11. Anti-inflammatory study

- Animal experiment was performed as per the protocol approved by the Institutional Animal Ethics Committee (Approval letter in Appendix 1).

- The study included ten groups containing six healthy adult Wistar albino rats (160-180 gms) in polypropylene cages layered with husk and maintained in a controlled room at a temperature (22±3°C) and light (12 hours light/dark cycle).

- Animals were provided free access to water and standard pellet diet. Animals were cared in accordance with the “Guide for the care and use of laboratory animals” (NIH 1985) and study was conducted in accordance with the committee for the purpose of control and supervision on experiments on animals (CPCSEA).

- The animals were randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

- Animals in the control group (Group 1) received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat. 1 h after the oral administration of distilled water (1ml).  

- Animals in the Group 2 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of pure Quercetin (50mg/kg of body weight).

- Animals in the Group 3 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of pure Rutin (50mg/kg of body weight).
Animals in the Group 4 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of pure Silibinin (50 mg/kg of body weight).

Animals in the Group 5 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of prepared Quercetin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight).

Animals in the Group 6 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of prepared Rutin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight).

Animals in the Group 7 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of prepared Silibinin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight).

Animals in the Group 8 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of Quercetin-Rutin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight).

Animals in the Group 9 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of Quercetin-Rutin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight).
inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of Quercetin-Silibinin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight)

- Animals in the Group 10 received an injection of 0.1 ml Freund’s complete adjuvant intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rat, 1 h after the oral administration of indomethacin standard drug (equivalent to 50 mg/kg of body weight).

- The degree of pedal edema was determined by measuring the volume of both hind paws by plethysmograph.

- Plethysmographic measurements were made after the adjuvant injection (on day 0), which was repeated again 3 days later at 3 hrs, 6 hrs (acute phase) and 7th, 14th to 28th day (chronic phase) after carrageenan injection.141,142

- For the control group, the vehicle used was saline solution. The inhibition percentage of edema was calculated using following formula for each animal group in comparison with the control group.

  Percentage of edema inhibition = \((V_c - V_t / V_c) \times 100\)

  \(V_c\)- Volume of paw edema in control group

  \(V_t\)- Volume of paw edema in treated group