# CHAPTER 3
# RESULTS

## 3.1 Anti-inflammatory activity of the GW extract

### 3.1.1 Effects of the GW extract and phenylbutazone on EPP-induced ear edema in rats

The inhibitory effect produced by the topical administration of the GW extract on EPP-induced ear edema was assessed. As shown in Table 3, the ear edema thickness of the rats in control group (received 5% DMSO in acetone) increased gradually and was at peak at 60 min after EPP application. The edema was maintained till 2 hours of assessment. Phenylbutazone at the dose of 1 mg/ear, used as positive control, significantly reduced the edema formation at all assessment times. The GW extract at the dose of 1 mg/ear produced significant inhibitory activity on edema formation at all determination times with slightly weaker intensity than phenylbutazone.

**Table 3.** Effects of the GW extract and phenylbutazone on EPP-induced ear edema in rats

| Group | Time after topical application of EPP | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 15 min | | 30 min | | 60 min | | 120 min | |
| | ED(μm) | EDI(%) | ED(μm) | EDI(%) | ED(μm) | EDI(%) | ED(μm) | EDI(%) |
| Control | 88.33 ± 4.77 | - | 98.33 ± 6 .00 | - | 101.67 ± 9.10 | - | 96.67 ± 11.15 | - |
| Phenylbutazone 1 mg/ear | 30.67 ± 3.65* | 66 | 45.00 ± 6.71* | 54 | 46.67 ± 7.60* | 54 | 56.67 ± 7.60* | 41 |
| GW extract 1 mg/ear | 23.33 ± 3.33* | 74 | 40.00 ± 3.65* | 59 | 50.00 ± 2.58 * | 51 | 65.00 ± 2.24* | 33 |

Test drugs were applied topically to both inner and outer surfaces of the ear. The control group received vehicle (5% DMSO in acetone) only.

Values represent the mean ± S.E.M. ($N = 6$).

* Significantly different from the control, $P < 0.05$

ED: edema thickness; EDI: edema inhibition

### 3.1.2 Effects of the GW extract and diclofenac on carrageenin-induced paw edema in rats.

The activity of the GW extract and diclofenac against carrageenin-induced paw edema are presented in Table 4. In control group (received distilled water), the edema volume of rats paw was found to increased gradually and reached its peak at 5$^{th}$ h after carrageenin injection.

The results show that diclofenac at dose of 10 mg/kg also possessed significant inhibitory effect on carrageenin-induced paw edema at all recorded times. Similarly to that of diclofenac, the inhibitory effect of the GW extract at a dose of 300 mg/kg on the edema formation was seen at all determination times. However, at doses of 75 and 150 mg/kg the GW extract exhibited slight but significant inhibition on the edema formation of rat paw at 3$^{rd}$ and 5$^{th}$ h, respectively, after carrageenin injection. The inhibitory effect of the GW extract on the edema formation gradually increased with the increased doses.

**Table 4.** Effects of the GW extract and diclofenac on carrageenin-induced paw edema in rats

| Group | Dose (mg/kg) | Edema volume (mL) | | | % Edema inhibition | | |
|---|---|---|---|---|---|---|---|
| | | 1 h | 3 h | 5 h | 1 h | 3 h | 5 h |
| Control | - | 0.18 ± 0.01 | 0.57 ± 0.02 | 0.60 ± 0.02 | - | - | - |
| Diclofenac | 10 | 0.08 ± 0.01* | 0.11 ± 0.01* | 0.16 ± 0.01* | 50 | 81 | 73 |
| GW extract | 75 | 0.14 ± 0.01 | 0.48 ± 0.02* | 0.52 ± 0.02* | 13 | 16 | 13 |
| GW extract | 150 | 0.10 ± 0.01 | 0.36 ± 0.01* | 0.43 ± 0.01* | 44 | 37 | 28 |
| GW extract | 300 | 0.08 ± 0.01* | 0.19 ± 0.04* | 0.32 ± 0.04* | 50 | 67 | 47 |

Test drugs were orally administered 1 h before carrageenin injection. The control group received vehicle (distilled water) only. Values represent the mean ± S.E.M. ($N = 6$). * Significantly different from the control, $P < 0.05$

### 3.1.3 Effects of the GW extract, diclofenac and prednisolone on AA-induced hind paw edema in rats.

The result of the GW extract on AA-induced hind paw edema in rats is shown in Table 5. One hour after AA injection, the average edema volume in the control group amounted to 0.50±0.01 mL. Diclofenac at the dose of 10 mg/kg did not show any inhibitory effect on AA-induced paw edema. By contrast, prednisolone at the dose of 5 mg/kg exhibited significant inhibitory activity on the edema of the rats paw when assessment was done 1 h after AA injection. The GW extract at doses of 75, 150 and 300 mg/kg dose-dependently exhibited significant reduction of the paw edema similarly to prednisolone.

**Table 5.** Effects of the GW extract, diclofenac and prednisolone on AA-induced hind paw edema in rats

| Group | Dose (mg/kg) | Edema volume (mL) | % Edema inhibition |
|---|---|---|---|
| Control | - | 0.50 ± 0.01 | - |
| Diclofenac | 10 | 0.47 ± 0.03 | 6 |
| Prednisolone | 5 | 0.15 ± 0.01* | 70 |
| GW extract | 75 | 0.41 ± 0.02* | 18 |
| GW extract | 150 | 0.33 ± 0.01* | 34 |
| GW extract | 300 | 0.21 ± 0.04* | 58 |

Test drugs were orally administered 2 h before AA injection. The control group received vehicle (distilled water) only.

Values represent the mean ± S.E.M. ($N = 6$).

* Significantly different from the control, $P < 0.05$

### 3.1.4 Effects of the GW extract, diclofenac and prednisolone on cotton pellet-induced granuloma formation in rats

#### 3.1.4.1 Effects on granuloma formation

The result of the GW extract on cotton pellet-induced granuloma formation in rats is demonstrated in Table 6 and 7. The results indicate that the GW extract at an oral dose of 300 mg/kg/day, similarly to prednisolone (5 mg/kg/day) significantly reduced transudative weight, granuloma formation, thymus weight and body weight gain of animals. In contrast, diclofenac (2.5 mg/kg/day) significantly reduced only granuloma formation.

#### 3.1.4.2 Effects on alkaline phosphatase activity

As shown in Table 8, cotton pellet implantation caused an increase in serum alkaline phosphatase. The GW extract at the dose of 300 mg/kg as well as prednisolone (5 mg/kg/day) could normalize alkaline phosphatase activity in rats whereas diclofenac (2.5 mg/kg/day) slightly reduced the increased serum alkaline phosphatase activity.

**Table 6.** Effects of the GW extract, diclofenac and prednisolone on body weight and thymus weight of cotton pellet-induced granuloma formation in rats

| Group | Dose (mg/kg/day) | Body weight (g) | | | Dry thymus weight (mg/100 g BW) |
|---|---|---|---|---|---|
| | | Initial | Final | Gain | |
| Control | - | 199.33 ± 3.63 | 244.67 ± 3.71 | 45.33 ± 6.18 | 49.51 ± 5.31 |
| Prednisolone | 5 | 190.00 ± 3.93 | 179.83 ± 12.89* | -10.16 ± 9.45* | 23.00 ± 2.26* |
| Diclofenac | 2.5 | 203.00 ± 4.02 | 230.66 ± 5.88 | 27.66 ± 4.33 | 47.52 ± 2.79 |
| GW extract | 300 | 195.67 ± 6.20 | 196.67 ± 7.84* | 1.00 ± 2.90* | 29.54 ± 1.70* |

Values represent the mean ± S.E.M. ($N = 6$).

* Significantly different from the control, $P < 0.05$

**Table 7.** Effects of the GW extract, diclofenac and prednisolone on cotton pellet-induced granuloma formation in rats

| Group | Dose (mg/kg/day) | Granuloma wet weight (mg) | Granuloma dry weight (mg) | Transudative weight (mg) | Granuloma weight (mg/mg cotton) | GI (%) |
|---|---|---|---|---|---|---|
| Control | - | 386.92 ± 16.58 | 71.67 ± 2.75 | 315.25 ± 17.24 | 2.59 ± 0.14 | - |
| Prednisolone | 5 | 178.50 ± 15.26* | 40.83 ± 2.20* | 137.66 ± 22.14* | 1.04 ± 0.11* | 60 |
| Diclofenac | 2.5 | 241.75 ± 33.77* | 49.33 ± 5.51* | 192.41 ± 22.14* | 1.46 ± 0.27* | 43 |
| GW extract | 300 | 243.50 ± 16.02* | 52.58 ± 3.67* | 190.92 ± 17.24* | 1.63 ± 0.18* | 37 |

Values represent the mean ± S.E.M. ($N = 6$).

* Significantly different from the control, $P < 0.05$

GI = granuloma inhibition

**Table 8** Effects of the GW extract, diclofenac and prednisolone on alkaline phosphatase activity in the serum of cotton pellet-induced granuloma formation in rats

| Group | Dose (mg/kg/day) | Alkaline phosphatase (U/L) | Total protein (g/dL) | Alkaline phosphatase activity ( U of enz/mg of total protein x $10^{-4}$ ) |
|---|---|---|---|---|
| Normal | - | 120.50 ± 11.41 | 4.20 ± 0.41 | 29.94 ± 4.36 |
| Control | - | 223.33 ± 10.64 | 4.98 ± 0.06 | 44.75 ± 1.79$^{\#}$ |
| Prednisolone | 5 | 158.33 ± 6.44 | 5.18 ± 0.14 | 30.83 ± 2.13$^{*}$ |
| Diclofenac | 2.5 | 164.50 ± 10.50 | 4.23 ± 0.16 | 39.15 ± 2.98$^{\#}$ |
| GW extract | 300 | 159.66 ± 4.99 | 5.08 ± 0.20 | 31.70 ± 1.71$^{*}$ |

Values represent the mean ± S.E.M. ($N = 6$).

*Significantly different from control, $P < 0.05$

$^{\#}$Significantly different from normal, $P < 0.05$

**3.2 Analgesic activity of the GW extract.**

**3.2.1 Effects of the GW extract and diclofenac on acetic acid-induced writhing response in mice.**

The results of the acetic acid-induced writhing response in mice are presented in Table 9. Diclofenac at the dose of 10 mg/kg orally showed marked inhibition of writhes. Similarly, GW extract at doses of 75, 150 and 300 mg/kg significantly exerted inhibitory effect on acetic acid-induced writhing response in mice. An increase in the doses of the GW extract resulted in a greater inhibition. Dose of 300 mg/kg of the GW extract produced marked analgesic effect by reducing the writhing response caused by acetic acid injection.

**3.2.2 Effects of the GW extract and reference drugs on the tail-flick test in rats**

The inhibitory effects of the GW extract and reference drugs on the tail-flick test in rats are shown in Table 10. It was found that the GW extract caused an inhibition on the tail-flick response in rats. An increase in the doses of the GW extract resulted in a greater inhibition. Dose of 300 mg/kg of the GW extract and morphine (10 mg/kg) showed marked inhibition on the tail-flick response in rats. In contrast, diclofenac at a dose of 10 mg/kg did not show any inhibitory effect on the avoiding response in tail-flick test in rats.

**Table 9** Effects of the GW extract and diclofenac on acetic acid-induced writhing response in mice

| Group | Dose (mg/kg) | No. of writhes | Inhibition of writhing response (%) |
|---|---|---|---|
| Control | - | 45.00 ± 3.73 | - |
| Diclofenac | 10 | 11.00 ± 1.08* | 76 |
| GW extract | 75 | 28.00 ± 0.93* | 36 |
| GW extract | 150 | 21.00 ± 0.80* | 53 |
| GW extract | 300 | 13.00 ± 0.98* | 71 |

Values represent the mean ± S.E.M. ($N = 6$).

* Significantly different from the control, $P < 0.05$

**Table 10** Effects of the GW extract and reference drugs on the tail-flick test in rats

| Group | Dose (mg/kg) | Tb (sec) | Tr (sec) | Inhibition (%) |
|---|---|---|---|---|
| Control | - | 2.48 ± 0.18 | 2.65 ± 0.15 | - |
| Diclofenac | 10 | 2.46 ± 0.13 | 3.28 ± 0.08 | 9 |
| Morphine | 10 | 2.43 ± 0.09 | 9.88 ± 0.11* | 98 |
| GW extract | 100 | 2.71 ± 0.14 | 4.03 ± 0.33* | 26 |
| GW extract | 200 | 2.63 ± 0.09 | 6.91 ± 0.49* | 58 |
| GW extract | 300 | 2.71 ± 0.08 | 9.43 ± 0.44* | 92 |

Values were expressed as mean ± S.E.M. ($N = 6$).

* Significantly different from the control, $P < 0.05$

Tb = baseline reaction time; Tr = reaction time after injection of test drugs.

### 3.3 Acute toxicity

A single administration of the GW extract by oral route at the high dose of 3000 mg/kg did not produce mortality or show any signs of toxicity or changes in general behavior or other physiological activities when compared with those of the control group.

On the 15$^{th}$ day, all rats were sacrificed to examine gross pathological changes of internal organs, the result showed that there were no detectable abnormalities and no differences between the control and the GW extract treated group.