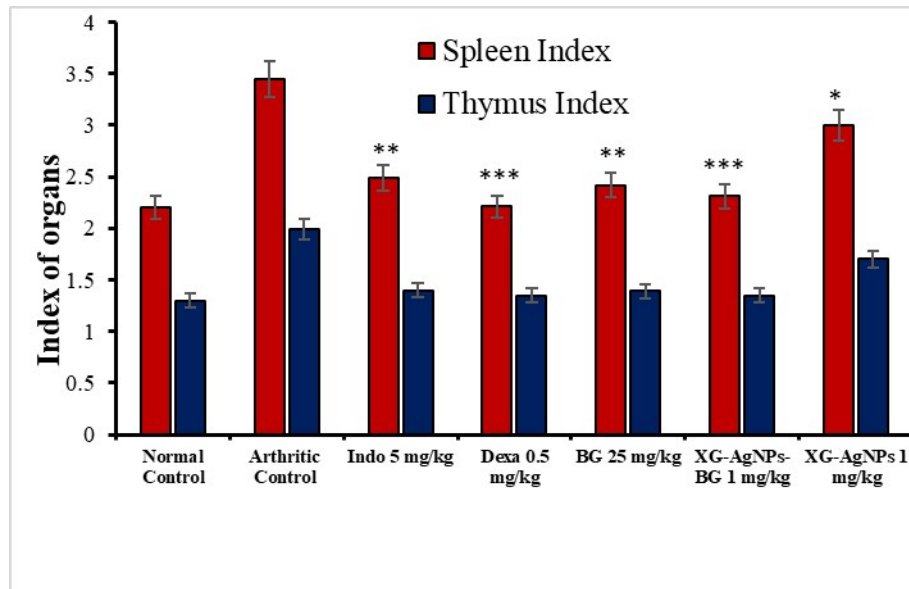


Figure S3. Effect of BG (25 mg/kg), GX-AgNPs-BG (1 mg/kg) and GX-AgNPs (1 mg/kg), Indo (5 mg/kg), Dexamethasone (0.5 mg/kg) on thymus and spleen indices in CFA induced arthritic rats. Asterisks indicate the significance difference at * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ with respect to arthritic control.

Taken together, the body weight and index of spleen and thymus can be considered as apparent indicators of arthritis incidence and disease progression in adjuvant arthritic model. Weight loss is a powerful predictor of health especially in pathological states [4]. In the case of RA, loss of body weight would be due to muscle loss, poor appetite and metabolic burden of inflammatory responses and its relevant immune cells and cytokines [5]. The high concentrations of TNF- α and IL-1 β exert a powerful influence on whole-body protein and energy metabolism. Although the specific mechanism(s) is not known, TNF- α is thought to stimulate muscle catabolism [6]. The increased catabolism raises resting energy expenditure, which leads to weight loss and reduced lean body mass, especially if energy and protein requirements are not met, which is recognized as “rheumatoid cachexia”. Changes in body weight, therefore, have also been used to assess the course of disease and response to therapy of anti-inflammatory drug [7]. The present study demonstrated potential body weight loss in arthritic rats that decline gradually whereas BG and GX-AgNPs-BG treatment significantly improved the body weight of arthritic rats similar to normal control, indicating a reduction of catabolism caused by the inflammatory cytokines and hence representing their therapeutic potential in the management of RA.

Furthermore, Thymus and spleen are two major organs of the body’s immune system and their weights are commonly used as the preliminary indicators to evaluate the immunoregulatory activity of the tested compounds. The spleen normally filters out and destroys old and damaged blood cells and removes antigens by phagocytosis, which plays a key role in preventing infection by producing white blood cells called lymphocytes and acting as a first line of defense against

invading pathogens [8]. Similar arguments apply to the thymus, which typically processes immature precursor T-lymphocytes (derived from bone marrow and initially located in the outer cortex of the thymus) into the mature immune competent T-cells of the medulla [9]. Our results clearly revealed that BG and GX-AgNPs-BG reversed splenomegaly of rats induced by CFA injection and exhibited thymus weight similar to normal, which suggested that BG and GX-AgNPs-BG played an important role in the immune system mainly by inhibiting the enlargement of the spleen and the systemic expansion of immune cells.

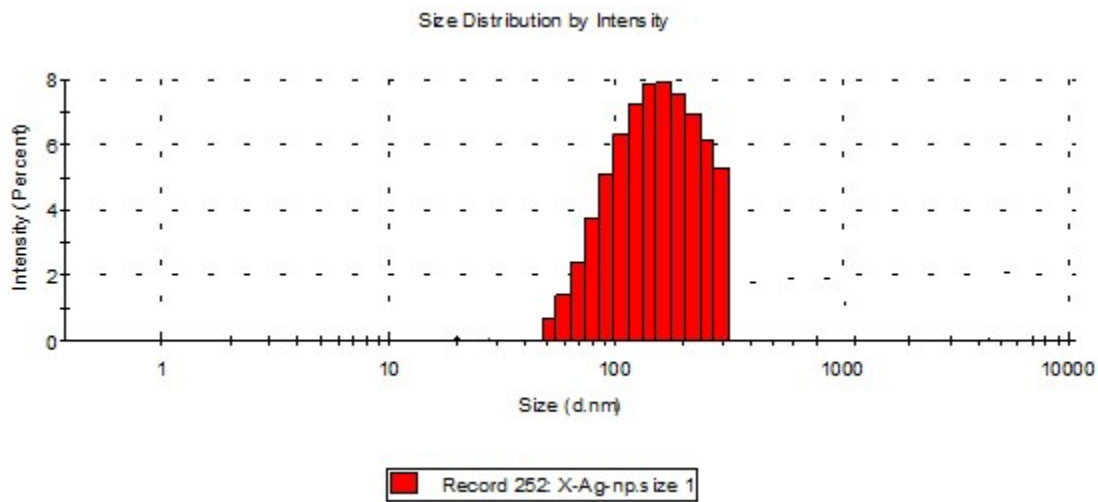


Figure S4: Zetasizer chromatogram of GX-AgNPs size and size distribution

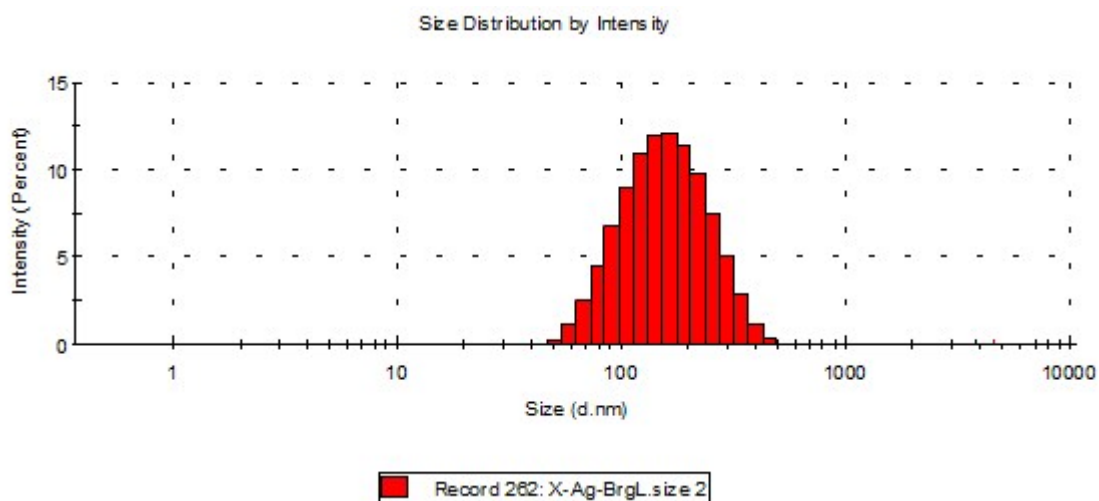


Figure S5: Zetasizer chromatogram of GX-AgNPs-BG size and size distribution

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