Chapter-4

Results

RESULTS

4.1. Analytical Characterization of *Andrographis paniculata* Extract and Andrographolide

Standardised *Andrographis paniculata* extract (KalmCold™) contained andrographolide (32.2%, w/w), isoandrographolide (0.5%, w/w), neoandrographolide 2.7%, w/w), andrograpanin (0.9%, w/w), 14-deoxy-11,12 didehydroandrographolide 4.7%, w/w) and skullcapflavone I (0.06%, w/w). HPLC fingerprints of *Andrographis paniculata* extract and isolated pure andrographolide are shown in **Figures 4.1** and **4.2** respectively. HPLC fingerprint of standardised root extract of *Withania somnifera* is shown in **Figure 4.3**.

4.2. Pilot Experiments with *Andrographis paniculata* Extract

4.2.1. Stress-induced hyperthermia: No statistically significant effects of AP treatments on foot shock stress triggered transient hyperthermia was detected after its acute oral doses, and no effects of the extract on basal core temperatures of unstressed animals (before foot stress) were observed after its single oral doses. It is apparent from the results summarized in **Figure 4.4** that the basal core temperature of the vehicle treated control group increased gradually on the subsequent days of the experiment. Such daily handling-induced hyperthermia were not observed in any of the AP treated groups, the mean values of which in these groups on days 1, 5, 7 and 10 were not significantly different from each other. Despite significant ($p<0.05$) increases in basal core temperature of the control group on days 5, 7 and 10 of the experiment, the observed foot shock triggered hyperthermia in this group remained constant in magnitude on all these test days **(Figure 4.5A)**. On the 5th day of the treatments, significant antagonistic effect ($p<0.05$) of AP against stress-induced hyperthermia was observed after its 100 and 200 mg/kg/day doses only. Continuing the treatments for seven days increased somewhat its efficacy, and significant treatment effects ($p<0.05$) on this day were observed after its doses between 100 to 600 mg/kg/day. However, on this day, the efficacies of 400 and 600 mg/kg doses of

AP were lower than that observed after its 200 mg/kg daily doses. On day 10 of the treatments significant ($p<0.05$) antagonistic effects of AP was observed after all its tested doses higher than 100 mg/kg **(Figure 4.5A)**. The dose-effect curves obtained from these values are shown in **Figures 4.5B, C** and **D.**

4.2.2. Ring test and inverted screen test: In the ring test, no effects of AP were observed after its single or 5 or 7 or 10 daily doses tested **(Table 4.1)**. Similarly, no effects of AP treatments were detected in the inverted screen test for detecting muscle relaxing, or for assessing potential neurotoxicity of test agents **(Table 4.2)**. During these tests, no effects of AP on body weight or on behaviour of animals in their home cages were observed. These observations reaffirm that oral doses of AP up to 800 mg/kg administered daily for ten consecutive days are well tolerated by the animals, and do not cause any extra pyramidal side effects.

4.2.3. Cage-climbing test: Many psychoactive agents stimulating central dopaminergic system induce stereotypic and other behavioural changes in rodents. Apomorphine is one such clinically used dopamine agonist commonly used to test the involvement of central dopaminergic system in the modes of actions of anxiolytic, sedative, or antipsychotic agents. The cage-climbing test used in this study is a convenient one for quantifying such effects. A single or 5 or 7 daily oral dose of AP up to 800 mg/kg does not influence apomorphine-induced cage-climbing behaviour in rats. However, statistically significant anti-dopaminergic effects ($p<0.05$) of the extract were observed after its 10 daily 100 mg/kg or higher oral doses. The results summarized in **Figure 4.6A** and the corresponding dose response curve shown in **Figure 4.6B**, clearly reveal that the maximal possible anti-dopaminergic effect of AP in this test was achieved after its 10 daily 200 mg/kg oral doses.

4.3. Pilot Experiments with Andrographolide

4.3.1. Stress-induced hyperthermia and pentobarbital-induced hypnosis: The body weights and rectal temperatures of all age-matched animals used in the three described experiments on the first experimental day were within physiological ranges. During course of the experiments the mean body weights of

all the three vehicle treated control groups decreased consistently, and their mean basal core temperatures recorded on the days 5, 7, 10 and 11 of the experiments were significantly higher ($p<0.05$) than those observed on the first experimental days **(Figures 4.7A** and **4.7B)**. Such elevations of basal core temperature of the control groups in the two experiments conducted only with male or female animals occurred gradually during the course of the experiments, and remain constant on the 10^{th} and 11^{th} experimental days. Such was not the case for the control group in the pilot experiment where equal numbers of male and female animals were used in all experimental groups. In this experiment, the mean basal rectal temperatures of the control group recorded on the 5^{th} day almost equal in magnitude to those recorded on subsequent observational days of the experiment **(Figure 4.7B)**. The magnitude of this elevation in basal core temperatures due to daily handling and transient foot-shocks on the 10^{th} and 11^{th} days of control groups were almost equal in all the three experiments, and such was also the case for the magnitude of foot-shock stress triggered transient hyperthermia in all the three vehicle treated control groups **(Figure 4.7C)**. The mean time of onset of sleep and duration of sleep observed after pentobarbital challenge on the 11^{th} day of the experiments observed in all the three control groups were also almost equal in magnitudes **(Figure 4.7D)**.

Observed effects of daily oral doses of andrographolide, and number of treatment days, on the four parameters quantified in the pilot experiment are summarized in **Figure 4.8**. These results revealed that even a very high single oral dose of andrographolide (300 mg/kg) do not alter the basal rectal temperature of the animals, and had no statistically significant effects on their acute responses to foot-shock triggered hyperthermia. However, significant inhibitory effects ($p<0.05$) of even the lowest tested andrographolide doses (3 mg/kg/day) on both these quantified parameters were apparent on the 5^{th} and subsequent days of the experiment. Body weight losses of the animals of the 3 mg/kg/day andrographolide treated group on the 5^{th} and subsequent experimental days were also less severe than that observed in the vehicle treated control group on these days. Mean body weights of all other andrographolide

treated groups on this day were significantly higher ($p<0.05$) than those recorded for the groups on the day 1 of the experiment, and remained almost constant during the subsequent experimental days **(Figure 4.8A)**. Mean basal core temperatures of the groups treated with 3, 10 and 30 mg/kg/day andrographolide on the 1st and 5th experimental days were almost identical to each other. Numerically, the mean basal core temperatures of the 100 and 300 mg/kg/day andrographolide treated groups on day 5 were somewhat higher than (but not statistically significantly different from) those recorded for the groups on day 1. Mean basal core temperatures of all andrographolide treated groups on 7th, 10th and 11th days remained almost constant, and were always significantly lower ($p<0.05$) than those of the control group on these days **(Figure 4.8B)**.

Statistical significant dose and duration of treatment dependent antagonistic effects ($p<0.05$) of andrographolide against foot-shock triggered hyperthermia were also observed on the 5th, 7th and 10th day of the pilot experiment **(Figure 4.8C)**. Such observed efficacy of andrographolide observed on the 5th day was much lower than the efficacies observed on the 7th and 10th days. Hereupon efficacies of the highest two tested doses were almost identical. However, even after the highest andrographolide dose tested (300 mg/kg/day) the foot-shock stress triggered hyperthermic responses were only partially antagonized on both these days. The estimated ED_{50} values of andrographolide in this test on the 7th and 10th days of the experiment were 10.88 and 10.35 mg/kg/day respectively. Dose-dependent potentiating effects of 10 daily andrographolide treatments on pentobarbital-induced sedation and hypnosis were observed on the 11th day of the experiment. Log-dose response curves for these effects of andrographolide are shown in **Figure 4.8D**. The estimated ED_{50} vales of andrographolide for prolongation of onset time and duration of sleep induced by pentobarbital were 13.76 and 12.36 mg/kg/day respectively.

The subsequent two experiments were conducted not only to verify the observations made in the pilot experiment, but also to test whether the observed efficacy of andrographolide could be differ in male and female animals. Results of

these two experiments are summarized in **Figures 4.9, 4.10** and **4.11**. Although somewhat lower mean body weights, higher basal rectal temperatures, and calculated values of foot-shock triggered hyperthermia of vehicle treated female control group on the first and subsequent observational days of the experiments. However, these mean values were not statistically significantly different from the corresponding values of the vehicle treated male control group **(Figure 4.9)**, and no statistically significant differences between the effects of daily handling and transient foot-shocks in male and female mice were observed during the entire experimental period. Such were also the cases for all dose-dependent effects of andrographolide, or those of the tested dose (5 mg/kg/day) of the anxiolytic diazepam used as a reference standard in these experiments.

4.4. General Neuropharmacological Screening

4.4.1. Potentiation of pentobarbital-induced hypnosis: Results summarized in **Figure 4.12** reveal that daily treatment with AP for 10 consecutive days shortens sleep induction period and prolongs duration of sleep induced by pentobarbital. These statistically significant ($p<0.05$) effects of the extract were observed after its two highest doses tested (i.e. 100 and 200 mg/kg/day), and qualitatively the efficacy of 200 mg/kg/kg dose of AP was almost equal to those of the anxiolytic diazepam (5 mg/kg).

4.4.2. Spontaneous locomotor activity: Significant dose-dependent inhibitory effect ($p<0.05$) of 10 daily oral 100 and 200 mg/kg doses of AP on locomotor activity counts were observed ($p<0.05$). Qualitatively, the observed effect of the highest AP dose tested was lower than that of 1 mg/kg dose of the standard anxiolytic lorazepam **(Figure 4.13)**.

4.4.3. Maximal electroshock (MES) seizures in rats: Ten daily oral 50 and 100 mg/kg doses of AP had no significant anticonvulsant activity in various phases of MES-induced seizures like tonic flexion, hind limb tonic extension (HLTE), clonus and stupor. However, the highest AP dose tested (200 mg/kg/day, p.o.) did show significant ($p<0.05$) anticonvulsant like efficacy in this test. Efficacy of the

standard anticonvulsant drug phenytoin (30 mg/kg, p.o.) was much higher than that of the extract **(Table 4.3)**.

4.4.4. Pentylenetetrazole (PTZ)-induced convulsions in mice: Oral daily doses of 50 and 100 mg/kg AP for ten days had no significant anticonvulsant activity quantified as latency in myoclonic jerk and percentage clonic seizure by PTZ challenged mice. However, significant ($p<0.05$) anticonvulsant activity of the highest daily AP dose tested (200 mg/kg/day) and that of the standard anxiolytic diazepam (10 mg/kg, p.o.) were apparent in this test **(Table 4.4)**.

4.5. Anti-diabesity Activity

4.5.1. Oral glucose tolerance test: Dose-dependent effect of AP was observed 30 min after the oral glucose administration compared to control rats ($p<0.05$). Blood glucose levels of different groups observed immediately before oral glucose load and 30, 60 and 120 min thereafter are summarized in **Figure 4.14**.

4.5.2. Effect on body weight, glucose, insulin level, lipids profile and anti-oxidant enzyme activity of type-2 diabetic rats: During the ten days treatment period, the vehicle treated normal control group gained body weight (5.00 ± 0.30 g), whereas loosed body weight of vehicle treated diabetic group (7.67 ± 0.62 g). Beneficial effects of AP or of glibenclamide treatments on body weight changes, plasma glucose and insulin levels of diabetic rats were apparent from the data summarized in **Table 4.5**. These dose-dependent effects of AP were qualitatively analogous to the anti-diabetic drug glibenclamide. AP treatments dose-dependently reduces the elevated plasma levels of total cholesterol, triglycerides and LDL, and increases the lowered plasma HDL levels observed in diabetic animals compared to diabetic control ($p<0.05$). These effects of AP treatments were also qualitatively analogous to those of glibenclamide treatment **(Figure 4.15)**. Analogous were also the effects of AP treatments on elevated MDA levels, and reduced SOD and CAT activities in the all the three studied organs of diabetic rats. In our experiment, STZ-nicotinamide-induced diabetic rats showed significant increase ($p<0.05$) in lipid peroxidation in liver, kidney and pancreas, as measured in terms of MDA when compared to nondiabetic normal control

rats**.** On the other hand the activity of superoxide dismutase and catalase reduced significantly in these organs of diabetic rats (p<0.05) compared to nondiabetic normal control rats. Subsequently treatment with AP or glibenclamide significantly normalised these parameters in liver and pancreas of diabetic rats and resulted in restoration of normal oxidative status **(Table 4.6)**.

4.5.3. Histological examinations in type-2 diabetic rats: Representative pictures of histological slides of pancreas, liver, kidney and spleen of different groups of diabetic animals are shown in **Figure 4.16**. Pancreatic tissues of vehicle treated diabetic rats had diminished glandular acini, islets of Langerhans (necrosis of beta cells), and atrophy in pancreatic duct. These pathologies were less severe in all AP or glibenclamide treated groups **(Figure 4.16A)**, and such protective effects of AP treatments were more prominent in the highest dose of AP treated animals. Such were not always the cases for other organ pathologies studied. No beneficial effects of glibenclamide treatment on hepatic pathologies encountered in diabetic animal were observed, whereas these pathologies were less severe, or almost absent, in different AP treated groups **(Figure 4.16B)**. Treatments with 50 mg/kg/day AP or with glibenclamide (10 mg/kg/day) had no observable effects on renal pathologies observed in diabetic rats. However, these pathologies observed in diabetic rats treated with 100 or 200 mg/kg/day were either less severe or completely absent **(Figure 4.16C)**. Although the spleen pathologies observed in the vehicle, glibenclamide, 50 or 100 mg/kg/day AP treated animals were qualitatively similar, the histological pictures of the spleens of the 200 mg/kg/day AP treated animals were like those of vehicle treated normal laboratory rats **(Figure 4.16D)**.

4.5.4. High fat fed obesity model: During the 10-day treatment period the vehicle treated obese rats continued to gain more body weight, and consumed more food and water than the vehicle treated normal control ones (p<0.05). Levels of plasma glucose, insulin, total cholesterol, triglycerides, and LDL were all elevated in the obese control group, and the mean plasma HDL levels in this group were significantly (p<0.05) lower than that of the normal control one. Results summarized in **Table 4.7** and **Figure 4.17** revealed dose-dependent

beneficial effects of AP treatments against all these parameters. Qualitatively, all observed effects of AP treatments were analogous to those of the standard anti-hyperlipidemic drug atorvastatin.

4.5.5. Fructose fed obesity model: Data summarized in **Table 4.8** revealed that the body weight gains of the obese control rats observed during the ten day treatment period were significantly ($p<0.05$) higher than those of the AP or atorvastatin treated groups. These effects of drug treatments were accompanied with significantly ($p<0.05$) lower plasma levels of glucose and insulin. Moreover, the elevated plasma levels of total cholesterol, triglycerides and LDL, and lower plasma levels of HDL observed in fructose fed rats were also dose-dependently antagonized by AP treatments **(Figure 4.18)**. All these observed effects of AP treatments were qualitatively analogous to that of the anti-hyperlipidemic drug atorvastatin.

4.6. Antidepressant Activity

4.6.1. Body weight: There was no significant effect of AP or imipramine treatments on body weight of nondiabetic rat compared to vehicle treated nondiabetic groups. Moreover, the vehicle treated as well as AP treated nondiabetic rats demonstrated normal body weight gain **(Table 4.9)**. Unlike in the nondiabetic control group, the mean body weight of the vehicle treated diabetic control group during the treatment period decreased considerably. Such body weight losses were less severe in the 50 and 100 mg/kg/day AP treated diabetic group, and 200 mg/kg/day AP treated diabetic animals gained some body weights during the 10 days of the treatment. Imipramine treatments had no significant effects on the body weight losses of diabetic animals **(Table 4.10)**.

4.6.2. Behavioural despair test: The extract at doses of 50, 100 and 200 mg/kg significantly ($p<0.05$) decreased the duration of immobility (sec) in nondiabetic rats in a dose-dependent manner compared to vehicle treated nondiabetic control rats. Qualitatively, the effect of AP at dose of 200 mg/kg was analogous to that of standard drug (imipramine 15 mg/kg) treated group **(Figure 4.19A)**. Mean immobility period of the diabetic control group was significantly ($p<0.05$)

higher than that of the nondiabetic control group. Ten daily AP treatments to diabetic rats at doses of 50, 100 and 200 mg/kg/day significantly ($p<0.05$) and dose-dependently decreased the duration of immobility. Numerically, the mean immobility period of 200 mg/kg/day AP treated group was somewhat lower than that of the group treated with 15 mg/kg/day imipramine. However, efficacies of the two higher AP doses tested were almost equal in magnitude to that of the standard antidepressant drug imipramine **(Figure 4.19B)**.

4.6.3. Learned helplessness test: The extract at doses of 50, 100 and 200 mg/kg significantly ($p<0.05$) decreased the escape failures in nondiabetic rats in a dose-dependent manner compared to vehicle treated nondiabetic control rats. Qualitatively, the effect of AP at dose of 200 mg/kg was analogous to that of standard drug (imipramine 15 mg/kg) treated group **(Figure 4.20A)**. Mean numbers of escape failures of the vehicle treated diabetic control group in this test on all the three observational days were significantly higher, than those observed for the nondiabetic control group. AP treatments significantly ($p<0.05$) and dose-dependently reduced the escape failures of diabetic rats on all test days, and its efficacy increased somewhat during the three experimental days. Efficacy of 100 mg/kg/day AP was similar in magnitude to that of 15 mg/kg/day of the standard antidepressant imipramine, and that of the 200 mg/kg/day AP treated group was somewhat higher than that of the antidepressant imipramine **(Figure 4.20B)**.

4.6.4. Blood glucose and insulin level: Mean blood glucose and insulin levels of the different test groups quantified just after completion of the learned helplessness test are summarized in **Tables 4.11** and **4.12**. There was no any significant effect of AP or imipramine treatments on blood glucose and insulin levels of nondiabetic rats (**Table 4.11**). Similarly, ten daily imipramine treatments had no effects on hyperglycemia or on insulin deficiency observed in diabetic animals. However, AP treatments significantly ($p<0.05$) and dose-dependently decreased the blood glucose levels of diabetic animals, and the blood insulin levels of the diabetic rats were also significantly ($p<0.05$) and dose-dependently increased by AP treatments (**Table 4.12**).

4.6.5. Monoamines level in hippocampus: AP treatments in nondiabetic rats demonstrated significant increased in all the three monoamines level in hippocampus part of nondiabetic rats. Qualitatively, the efficacy of higher dose of AP (200 mg/kg) was higher than imipramine (15 mg/kg) treated group **(Figure 4.21A).** Hippocampal levels of all the three monoamines quantified (NA, DA, 5-HT) in vehicle treated diabetic control rats were lower than those observed in nondiabetic control rats. AP (50, 100 and 200 mg/kg) treatments to diabetic rats significantly and dose-dependently increased the levels of all the three monoamines in diabetic animals, and their levels observed in the 100 and 200 mg/kg/day AP treated diabetic groups rats were higher than those of the nondiabetic control group. Such efficacies of imipramine (15 mg/kg/day) in diabetic rats were somewhat lower than the efficacy observed after the intermediate dose of AP (100 mg/kg/day) treated diabetic group **(Figure 4.21B).**

4.6.6. Monoamine oxidase activity in hippocampus: Results of the MAO-A and MAO-B assays conducted with mitochondria preparations from rat hippocampus of different groups of animals used in the learned helplessness test are summarized in **Figure 4.23**. Unlike imipramine treated group, the MAO-A and MAO-B activities in AP treated nondiabetic rats were significantly decreased **(Figure 4.22A).** Mean enzymatic activities observed in the diabetic control group were significantly higher than the enzyme activity of the nondiabetic control group. Imipramine treatment had no significant effects on these enzymatic activities in diabetic animals. AP treatments dose-dependently decreased enzymatic activities of both MAO-A and MAO-B in diabetic animals, and the MAO-A activity levels quantified in the 100 and 200 mg/kg/day AP treated groups were even lower than that estimated in the vehicle treated nondiabetic control group **(Figure 4.22B)**.

4.6.7. Antioxidative status in frontal cortex: Unlike imipramine (15 mg/kg), AP treatments dose-dependently and significantly lowered LPO level in nondiabetic rats, and the SOD and CAT activities of AP treated nondiabetic animals were significantly higher than those of the nondiabetic control group **(Figure 4.23A)**.

The results summarized in **Figure 4.23B** revealed that in comparison to the nondiabetic control group, mean LPO level and SOD and CAT activities in frontal cortex of the diabetic control group were significantly altered. Imipramine treatments had no significant effects on any of these assayed parameters. AP treatments dose-dependently and significantly lowered LPO level in diabetic animals, and the SOD and CAT activities of AP treated diabetic animals were significantly higher than those of the diabetic control group. Mean LPO value of the higher two AP dose (100 and 200 mg/kg/day) treated diabetic groups was lower than that of the nondiabetic control group, and the SOD and CAT activities of these two AP treated groups were either higher or equal to those of the nondiabetic control one.

4.7. Anxiolytic Activity

4.7.1. Body weight: There was no any significant effect of AP or lorazepam treatments on body weight of nondiabetic rats compared to vehicle treated nondiabetic groups. Moreover, the vehicle treated as well as AP treated nondiabetic rats demonstrated normal body weight gain (**Table 4.13**). Unlike in the nondiabetic control group, the mean body weights of the vehicle treated diabetic control group during the treatment period decreased considerably. Such body weight losses were less severe in the 50 and 100 mg/kg/day AP treated diabetic group, and 200 mg/kg/day AP treated diabetic animals gained some body weights during the 10 days of the treatment. Imipramine treatments had no significant effects on the body weight losses of diabetic animals **(Table 4.14)**.

4.7.2. Social interaction test: During this test, total time spent by the rat pair in 'social interaction', including sniffing, following, grooming, kicking/boxing, biting and crawling under or over the partner, was recorded. There was significant ($p<0.05$) increase in the total time of interactions of nondiabetic rats treated with AP (50, 100 and 200 mg/kg), or lorazepam (1 mg/kg) compared to nondiabetic control rats **(Figure 4.24A)**. The diabetic control rats demonstrated significant ($p<0.05$) less interaction time compared to nondiabetic control rats. Similar to lorazepam, AP treatments in diabetic rats demonstrated significant

($p<0.05$) and dose-dependent increase in total time of interactions compared to diabetic control rats **(Figure 4.24B)**.

4.7.3. Light-dark box (LDB) test: Mean latencies of the all AP treated groups (50, 100 and 200 mg/kg/day for 10 consecutive days) to enter the light chamber of the test box were significantly ($p<0.05$) lower than that of the vehicle treated nondiabetic control group. The mean numbers of entries and duration of residence time of the all AP treated groups were also significantly ($p<0.05$) higher than the control group **(Figure 4.25A)**. Qualitatively, the efficacy of 200 mg/kg dose of AP for all the three parameters quantified were similar to those observed for 1 mg/kg daily oral doses of the standard anxiolytic lorazepam. Diabetic control rats demonstrated significant ($p<0.05$) increase in mean latencies to entry, decrease in numbers of entries and decrease in residence time in light box compared to nondiabetic control rats. Similar to lorazepam, AP treatments in diabetic rats demonstrated significant ($p<0.05$) increase in mean latencies, increase in numbers of entries and increase in residence time in light box compared to diabetic control rats **(Figure 4.25B).**

4.7.4. Elevated plus maze test: Statistically significant ($p<0.05$) increase in mean numbers of entries and mean time spent in the open arm of the all the AP (50, 100 and 200 mg/kg), or lorazepam (1 mg/kg) treated nondiabetic groups were observed. These observations reconfirm that ten daily 50 mg/kg oral doses of AP is high enough to observe its significant anxiolytic like activities in rat models **(Figure 4.26A)**. Vehicle treated diabetic rats shown significant decrease in mean numbers of entries and mean time spent in the open arm compared to nondiabetic control. AP treated diabetic rats demonstrated significant and dose-dependent increase in mean numbers of entries and mean time spent in the open arm compared to nondiabetic control. Qualitatively, observed anxiolytic-like effects of AP (100 mg) were qualitatively comparable to lorazepam **(Figure 4.26B).**

4.7.5. Blood glucose and insulin level: Mean blood glucose and insulin levels of the different test groups quantified just after completion of elevated plus maze test are summarized in **Tables 4.15** and **4.16**. There was no any significant effect

of AP or lorazepam treatments on blood glucose and insulin levels of nondiabetic rats (**Table 4.15**). Similarly, ten daily lorazepam treatments had no effect on hyperglycemia or on insulin deficiency observed in diabetic animals. However, AP treatments significantly (p<0.05) and dose-dependently decreased the blood glucose levels of diabetic animals, and the blood insulin levels diabetic rats were also significantly (p<0.05) and dose-dependently increased by AP treatments (**Table 4.16**).

4.7.6. Antioxidative status in frontal cortex: Unlike lorazepam (1 mg/kg), AP treatments dose-dependently and significantly (p<0.05) lowered LPO level in nondiabetic rats, and the SOD and CAT activities of AP treated nondiabetic animals were significantly (p<0.05) higher than those of the nondiabetic control group **(Figure 4.27A)**. The results summarized in **Figure 4.27B** revealed that in comparison to the nondiabetic control group, mean LPO level and SOD and CAT activities in frontal cortex of the diabetic control group were significantly (p<0.05) altered. AP treatments dose-dependently and significantly (p<0.05) lowered LPO level in diabetic animals, and the SOD and CAT activities of AP treated diabetic animals were significantly (p<0.05) higher than those of the diabetic control group.

4.8. Nootropic Activity

4.8.1. Body weight: There was no any significant effect of AP, andrographolide or piracetam treatments on body weight of nondiabetic rat compared to vehicle treated nondiabetic one. Moreover, the vehicle treated as well as AP or andrographolide treated nondiabetic rats demonstrated normal body weight gain (**Table 4.17**). Unlike in the nondiabetic control group, the mean body weight of the vehicle treated diabetic control group during the treatment period decreased considerably. Such body weight losses were less severe in the AP or andrographolide treated diabetic group, and gained some body weight during the 10 days of the treatment. Piracetam treatments had no significant effects on the body weight losses of diabetic animals **(Table 4.18)**.

4.8.2. Morris water-maze (MWM) task: Escape latencies to find the platform by AP, andrographolide or piracetam treated nondiabetic rats were significantly decreased on days 8 and 9 compared to nondiabetic control on day 7 indicating increased learning activity. There were also significant differences in escape latencies between groups in the doses tested on same days **(Figure 4.28A)**. Twenty-four hours after the spatial reference memory task in the MWM (on day 10), probe trial was conducted to evaluate the retention of memory. Observation data revealed that there were significant differences among the groups on probe trial day. Rat treated with AP, andrographolide or piracetam showed significant longer mean time spent in the target quadrant of the maze compared to nondiabetic controls **(Figure 4.29A)**. Diabetic control rats showed significant increased escape latencies to find the platform during test days and less time spent in targeted quadrant compared to nondiabetic control rats showing amnesic-like behaviour. However, treatments with AP, andrographolide or piracetam successfully reverse the amnesic behaviour on day 8 and 9 as significant decreased escape latencies were observed compared to diabetic control on day 7 and between doses groups on the same day. In targeted quadrant, drugs treated rats demonstrated significantly increase in duration of time spent compared to diabetic control rats **(Figures 4.28B** and **4.29B)**. The efficacy of all the three doses of andrographolide is somewhat quantitatively comparable to AP treatment doses.

4.8.3. Blood glucose and insulin level: Mean blood glucose and insulin levels of the different test groups quantified just after completion of water-maze task (prob trial) are summarized in **Tables 4.19** and **4.20**. There was no significant effect of AP, andrographolide or piracetam treatments on blood glucose and insulin levels of nondiabetic rats (**Table 4.19**). Similarly, ten daily piracetam treatments had no effects on hyperglycemia or on insulin deficiency observed in diabetic animals. However, AP or andrographolide treatments significantly ($p<0.05$) and dose-dependently decreased the blood glucose levels of diabetic animals, and the blood insulin levels diabetic rats were also significantly

(p<0.05) and dose-dependently increased by AP or andrographolide treatments (**Table 4.20**).

4.8.4. Antioxidative status in frontal cortex: AP or andrographolide treatments dose-dependently and significantly lowered LPO level in nondiabetic rats, and the SOD and CAT activities of these drugs treated nondiabetic animals were significantly higher than the enzyme activity of the nondiabetic control group **(Figure 4.30A)**. The results summarized in **Figure 4.30B** revealed that in comparison to the nondiabetic control group, mean LPO level and SOD and CAT activities in frontal cortex of the diabetic control group were significantly altered. AP or andrographolide treatments dose-dependently and significantly lowered LPO level in diabetic animals, and the SOD and CAT activities of these drugs treated diabetic animals were significantly higher than the enzyme activity of the diabetic control group. The efficacies of AP treatments were quantitative comparable to corresponding doses of andrographolide. Piracetam treated nondiabetic and diabetic rats demonstrated significant decreased in LPO and increase in SOD and CAT activities.

4.8.5. Acetylcholinesterase (AChE) enzyme activity: AChE activity of the different test groups rats were quantified just after completion of water-maze task (prob trial). The effect of AP, andrographolide or piracetam on cholinesterase activity in distinct brain regions of nondiabetic and diabetic rats are shown in **Figure 4.31**. The analysis of data showed that AChE activity was significantly different between groups. AP or andrographolide dose-dependently decreased AChE activity in prefrontal cortex and hippocampus compared to nondiabetic control rats **(Figure 4.31A)**. The diabetic rats showed significant increase in AChE enzyme activity in both brain regions compared to nondiabetic control rats. However, AP, andrographolide or piracetam treated diabetic rats showed significant decrease in AChE enzyme activity in prefrontal cortex and hippocampus compared to diabetic control rats **(Figure 4.31B)**.

4.9. Other Neuropsychopharmacological Tests

4.9.1. Spontaneous locomotor activity: The body weight and glucose level data of animals from spontaneous locomotor activity are shown in **Tables 4.21** and **4.22.** There was no any significant effect of AP, andrographolide or lorazepam treatments on body weight or blood glucose level of nondiabetic rats compared to vehicle treated nondiabetic rats. However, AP or andrographolide treatments significantly (p<0.05) reverse the body weight losses and decreased the elevated blood glucose levels in diabetic animals compared to diabetic control rats. There was no significant effect of lorazepam treatments on body weight or blood glucose level of diabetic rats.

AP (100 and 200 mg/kg) and andrographolide (15, 30 and 60 mg/kg) treatment in nondiabetic rats showed significant decrease in locomotor activity similar to standard drug lorazepam compared to nondiabetic control rats **(Figure 4.32A)**. The diabetic rats showed significant increase in locomotor activity compared to nondiabetic control rats. However, lorazepam-like decrease in locomotor activity was observed in AP (100 and 200 mg/kg) and andrographolide (15, 30 and 60 mg/kg) treated diabetic rats **(Figure 4.32B).**

4.9.2. 5-Hydroxytryptophan (5-HTP) head twitches test in mice: The body weight and glucose level data of animals from 5-Hydroxytryptophan (5-HTP) head twitches test are shown in **Tables 4.23** and **4.24.** There was no significant effect of AP, andrographolide or imipramine treatments on body weight or blood glucose level of nondiabetic mice compared to vehicle treated nondiabetic mice. However, AP or andrographolide treatments significantly (p<0.05) reversed the body weight losses and decreased the elevated blood glucose levels in diabetic mice compared to diabetic control mice. There was no significant effect of imipramine treatments on body weight or blood glucose level of diabetic mice.

AP (50, 100 and 200 mg/kg) and andrographolide (15, 30 and 60 mg/kg) treatment in nondiabetic rats showed significant increase in head twitches similar to standard drug imipramine compared to nondiabetic control rats **(Figure 4.33A)**. The diabetic rats showed significant decreased head twitches

compared to nondiabetic control rats. However, imipramine like increased head twitches were observed in AP (50, 100 and 200 mg/kg) and andrographolide (15, 30 and 60 mg/kg) treated diabetic rats **(Figure 4.33B).**

4.9.3. L-Dopa potentiation test in mice: The body weight and blood glucose level data of animals from L-Dopa potentiation test are shown in **Tables 4.25** and **4.26.** There was no significant effect of AP, andrographolide or imipramine treatments on body weight or blood glucose level of nondiabetic mice compared to vehicle treated nondiabetic mice. However, AP or andrographolide treatments significantly (p<0.05) reversed the body weight losses and decreased the elevated blood glucose levels in diabetic mice compared to diabetic control mice. There was no significant effect of imipramine treatments on body weight or blood glucose level of diabetic mice.

AP (100 and 200 mg/kg) and andrographolide (30 and 60 mg/kg) treatment in nondiabetic rats showed significant increase in behaviour score (potentiation) similar to standard drug imipramine compared to nondiabetic control rats **(Figure 4.34A)**. The diabetic rats showed significant decrease in behaviour score compared to nondiabetic control rats. However, imipramine-like increased behaviour score (potentiation) were observed in AP (100 and 200 mg/kg) and andrographolide (30 and 60 mg/kg) treated diabetic rats **(Figure 4.34B).**

4.9.4. Apomorphine-induced cage-climbing test in mice: The body weight and glucose level data of animals from apomorphine-induced cage-climbing test are shown in **Tables 4.27** and **4.28.** There was no significant effect of AP, andrographolide or imipramine treatments on body weight or blood glucose level of nondiabetic mice compared to vehicle treated nondiabetic mice. However, AP or andrographolide treatments significantly (p<0.05) reversed the body weight losses and decreased the elevated blood glucose levels in diabetic mice compared to diabetic control mice. There was no significant effect of imipramine treatments on body weight or blood glucose level of diabetic mice.

AP (100 and 200 mg/kg) and andrographolide (30 and 60 mg/kg) treatment in nondiabetic rats showed significant decrease in climbing score similar to standard

drug imipramine compared to nondiabetic control rats **(Figure 4.35A)**. The diabetic rats showed significant increase in climbing score compared to nondiabetic control rats. However, imipramine-like decreased climbing score were observed in AP (100 and 200 mg/kg) and andrographolide (30 and 60 mg/kg) treated diabetic rats **(Figure 4.35B)**.

4.10. Anti-inflammatory and Analgesic Activity

4.10.1. Cotton pellet-induced granuloma in rats: The body weight and blood glucose level of animals from cotton pellet-induced granuloma test are shown in **Tables 4.29** and **4.30.** There was no significant effect of AP or andrographolide treatments on body weight or blood glucose level of nondiabetic rats compared to vehicle treated nondiabetic rats. However, AP or andrographolide treatments significantly (p<0.05) reversed the body weight losses and decreased the elevated blood glucose levels in diabetic rats compared to diabetic control rats.

AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) treatments in nondiabetic rats showed significant (p<0.05) decrease in cotton pellets-induced granuloma tissues weight similar to standard drug indomethacin compared to nondiabetic control rats **(Figure 4.36A).** The diabetic rats showed significant (p<0.05) increase in cotton pellets-induced granuloma tissues weight compared to nondiabetic control rats. However, diabetic rats treated with AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) demonstrated decreased cotton pellets-induced granuloma tissues weight similar to standard drug indomethacin **(Figure 4.36B)**.

4.10.2. Carrageenan-induced pedal oedema in rats: Unlike on day 1, AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) treatments in nondiabetic rats after 10 daily treatments showed significant (p<0.05) decrease in pedal oedema volume observed at different time interval up to six hours compared to nondiabetic control rats **(Figures 4.37A** and **4.38A).** The diabetic rats showed significant (p<0.05) increased pedal oedema volume compared to nondiabetic control rats on day 1 as well as on day 10. However unlike day 1, diabetic rats treated with 10 repeated daily dose of AP (100, 200 and 400

mg/kg), or andrographolide (30, 60 and 120 mg/kg) demonstrated decreased pedal oedema volume **(Figures 4.37B** and **4.38B).** Qualitatively, the efficacies of AP and andrographolide were similar to standard drug indomethacin. The percent inhibitions data of peal oedema test on day 10 in nondiabetic and diabetic rats are shown in **Figures 4.39A** and **4.39B.**

4.10.3. Tail flick test in rats: The body weight and blood glucose level data of animals from cotton pellet-induced granuloma test are shown in **Tables 4.31** and **4.32.** There was no significant effect of AP or andrographolide treatments on body weight or blood glucose level of nondiabetic rats compared to vehicle treated nondiabetic rats. However, AP or andrographolide treatments significantly (p<0.05) reversed the body weight losses and decreased the elevated blood glucose levels in diabetic rats compared to diabetic control rats.

Unlike on day 1, AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) treatments in nondiabetic rats after 10 daily treatments showed significant (p<0.05) increase in tail flick reaction time compared to nondiabetic control rats **(Figure 4.40A).** The diabetic rats showed significant (p<0.05) decrease in tail flick reaction time compared to nondiabetic control rats on day 1 as well as on day 10. However unlike day 1, diabetic rats treated with 10 repeated daily dose of AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) demonstrated increase in tail flick reaction time **(Figure 4.40B).** Qualitatively, the efficacies of AP and andrographolide were similar to standard drug pentazocine.

4.10.4. Hot plate reaction time in mice: The body weight and blood glucose level of animals from cotton pellet-induced granuloma test are shown in **Tables 4.33** and **4.34.** There was no significant effect of AP or andrographolide treatments on body weight or blood glucose level of nondiabetic mice compared to vehicle treated nondiabetic mice. However, AP or andrographolide treatments significantly (p<0.05) reversed the body weight losses and decreased the elevated blood glucose levels in diabetic mice compared to diabetic control mice.

Unlike on day 1, AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) treatments in nondiabetic mice after 10 daily treatments showed significant (p<0.05) increase in reaction time on hot plate compared to nondiabetic control mice **(Figure 4.41A).** The diabetic mice showed significant (p<0.05) decrease in reaction time on hot plate compared to nondiabetic control mice on day 1 as well as on day 10. However unlike day 1, diabetic mice treated with 10 repeated daily dose of AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) demonstrated increase in reaction time **(Figure 4.41B).** Qualitatively, the efficacies of AP and andrographolide were similar to standard drug pentazocine.

4.10.5. Formalin test in rats: AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) treatments in nondiabetic mice after 10 daily treatments showed significant (p<0.05) decrease in number of spontaneous flinch per minute compared to nondiabetic control mice at different time points **(Figure 4.42A).** The diabetic mice showed significant (p<0.05) increased number of spontaneous flinch per minute compared to nondiabetic control mice on day 10 at different time points. However, diabetic mice treated with 10 repeated daily dose of AP (100, 200 and 400 mg/kg), or andrographolide (30, 60 and 120 mg/kg) demonstrated decrease in number of spontaneous flinch per minute **(Figure 4.42B).** Qualitatively, the efficacies of AP and andrographolide were similar to standard drug pentazocine.

4.11. Anti-stress Activity

4.11.1. Body weight: Vehicle treated unstressed rats gained ca. 7 g body weight during the experimental period, whereas stressed controls continuously lost their body weight during the same period. Dose dependant and statistically significant beneficial effects (p<0.05) of treatments with AP (50, 100 and 200 mg/kg/day), or andrographolide (30 and 60 mg/kg/day), and that with WS (100 mg/kg/day) on body weight changes in stressed rats were observed **(Figure 4.43)**.

4.11.2. Adrenal gland and spleen weights: Significant (p<0.05) adrenal hypertrophy and spleen hypotrophy were apparent in stressed rats. AP (50, 100 and 200 mg/kg/day), andrographolide (30 and 60 mg/kg/day), or WS (100 mg/kg/day) treatments successfully reversed such stress-induced abnormality in weights of adrenal gland and spleen (p<0.05) towards normal value **(Figure 4.44A)**.

4.11.3. Plasma corticosterone: In comparison to vehicle treated control animals, elevated plasma corticosterone level was observed in stressed control rats. Treatments with AP (50, 100 and 200 mg/kg/day), or andrographolide (30 and 60 mg/kg/day), or WS (100 mg/kg/day) significantly (p<0.05) suppressed the elevation of plasma corticosterone level in stressed animals toward normal values **(Figure 4.44B)**.

4.11.4. Gastric ulceration: Results summarized in **Table 4.35** revealed that vehicle treated control animals had developed gastric ulcers, and that mean number of ulcers and their severity in stressed rats were significantly (p<0.05) lower in AP (50, 100 and 200 mg/kg/day), or andrographolide (30 and 60 mg/kg/day), or WS (100 mg/kg/day) treated groups. All quantified effects of AP and andrographolide against stress-induced ulcers were increased with their increasing daily doses.

4.11.5. Learned helplessness test: Results summarized in **Figure 4.45A** revealed that in comparison to non-stressed rats, the stressed ones had exaggerated depressive state (p<0.05) as judged by increased numbers of escape failures. Stressed rats treated with AP (50, 100 and 200 mg/kg/day), or andrographolide (30 and 60 mg/kg/day), or WS (100 mg/kg/day) had significantly (p<0.05) decreased numbers of escape failures. Efficacy of 100 mg/kg/day AP or of 30 mg/kg/day andrographolide was similar in magnitude to that of 100 mg/kg/day of WS used as a reference standard in this study.

4.11.6. Sexual behaviour: In comparison to normal rats, mounting behaviour of chronically stressed male rats was drastically reduced **(Figure 4.45B)**. Daily WS (100 mg/kg/day) treatments to stressed rats almost completely reversed such

behavioural deficits. Mean numbers of mount in the AP (50, 100 and 200 mg/kg/day), or andrographolide (30 and 60 mg/kg/day) treated groups also increased ($p<0.05$) with their increasing daily doses. These numbers for the highest AP or andrographolide dose tested were almost equal to those observed for the WS treated group.

4.11.7. Behavioural despair test: In comparison to the mean immobility period of non-stressed group, the mean immobility periods of the vehicle-treaded stressed one were significantly ($p<0.05$) higher. AP (50, 100 and 200 mg/kg/day) or andrographolide (30 and 60 mg/kg/day) treatment dose dependently reduced ($p<0.05$) the mean immobility periods in stressed rats. Observed efficacies of both andrographolide and AP were again qualitatively similar to those observed in the WS (100 mg/kg/day)-treated group **(Figure 4.45C)**.

4.11.8. Cytokine expressions: It is apparent from the results summarized in **Figure 4.46** that chronic stress procedure used in this study up-regulate the expressions of pro-inflammatory (TNF-α, IL-1β) as well as anti-inflammatory (IL-10) cytokines in both blood and brain of stressed rats. Except for blood IL-10 RNA levels in WS treated stressed rats, all other quantified RNA levels were not elevated in stressed rats, and some of these levels in this group were even lower than that quantified for the vehicle treated non-stressed control groups. However, blood IL-10 expression in WS treated stressed rats was also slightly but significantly suppressed ($p<0.05$) in stressed rats. Blood TNF-α and brain IL-1β expressions in stressed rats were dose dependently lowered by daily AP treatments. Observed effect of the higher andrographolide dose (60 mg/kg/day) in stressed rats on blood TNF-α expression was also somewhat higher than that observed after its lower dose tested (30 mg/kg/day). Elevated RNA levels of all other cytokines observed in stressed rats were always maximally suppressed even by the lowest tested daily doses of AP (50 mg/kg/day) or of andrographolide (30 mg/kg/day). Some of these RNA levels quantified in AP or andrographolide treated stressed groups were even lower than those of their levels observed in non-stressed rats. Statistically significant ($p<0.05$)

suppressive effects on stress triggered elevations of all cytokine expressions quantified were observed even after the lowest daily doses of AP or andrographolide tested in blood and in brain tissue.

4.12. HL-60 Cell-Line Study

4.12.1. In-vitro cell toxicity study: *In-vitro* cell cytotoxic effects of AP and andrographolide were assessed at different concentrations by MTT assay in HL-60 cells. Calculated IC_{50} value from percentage relative graph for AP and andrographolide was 48.50 µg/ml and 38.62 µM respectively **(Figures 4.47A** and **4.47B)**.

4.12.2. Cytokines and toll like receptors (TLRs) expression in HL-60 cell line: The non-cytotoxic concentration of andrographolide (10 µM) was chosen for the cytokines viz. TNF-α, IL-1β and IL-10 and toll like receptors (TLRs) expression viz. TLR-3, TLR-7 and TLR-8 study in HL-60 cell line. The Gel pictures showing mRNA expression of cytokines and TLRs normalised with β-actin (house-keeping gene) in **Figures 4.48A** and **4.49A.** HL-60 cell lines treated with andrographolide were demonstrated significant (p<0.05) decrease in fold change of TNF-α, IL-1β cytokines mRNA expression and significant (p<0.05) increase in fold change of IL-10 cytokine mRNA **(Figure 4.48B)**. Moreover unlike TLR-3, the fold change in mRNA expression of TLR-7 and TLR-8 were significantly (p<0.05) decreased in andrographolide treated cells compared to TLRs ligand control **(Figure 4.49B)**.

Figures and Tables

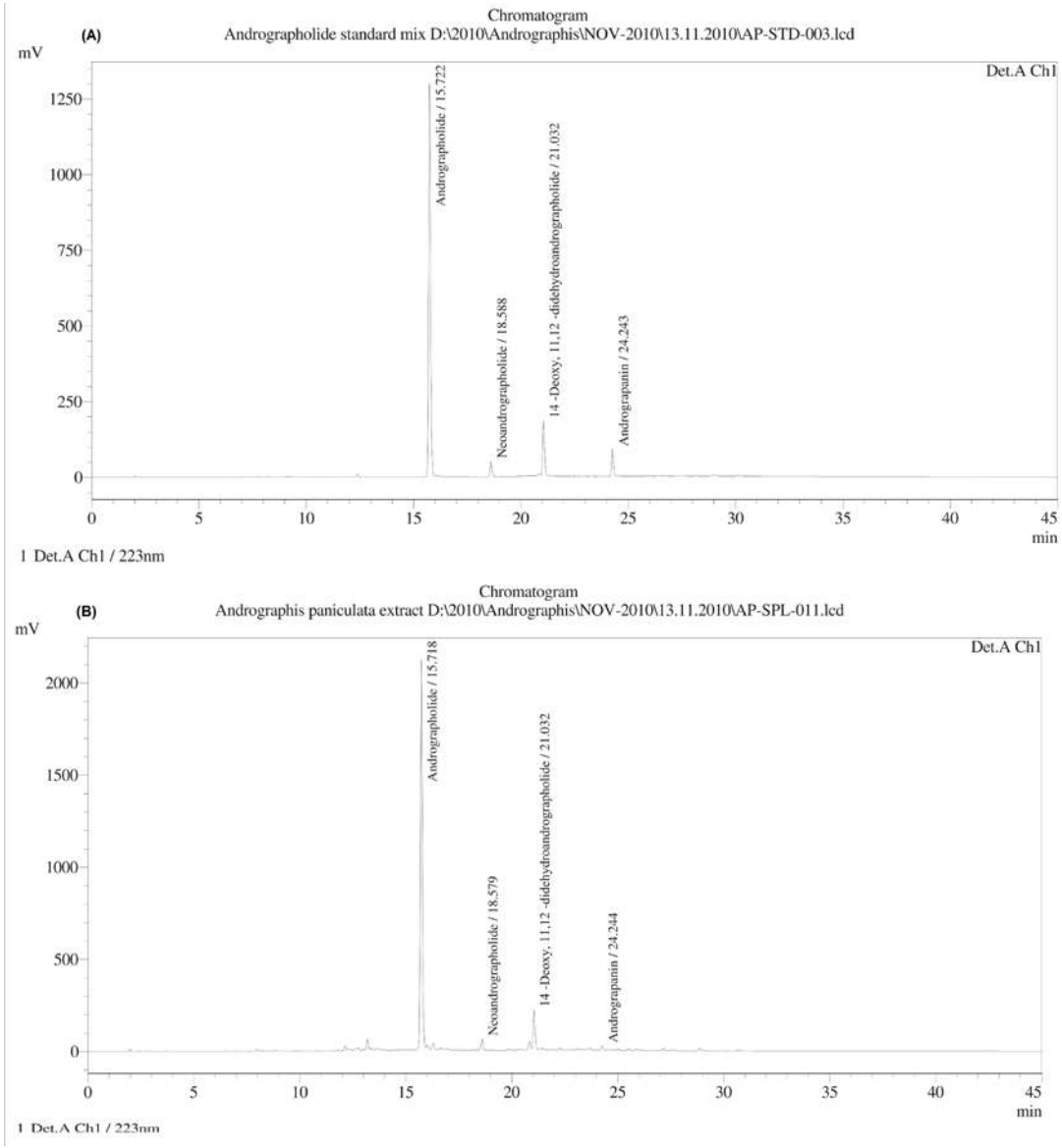

Figure 4.1: HPLC fingerprint of **(A)** standard mixtures and **(B)** *Andrographis paniculata* extract (AP; KalmCold™)

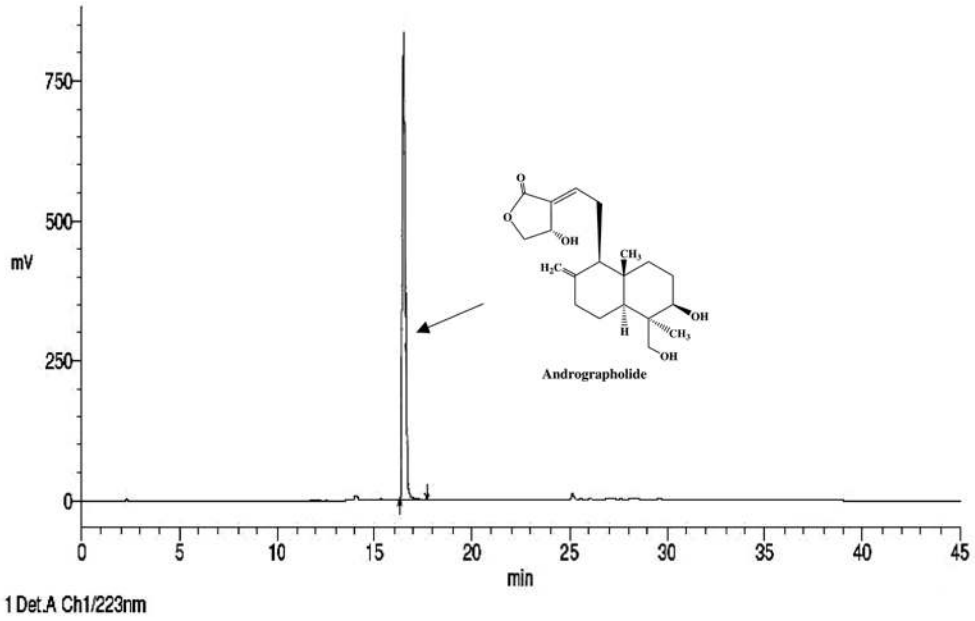

Figure 4.2: HPLC fingerprint of isolated pure andrographolide from *Andrographis paniculata* extract

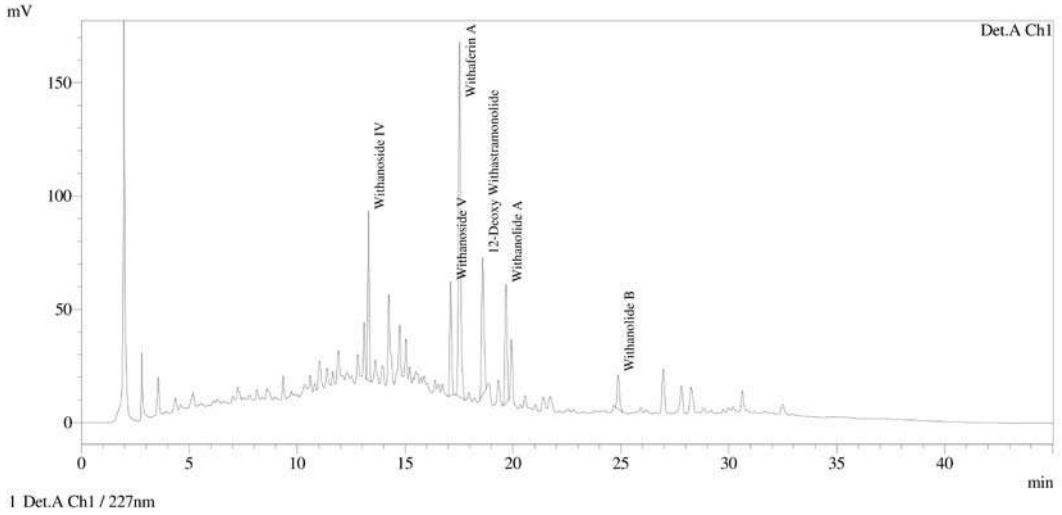

Figure 4.3: HPLC fingerprint of standardised root extract of *Withania somnifera*.

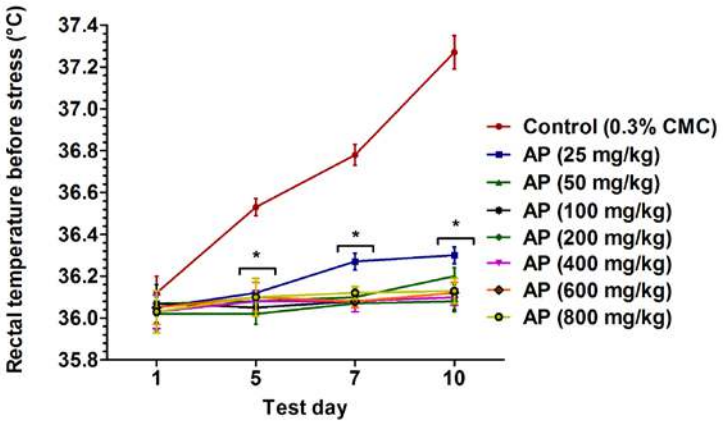

Figure 4.4: Effect of different dose of *Andrographis paniculata* extract (AP) on basal core temperatures of mice. *=p<0.05 vs. control

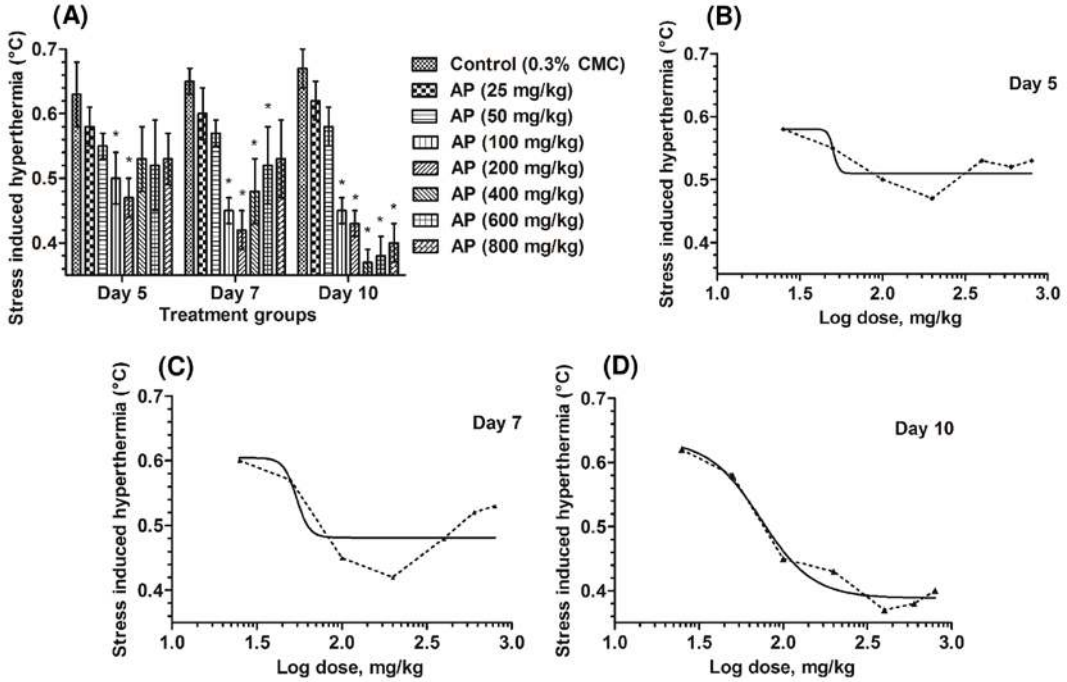

Figure 4.5: Effect of repeated treatment of *Andrographis paniculata* extract (AP) on **(A)** stress-induced hyperthermia in mice. Log dose response curves obtained from the data summarized in this figure are shown in **(B)**, **(C)** and **(D)**. *=p<0.05 vs. control. Dotted line represents the points of respective temperature values and continuous line represents a curve-fit (ED_{50} shift) line for log-dose response

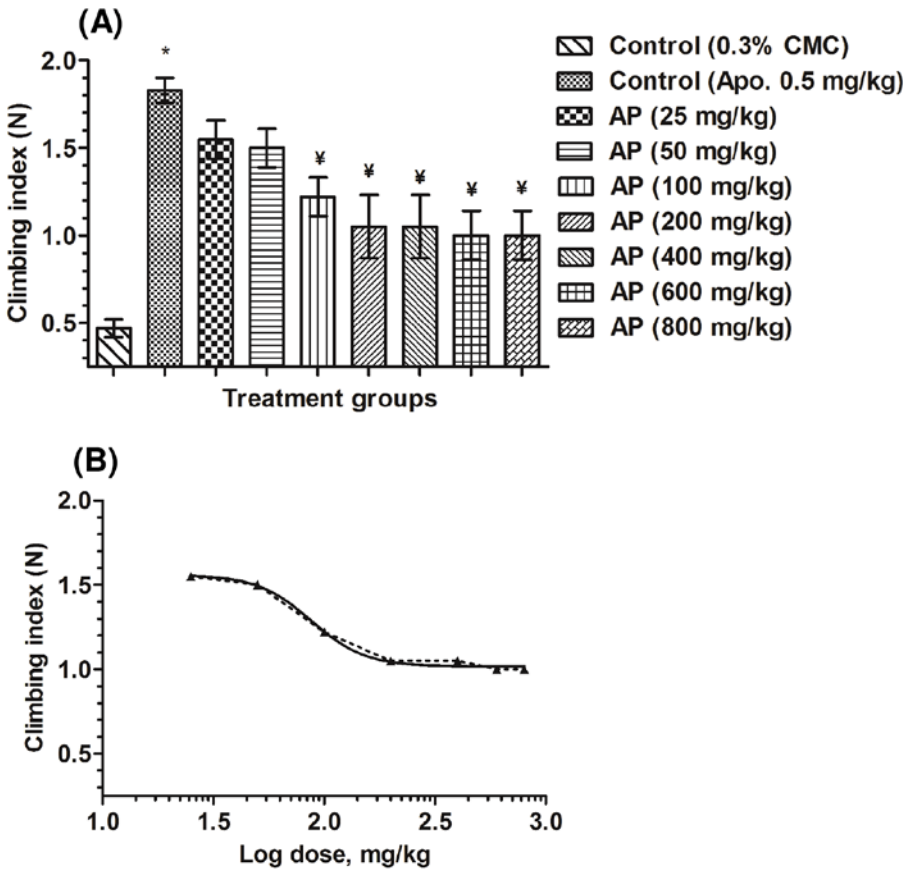

Figure 4.6: Effect of 10 daily oral doses of *Andrographis paniculata* extract (AP) on **(A)** apomorphine (Apo)-induced rats and **(B)** Log dose response curve for 10 daily oral doses of AP on apomorphine (Apo)-induced rats in cage-climbing test. n= 6/group, *=$p<0.05$ vs. control (CMC), and ¥=$p<0.05$ vs. control (Apo). Dotted line represents the points of respective temperature values and continuous line represents a curve-fit (ED_{50} shift) line for log-dose response

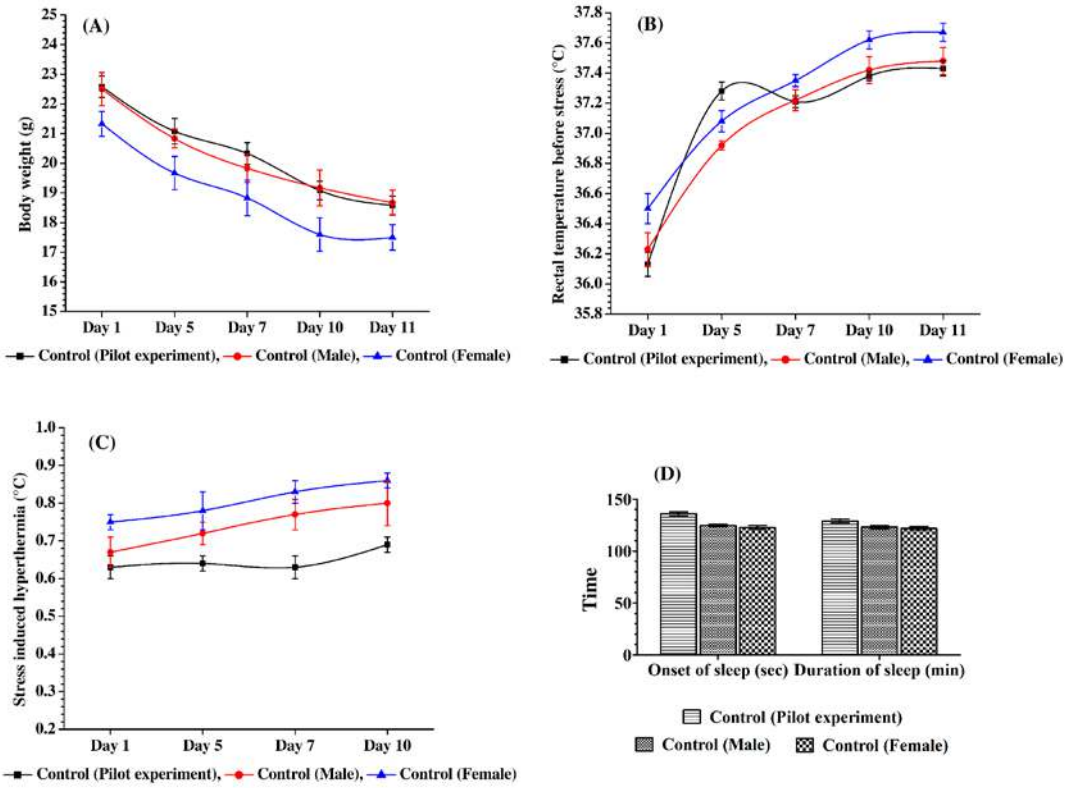

Figure 4.7: Mean (± SEM) of **(A)** body weight, **(B)** basal rectal temperatures, **(C)** foot-shock stress-induced hyperthermic responses, and **(D)** sleep onset periods and durations of sleep induced pentobarbital in the vehicle treated control groups used in the three experiments. Number of control animals used in the pilot experiment was 12, and in the two confirmatory experiments were 6 animals each. No statistically significant differences (two-tailed t-test) between the values observed in separate groups of male and female animals used in the confirmatory experiments were detected

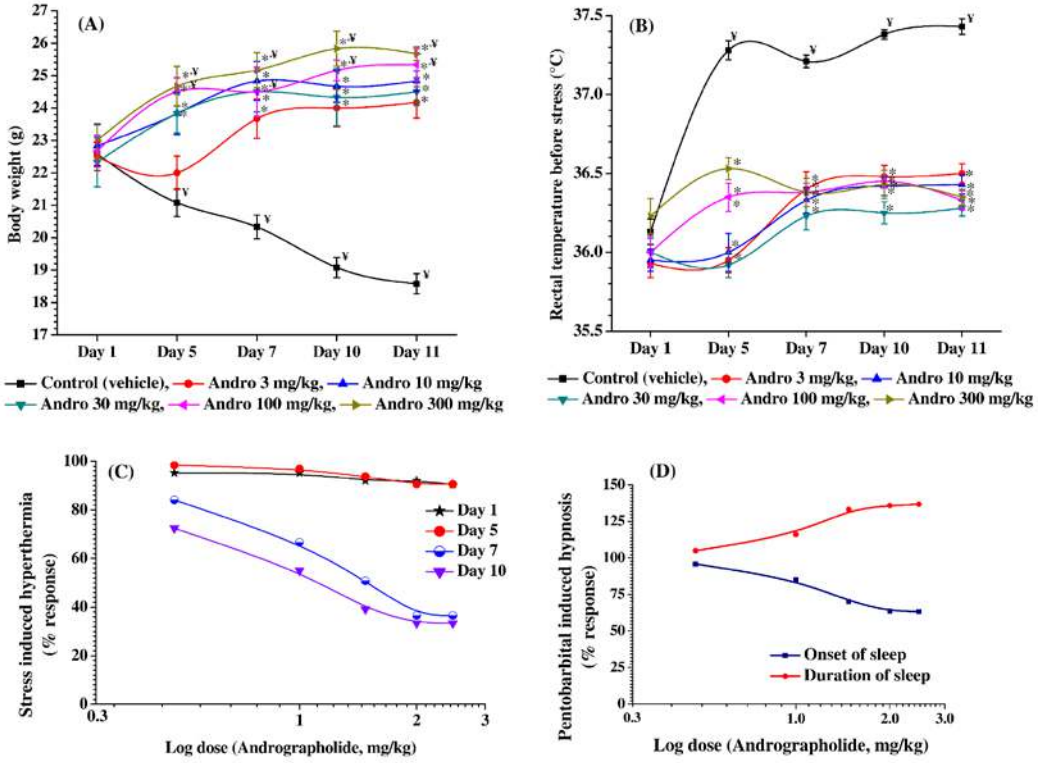

Figure 4.8: Effect of once daily oral doses of andrographolide for 10 consecutive days observed in the pilot experiment. **(A)** Mean body weight, and **(B)** basal core temperature quantified on different days of the experiment. Andrographolide dose response curves in the foot-shock stress-induced hyperthermia test conducted on days 1, 5, 7 and 10 of the experiment **(C)** and in the pentobarbital sleep test conducted on day 11 of the test **(D)** are shown in this figure. Observed statistically significant ($p<0.05$) mean values of andrographolide treated groups against the mean values of the control group on the same day are marked with *, and such differences ($p<0.05$) between the mean values of a group on day 1 and on subsequent days of the experiment are marked with the symbol ¥. Percent response values used in the dose response curves were calculated by considering the responses of the control group on the day of the experiment as 100%

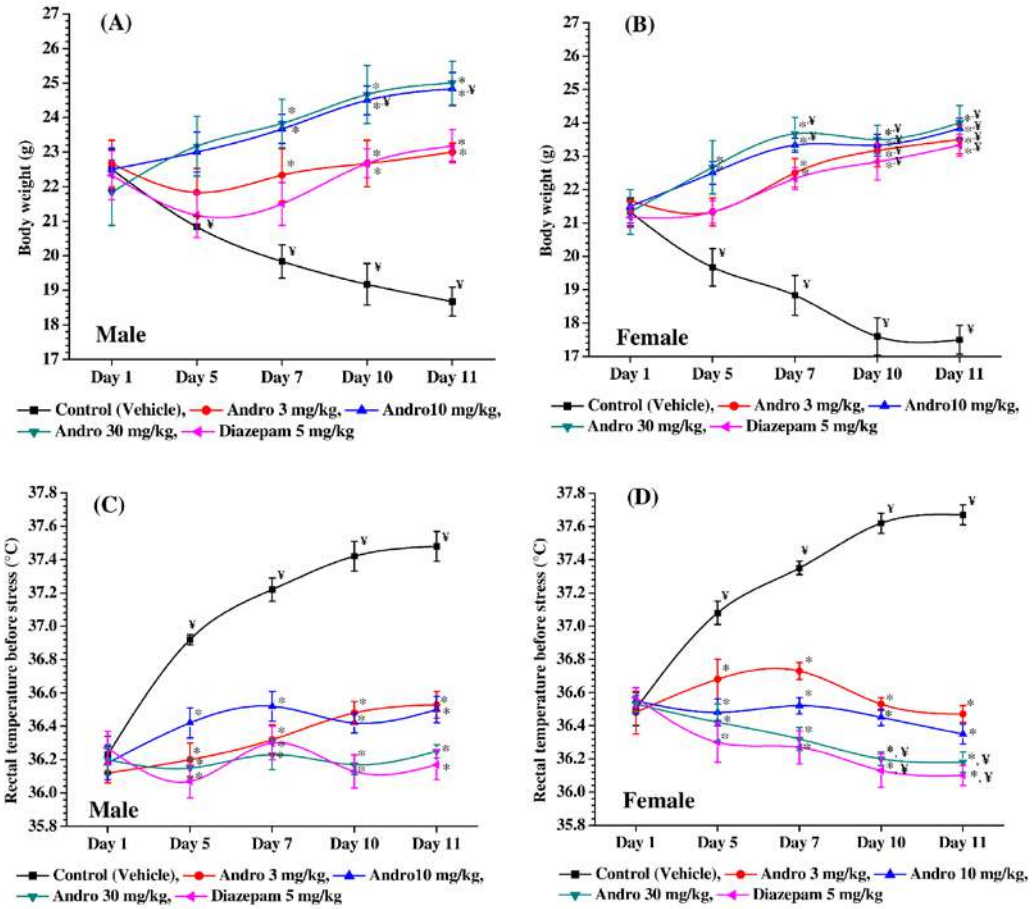

Figure 4.9: Effect of intermittent foot-shocks and daily handling on **(A** and **B)** body weight and **(C** and **D)** basal rectal temperature of male and female mice treated with vehicle, andrographolide, or diazepam. *=p<0.05 vs. control values on the same day, and ¥=p<0.05 vs. day 1 values of the same group

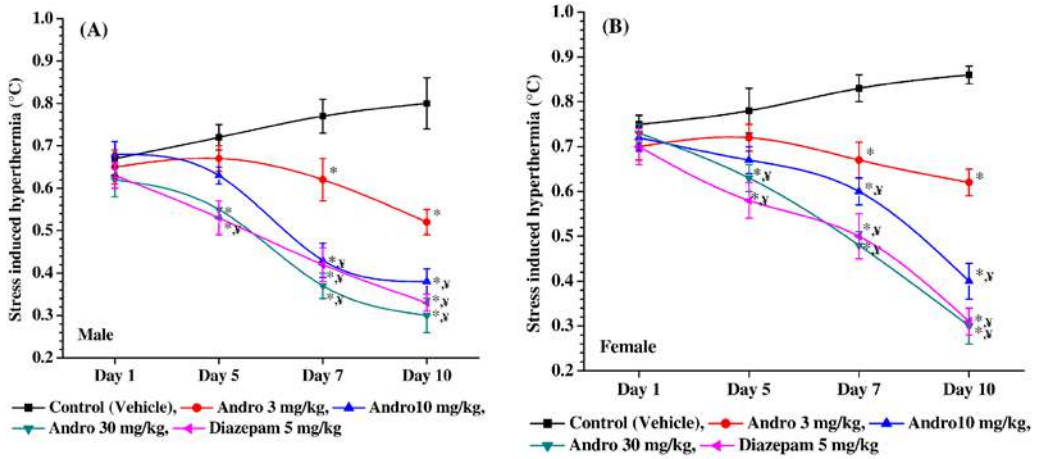

Figure 4.10: Effect of daily handling on foot-shock stress triggered hyperthermia on days 1, 5, 7 and 10 in **(A)** male and **(B)** female mice treated with vehicle, andrographolide, or diazepam. *=p<0.05 vs. control values on the same day, and ¥=p<0.05 vs. day 1 values of the same group

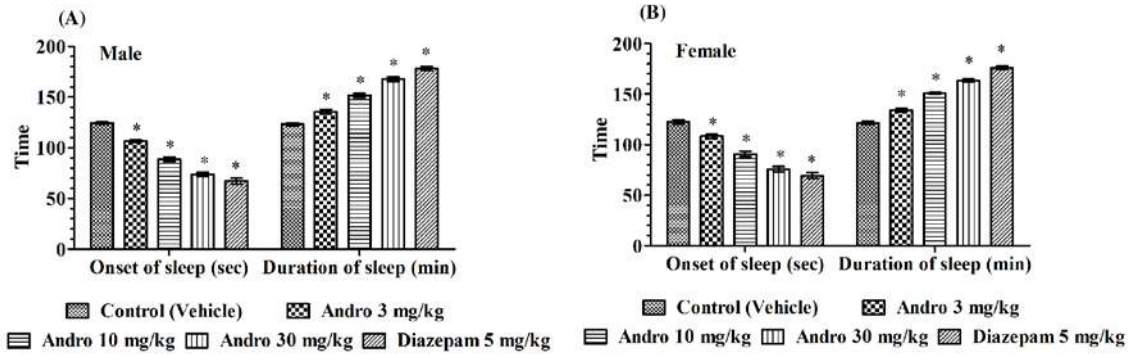

Figure 4.11: Effect of andrographolide or diazepam treatments on pentobarbital-induced sleep parameters quantified on day 11 of treatments in **(A)** male and **(B)** female mice. *=p<0.05 vs. control

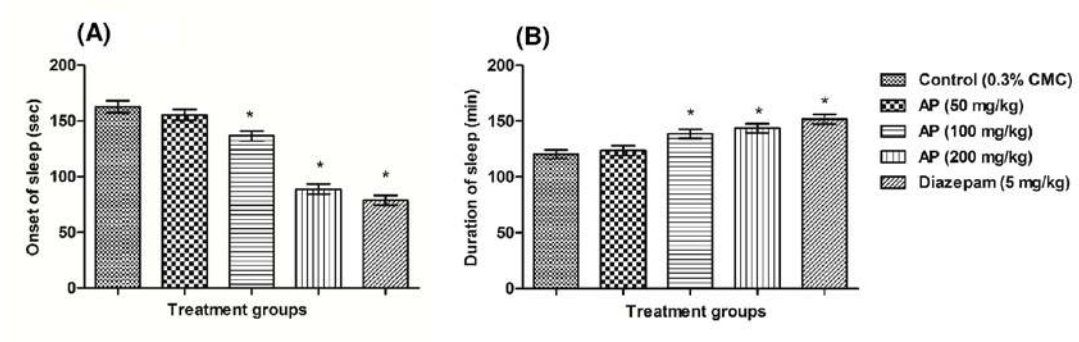

Figure 4.12: Effect of 10 daily oral doses of *Andrographis paniculata* extract (AP) on **(A)** onset of sleep, and **(B)** duration of sleep induced by pentobarbital sodium (40 mg/kg, i.p.) in rats. n= 6/group, *=p<0.05 vs. control

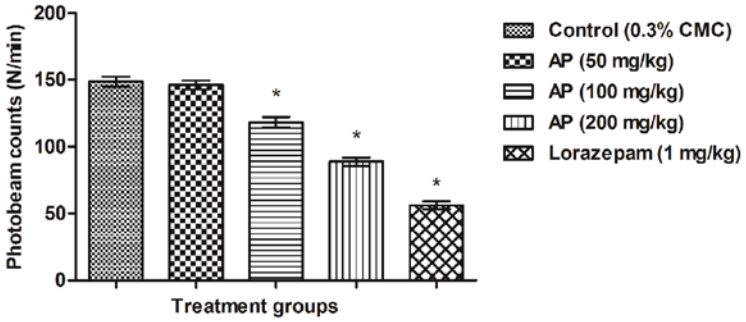

Figure 4.13: Effect of 10 daily oral doses of *Andrographis paniculata* extract (AP) on spontaneous locomotor activity in rats. n= 6/group, *=p<0.05 vs. control

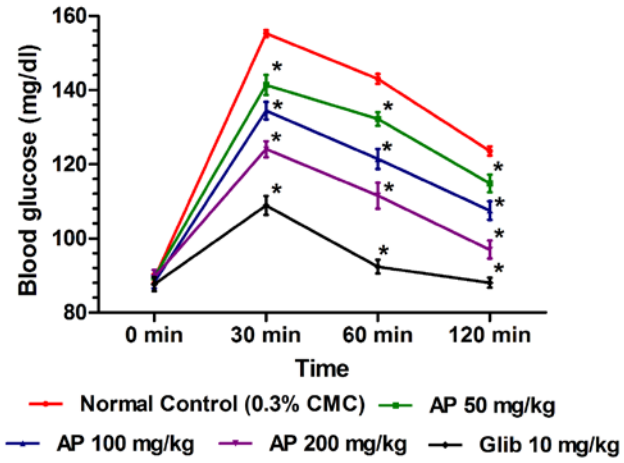

Figure 4.14: Effect of *Andrographis paniculata* extract (AP) on blood glucose level of rats in oral glucose tolerance test. *=p<0.05 vs. normal control (Two way ANOVA followed by Bonferroni post tests). Glib= Glibenclamide

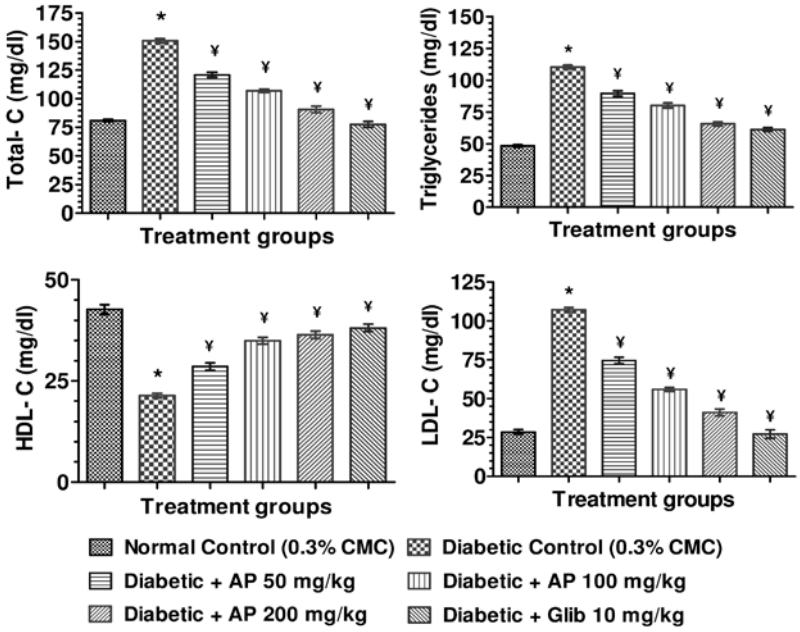

Figure 4.15: Effect of 10 consecutive daily oral doses of *Andrographis paniculata* extract (AP) or of glibenclamide, on plasma lipid profiles of type-2 diabetic rats. *=p<0.05 vs. normal control, ¥=p<0.05 vs. diabetic control. Glib= Glibenclamide

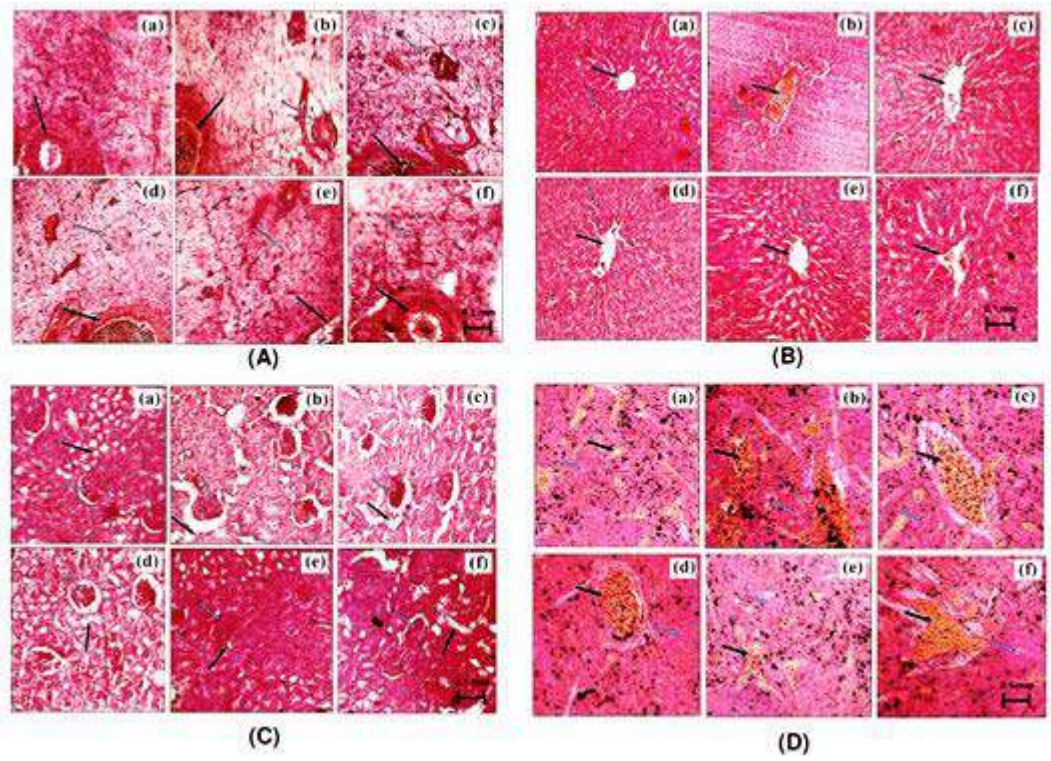

Figure 4.16: Histological sections (Haematoxylin & Eosin staining; X100) of **(A)** pancreases (solid arrows: pancreatic duct, and hollow arrows: islet of Langerhans), **(B)** livers (solid arrows: central vein and hollow arrows: hepatocyte), **(C)** kidneys (solid arrows: tubule and hollow arrows: glomerulus), and **(D)** Spleens (solid arrows: white pulp and hollow arrows: red pulp) of representative rats from each group. In all figures the ones marked with (a) represent a vehicle treated normal control rat, (b) represent a vehicle treated diabetic control rats, (c) represents a diabetic rat treated with AP (50 mg/kg/day), (d) represents a diabetic rat treated with AP (100 mg/kg/day), (e) represent a diabetic rat treated with AP (200 mg/kg/day), and (f) represents a diabetic rat treated with glibenclamide (10 mg/kg/day)

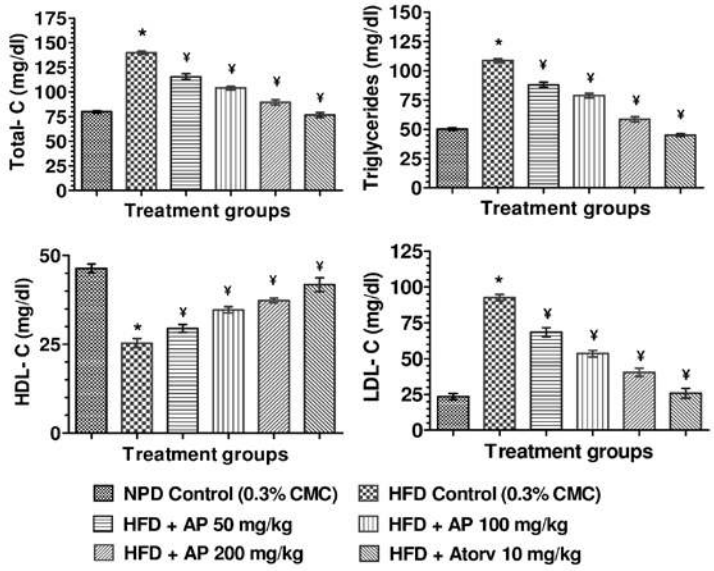

Figure 4.17: Effect of *Andrographis paniculata* extract (AP) or of atorvastatin treatments on plasma lipid profile in high fat fed rats. *=p<0.05 vs. normal control, ¥=p<0.05 vs. HFD control. Atorv= Atorvastatin

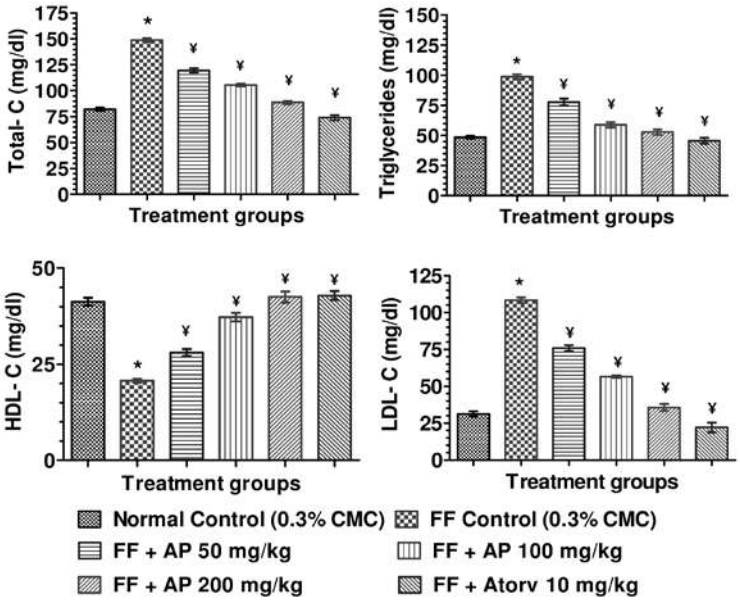

Figure 4.18: Effect of *Andrographis paniculata* extract (AP) or of atorvastatin treatments on plasma lipid profiles of in fructose fed obese rats. *=p<0.05 vs. normal control, ¥=p<0.05 vs. FF control. Atorv= Atorvastatin

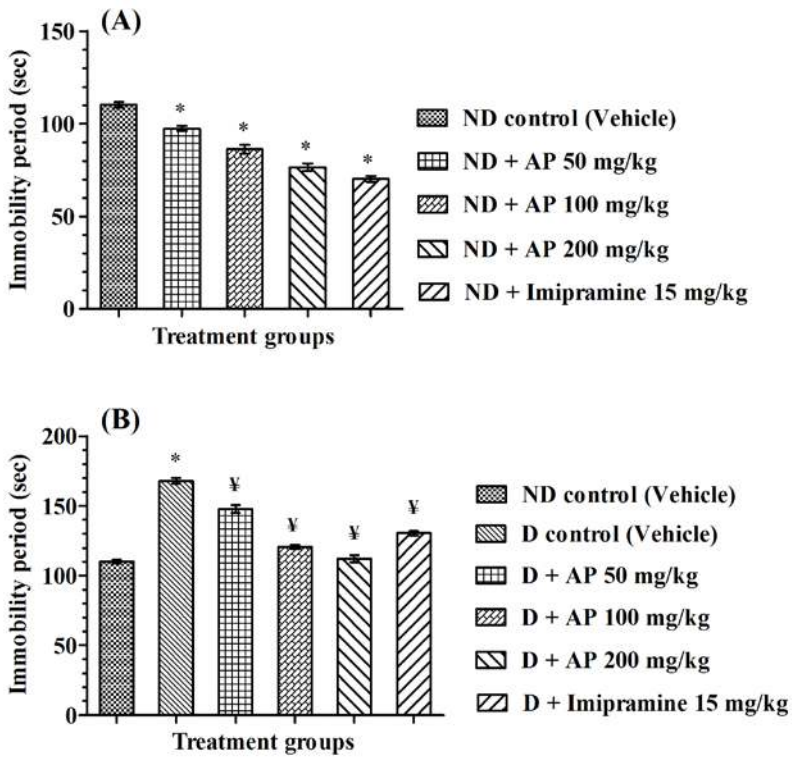

Figure 4.19: Effect of *Andrographis paniculata* extract (AP) on **(A)** nondiabetic and **(B)** diabetic rats in behaviour despair test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

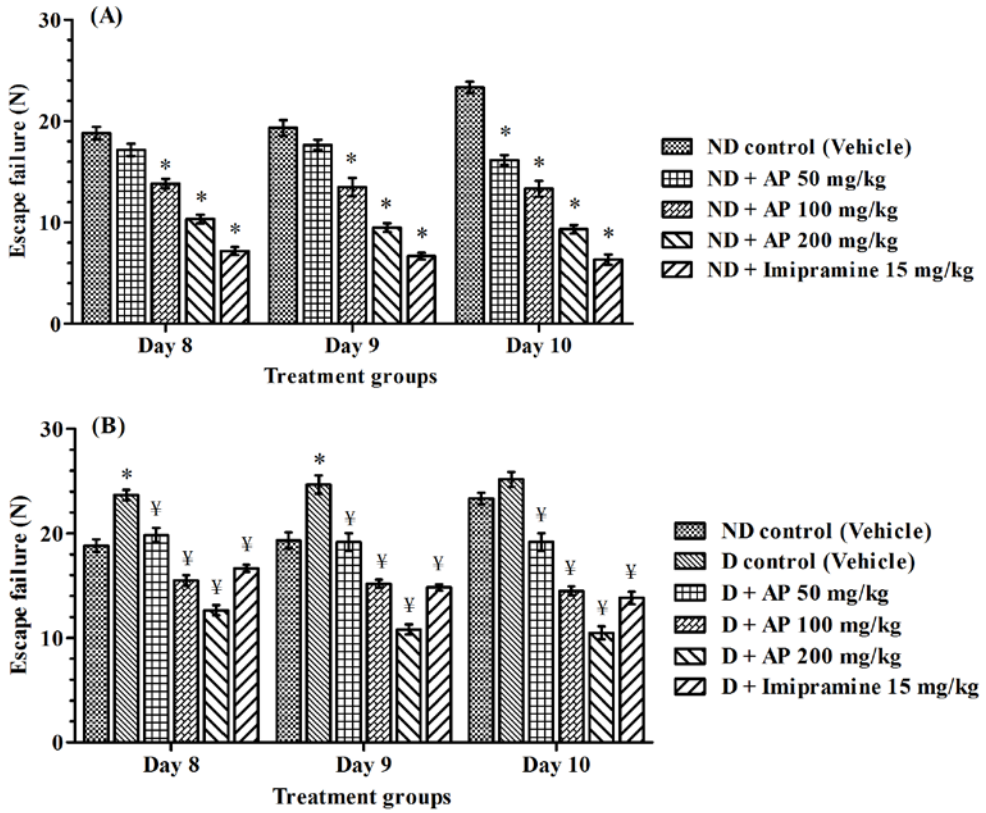

Figure 4.20: Effect of *Andrographis paniculata* extract (AP) on **(A)** nondiabetic and **(B)** diabetic rats in learned helplessness test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control (Two way ANOVA followed by Bonferroni post tests)

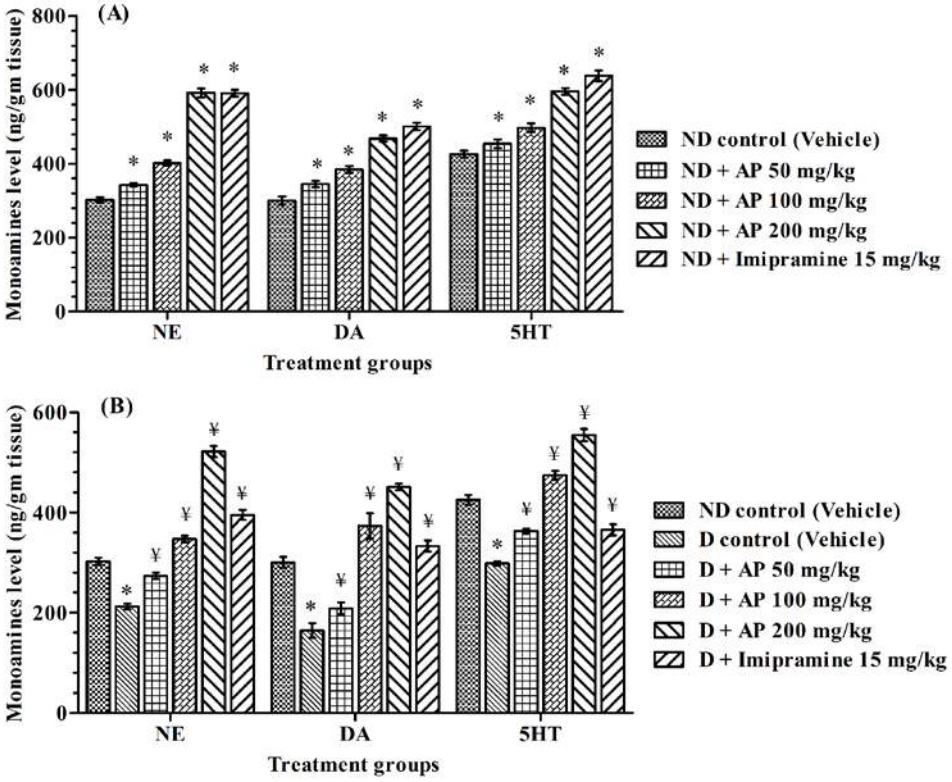

Figure 4.21: Effect of *Andrographis paniculata* extract (AP) on brain monoamines level of **(A)** nondiabetic and **(B)** diabetic rats after performing learned helplessness test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

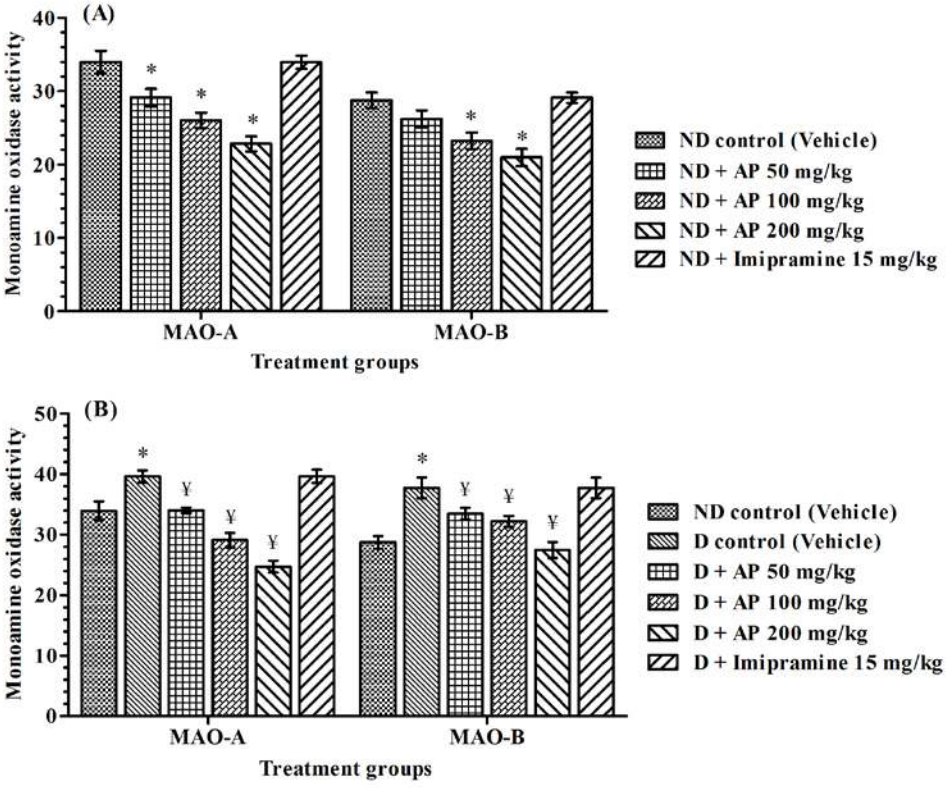

Figure 4.22: Effect of *Andrographis paniculata* extract (AP) on brain MAO-A (nmol 5-HT/mg protein/h) and MAO-B (nmol β-PEA/mg protein/h) enzyme activity of **(A)** nondiabetic and **(B)** diabetic rats after performing learned helplessness test. *=$p<0.05$ vs. nondiabetic (ND) control; ¥=$p<0.05$ vs. diabetic (D) control

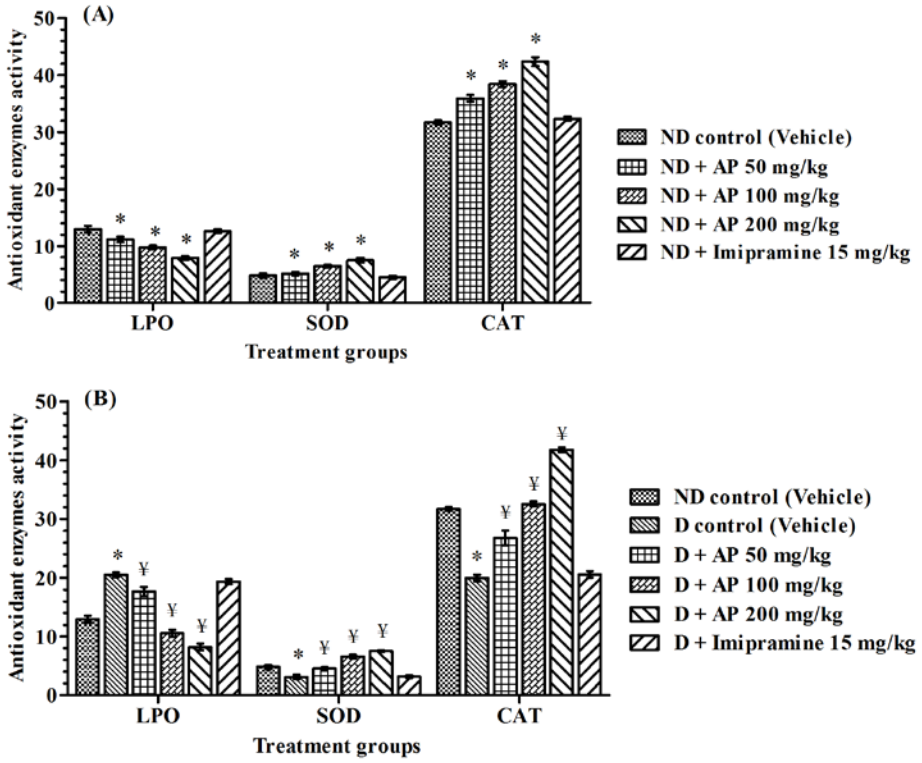

Figure 4.23: Effect of *Andrographis paniculata* extract (AP) on brain antioxidant enzymes activity viz. LPO (nmol MDA/mg protein), SOD (Units/mg protein), and CAT (µmol H_2O_2/min/mg protein) of **(A)** nondiabetic and **(B)** diabetic rats after performing learned helplessness test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

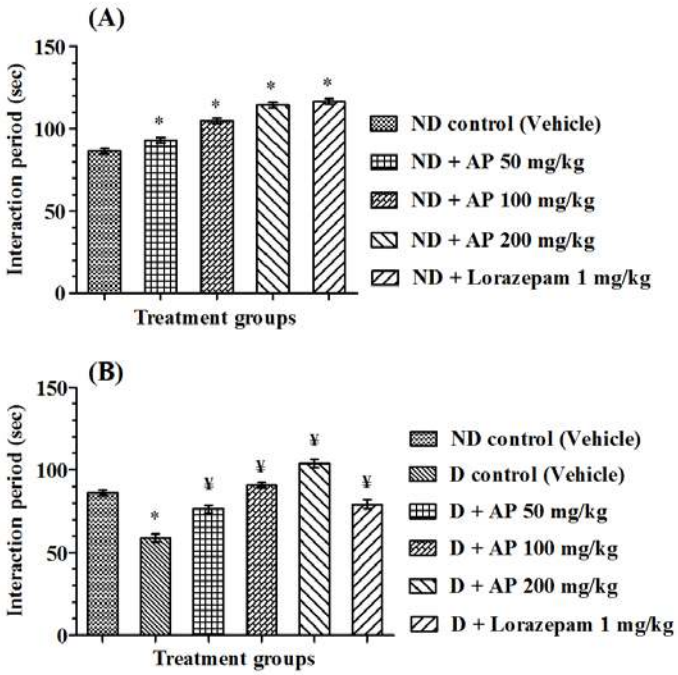

Figure 4.24: Effect of *Andrographis paniculata* extract (AP) on **(A)** nondiabetic and **(B)** diabetic rats in social interaction test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

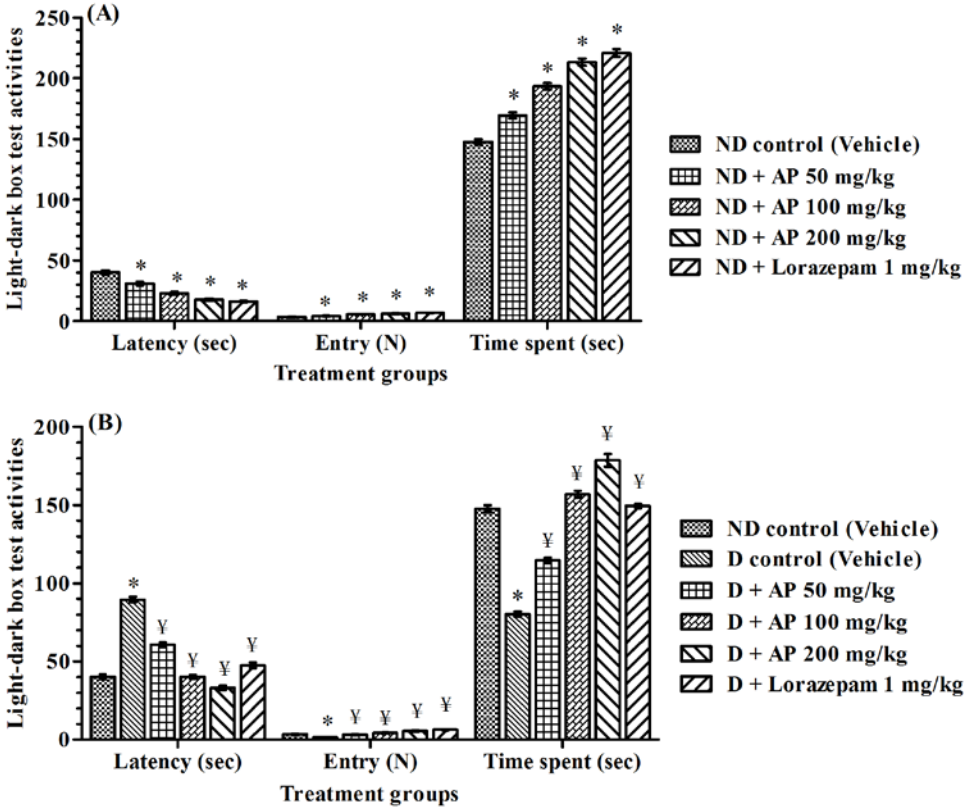

Figure 4.25: Effect of *Andrographis paniculata* extract (AP) on latency to entry in light box (sec), number of entry in light box (N), and time spent in light box (sec) by **(A)** nondiabetic and **(B)** diabetic rats in light-dark box test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

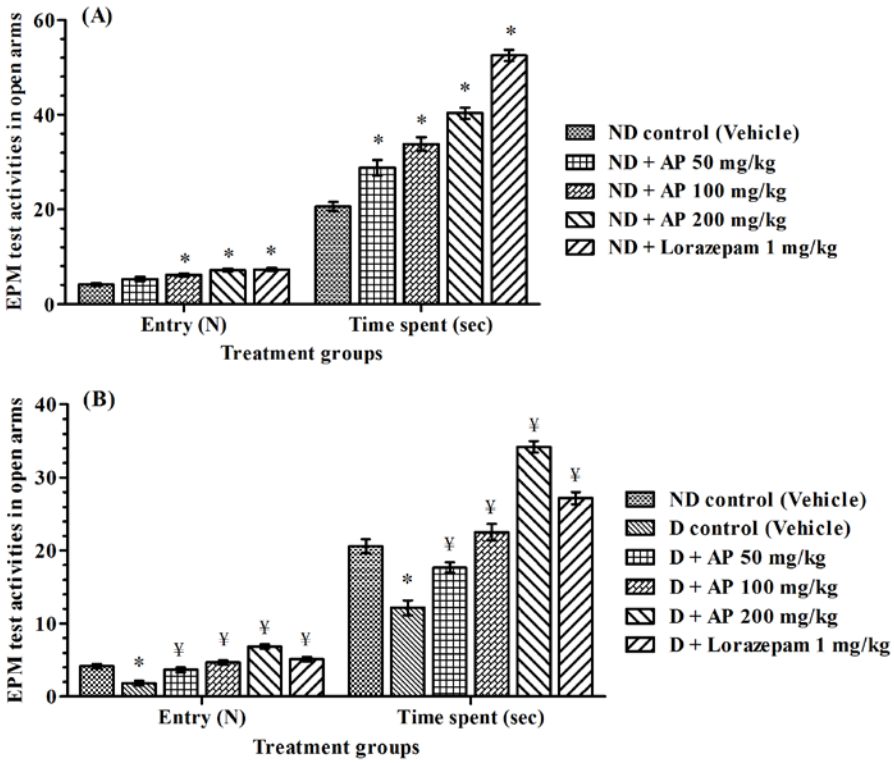

Figure 4.26: Effect of *Andrographis paniculata* extract (AP) on **(A)** nondiabetic and **(B)** diabetic rats on open arms activities during elevated plus maze test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

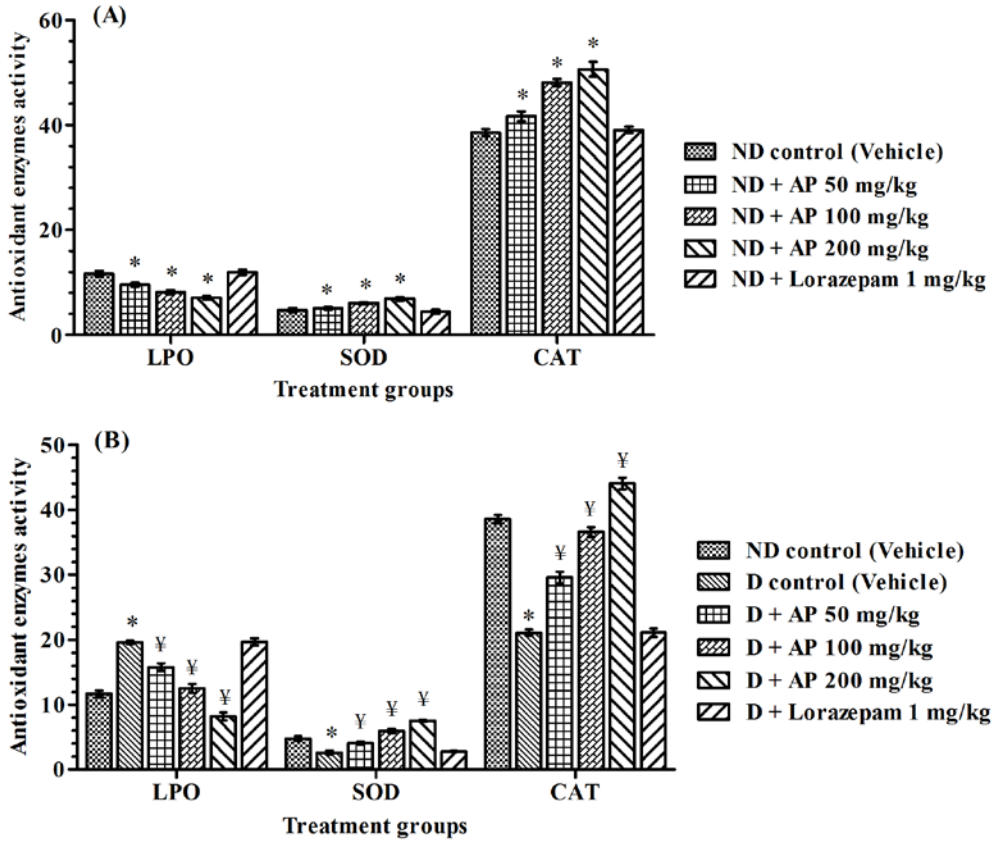

Figure 4.27: Effect of *Andrographis paniculata* extract (AP) on brain antioxidant enzymes activity viz. LPO (nmol MDA/mg protein), SOD (Unit/mg protein), and CAT (µmol H_2O_2/min/mg protein) of **(A)** nondiabetic and **(B)** diabetic rats after performing elevated plus maze test. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

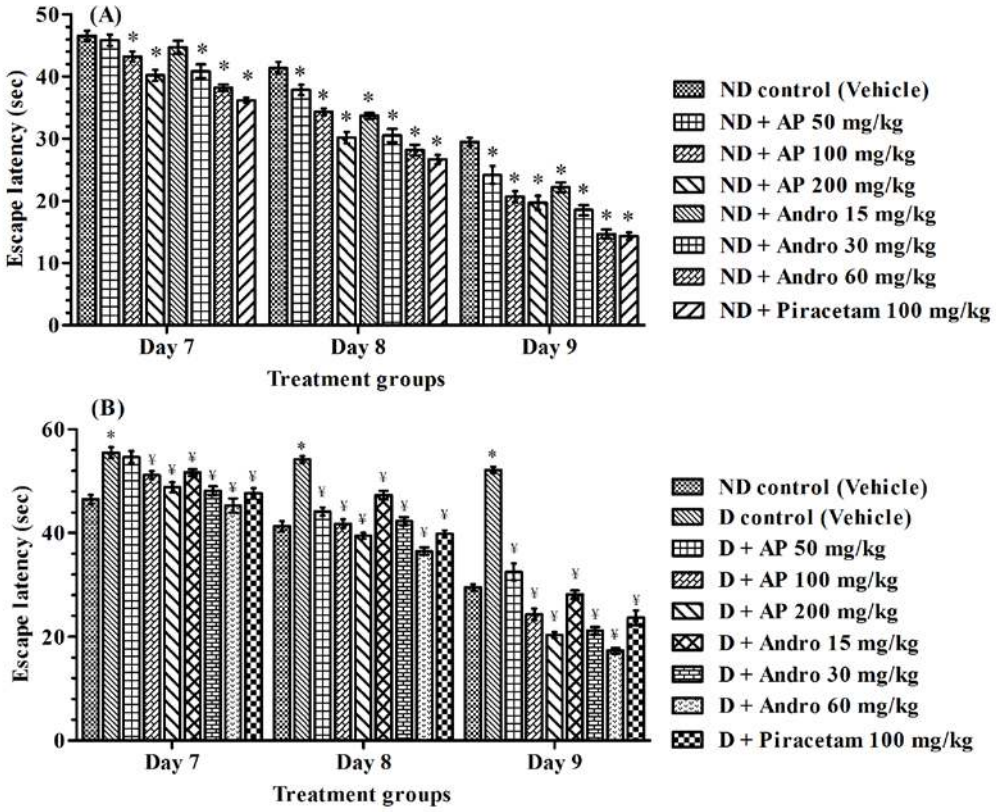

Figure 4.28: Effect of *Andrographis paniculata* extract (AP) and andrographolide on escape latency to find the platform (on days 7-9) by **(A)** nondiabetic and **(B)** diabetic rats during Morris water-maze task. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control (Two way ANOVA followed by Bonferroni post tests). Andro= Andrographolide

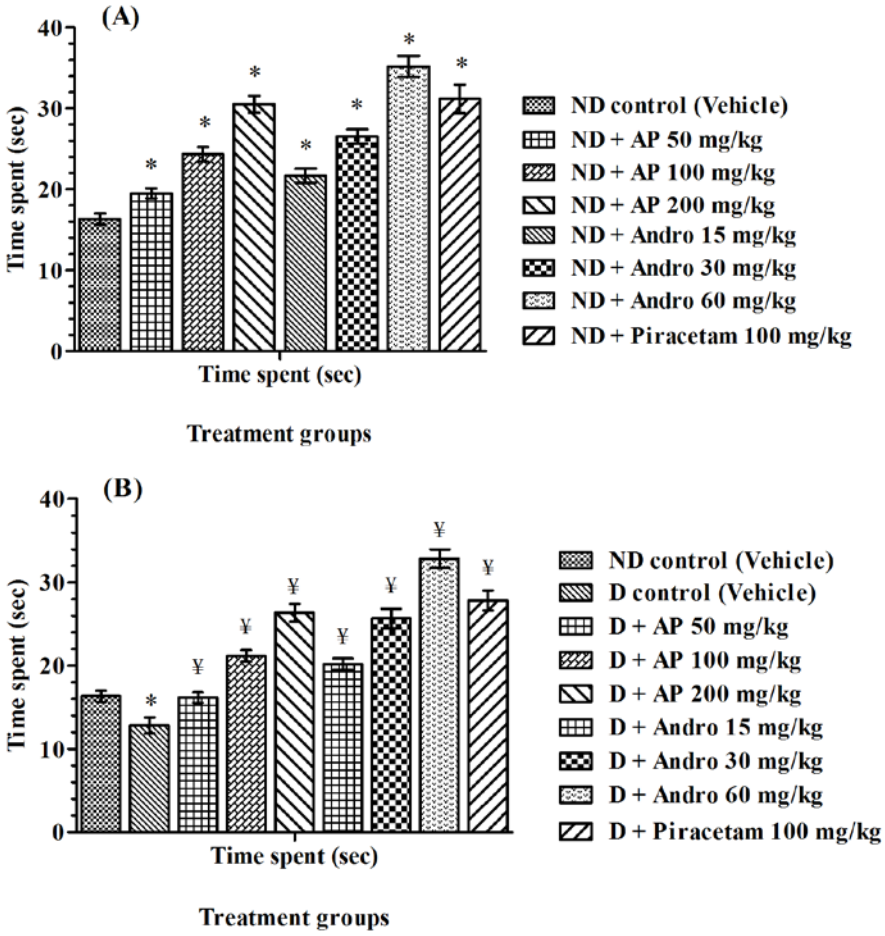

Figure 4.29: Effect of *Andrographis paniculata* extract (AP) and andrographolide on time spent (sec) in targeted quadrant by (A) nondiabetic and (B) diabetic rats during probe trial in Morris water-maze task. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

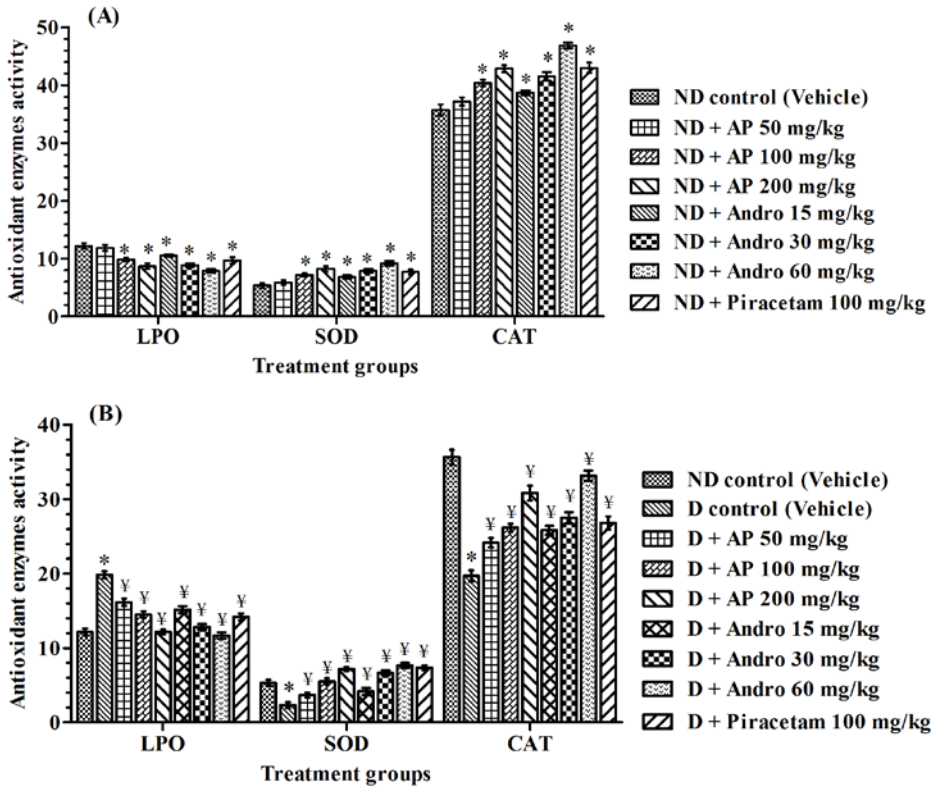

Figure 4.30: Effect of *Andrographis paniculata* extract (AP) and andrographolide on brain antioxidant enzymes activity viz. LPO (nmol MDA/mg protein), SOD (Unit/mg protein), and CAT (µmol H_2O_2/min/mg protein) of **(A)** nondiabetic and **(B)** diabetic rats of nondiabetic rats after performing probe trial. *=p<0.05 vs. nondiabetic control; ¥=p<0.05 vs. diabetic control. Andro= Andrographolide

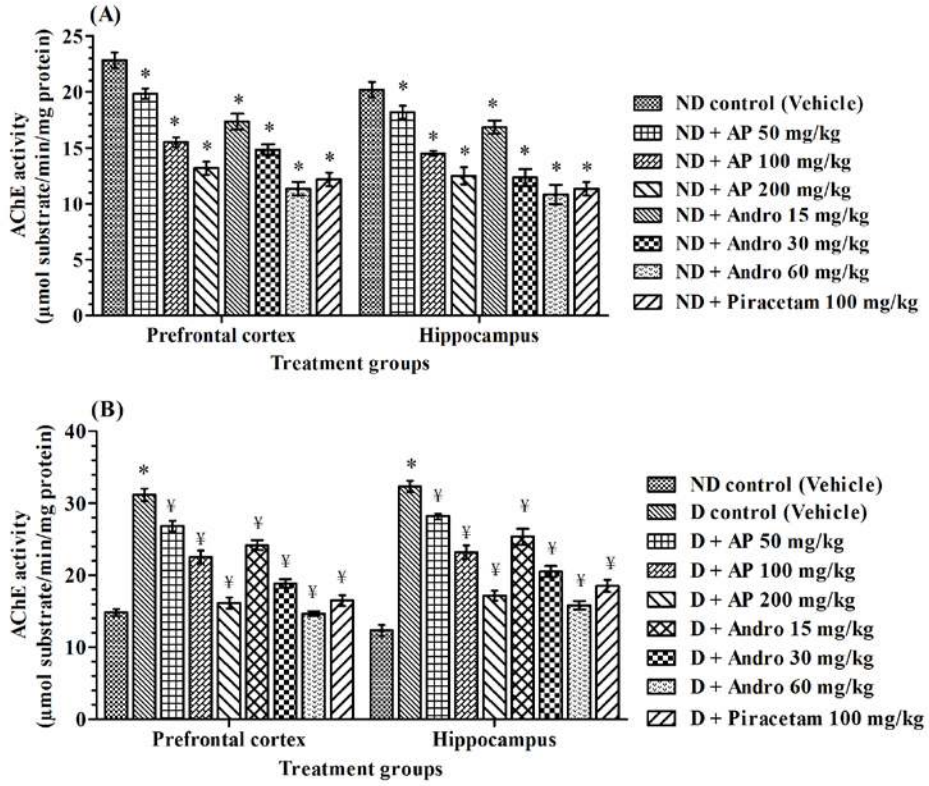

Figure 4.31: Effect of *Andrographis paniculata* extract (AP) and andrographolide on brain acetylcholinesterase enzyme activity of **(A)** nondiabetic and **(B)** diabetic rats after performing probe trial in Morris water-maze task. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

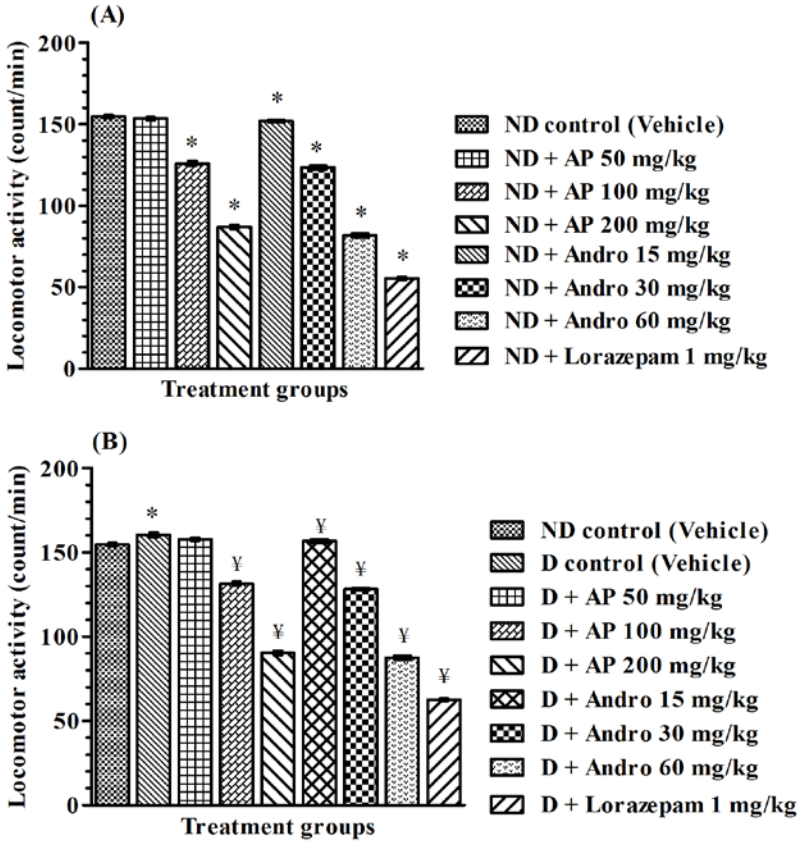

Figure 4.32: Effect of *Andrographis paniculata* extract (AP) and andrographolide on locomotor activity of **(A)** nondiabetic and **(B)** diabetic rats. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

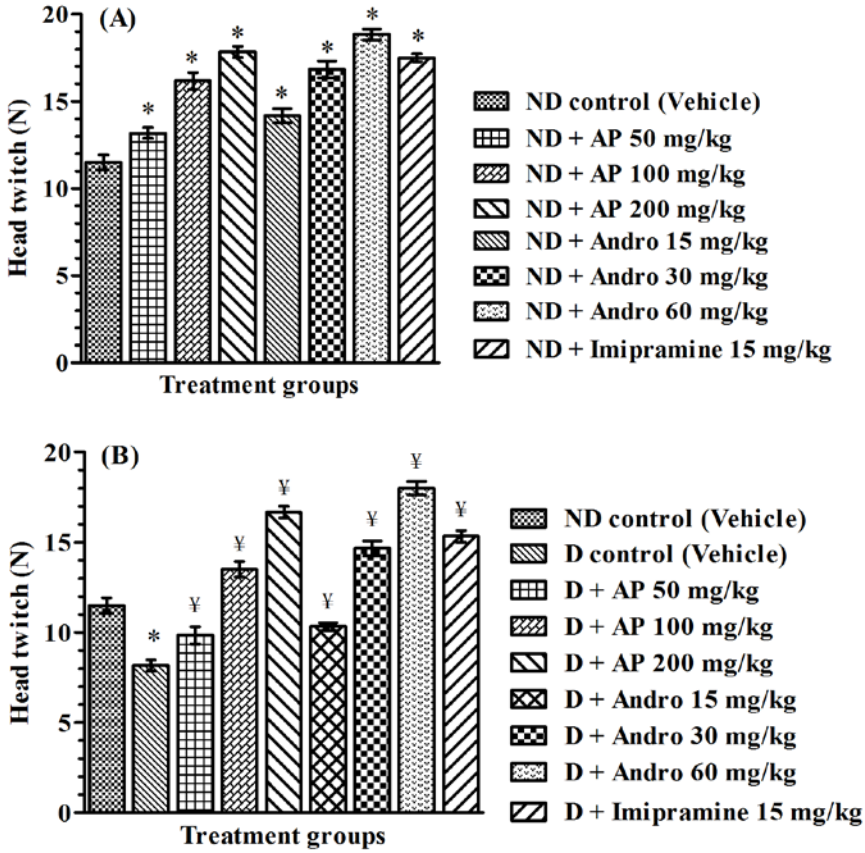

Figure 4.33: Effect of *Andrographis paniculata* extract (AP) and andrographolide on 5-HTP-induced head twitches of **(A)** nondiabetic and **(B)** diabetic mice. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

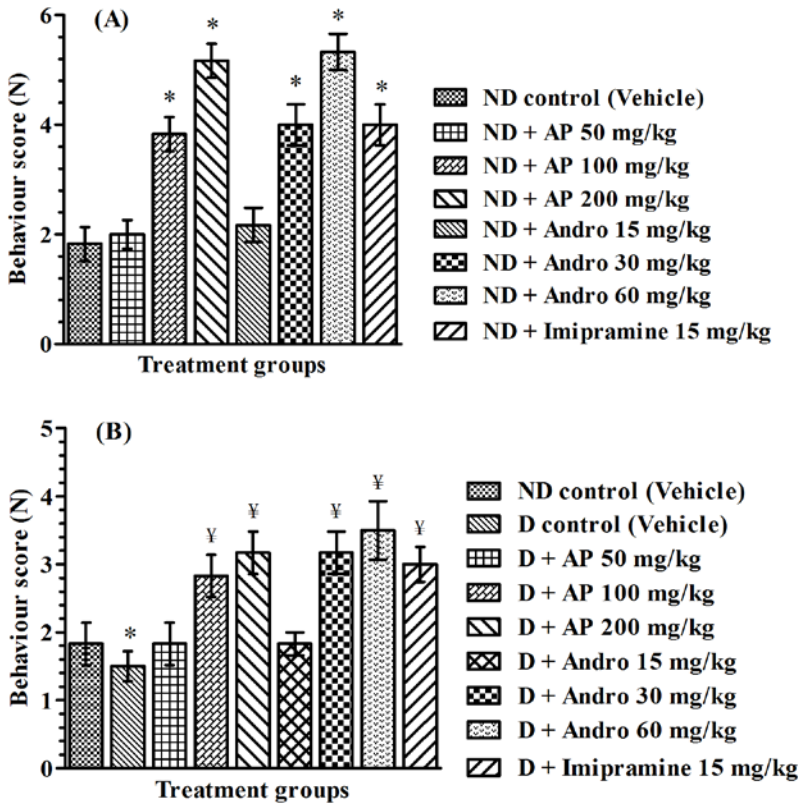

Figure 4.34: Effect of *Andrographis paniculata* extract (AP) and andrographolide on L-DOPA-induced hyperactivity of **(A)** nondiabetic and **(B)** diabetic mice. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

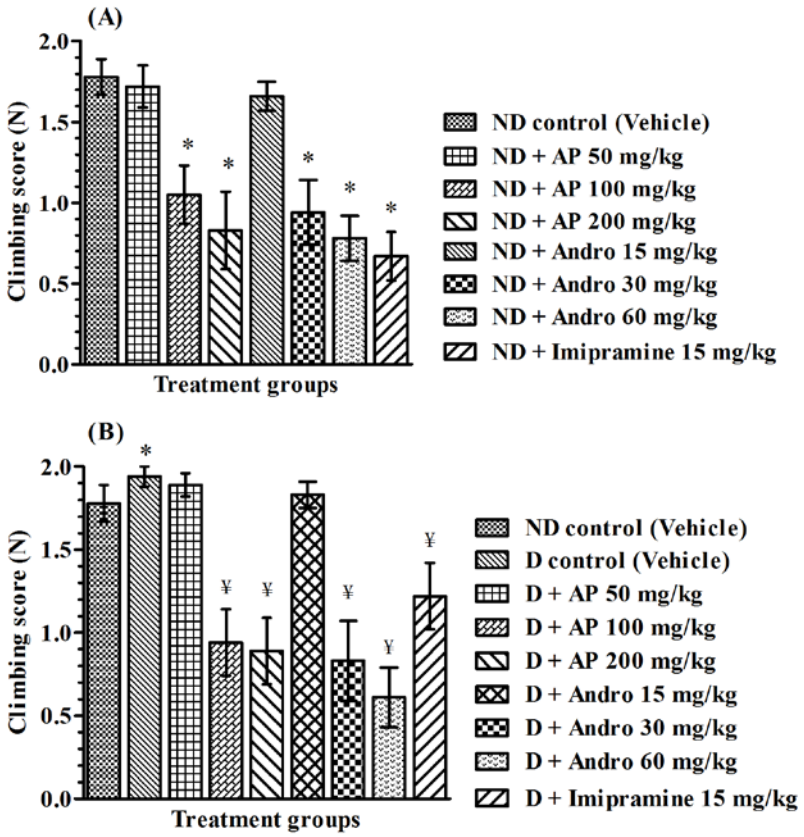

Figure 4.35: Effect of *Andrographis paniculata* extract (AP) and andrographolide on apomorphine-induced hyperactivity of **(A)** nondiabetic and **(B)** diabetic mice. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

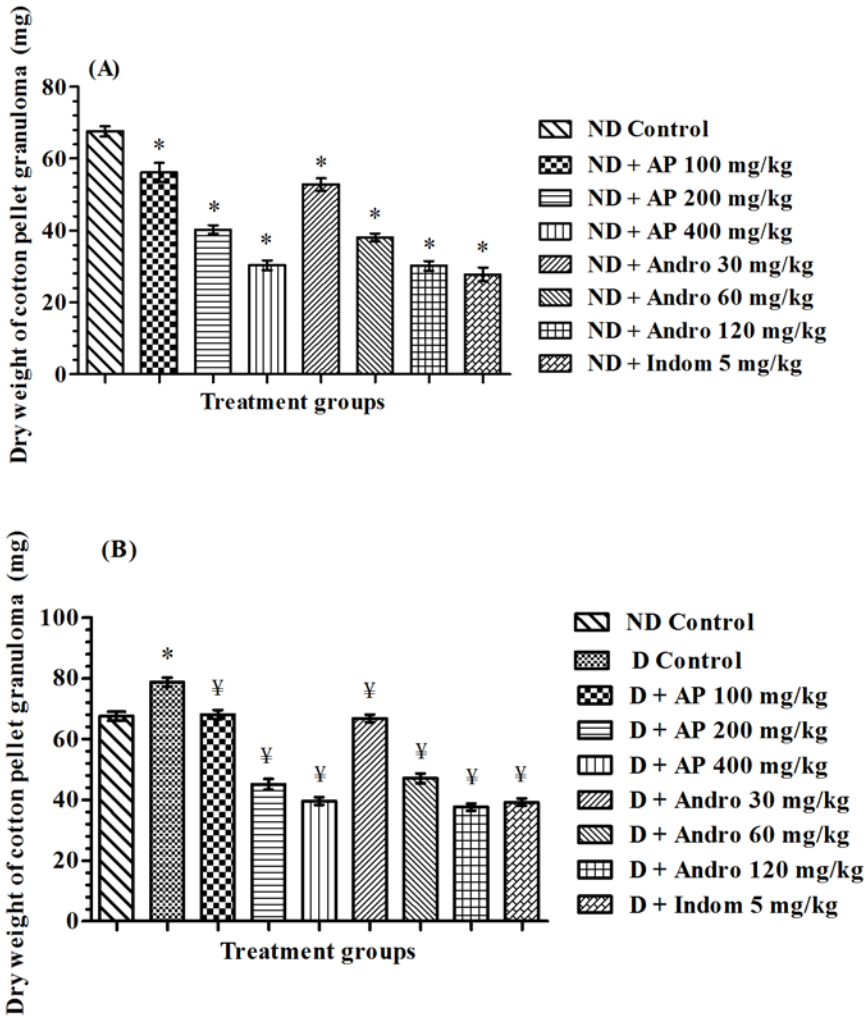

Figure 4.36: Effect of *Andrographis paniculata* extract (AP) and andrographolide on cotton pellet-induced granuloma in **(A)** nondiabetic and **(B)** diabetic rats. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Indom= Indomethacin

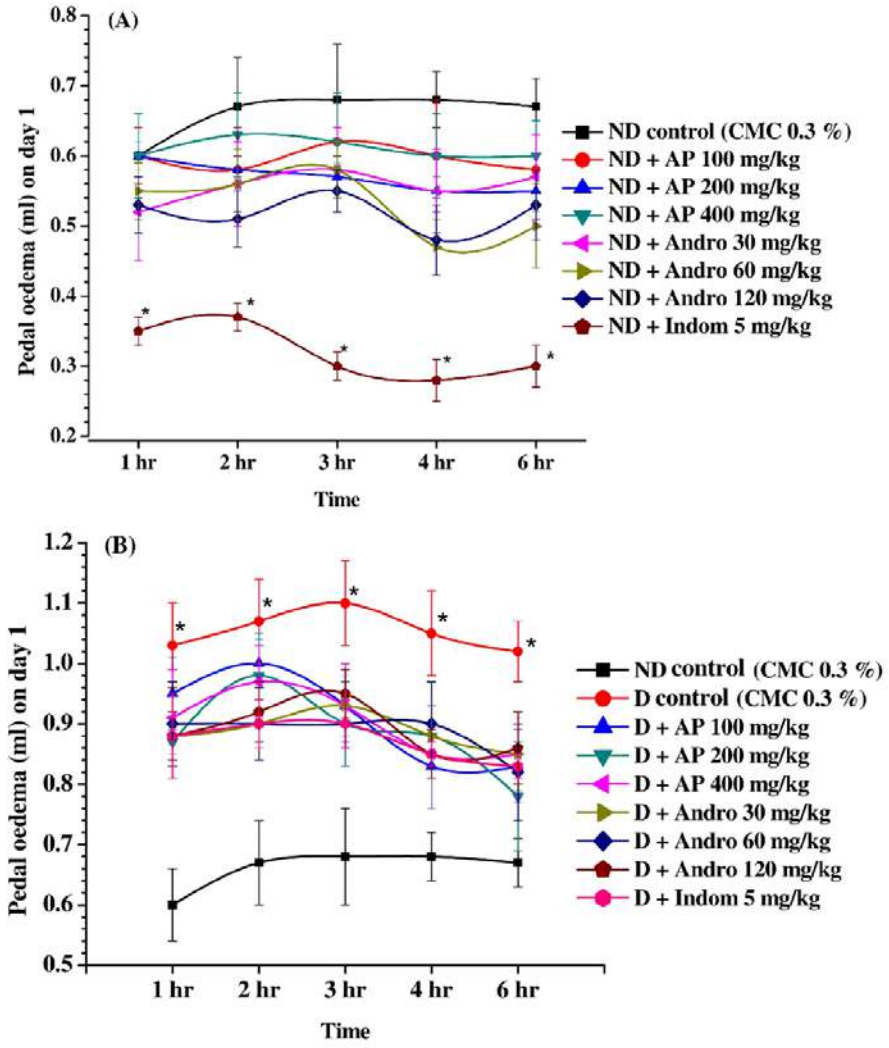

Figure 4.37: Effect of *Andrographis paniculata* extract (AP) and andrographolide on day 1 of carrageenan-induced pedal oedema in **(A)** nondiabetic and **(B)** diabetic rats. *=p<0.05 vs. nondiabetic (ND) control (Two way ANOVA followed by Bonferroni post tests). Andro= Andrographolide, Indom= Indomethacin

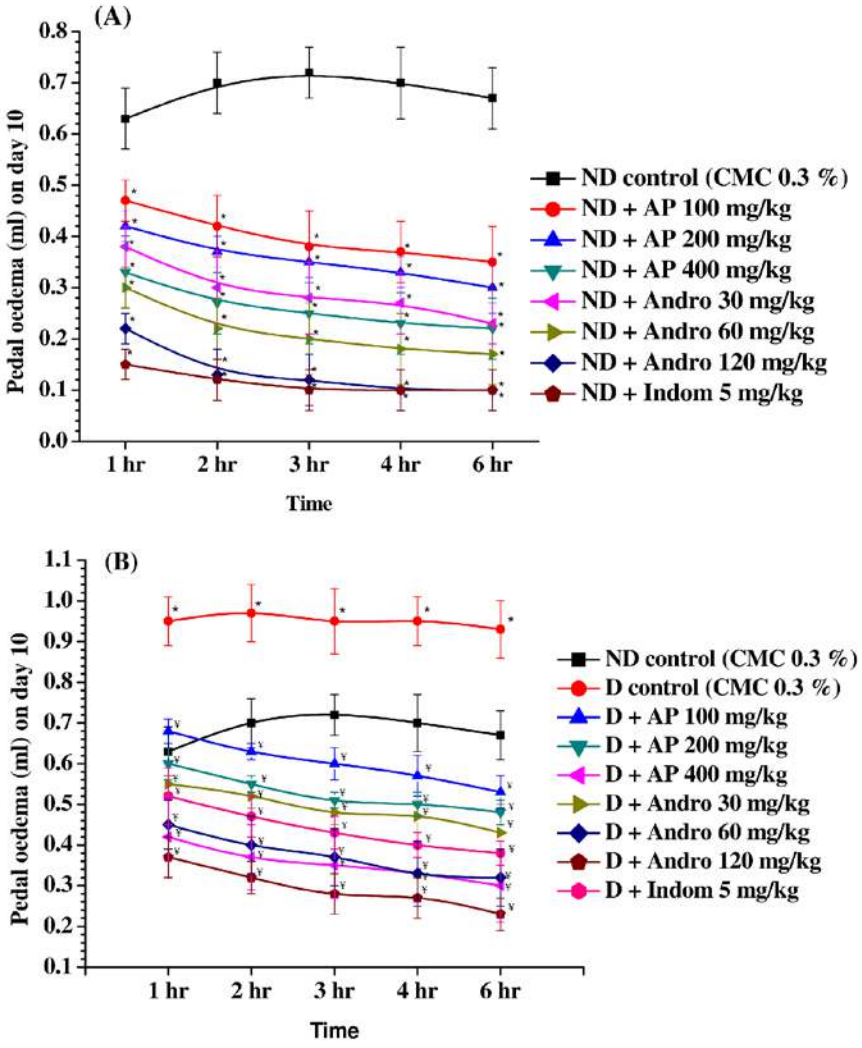

Figure 4.38: Effect of *Andrographis paniculata* extract (AP) and andrographolide on day 10 of carrageenan-induced pedal oedema in **(A)** nondiabetic and **(B)** diabetic rats. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control (Two way ANOVA followed by Bonferroni post tests). Andro= Andrographolide, Indom= Indomethacin

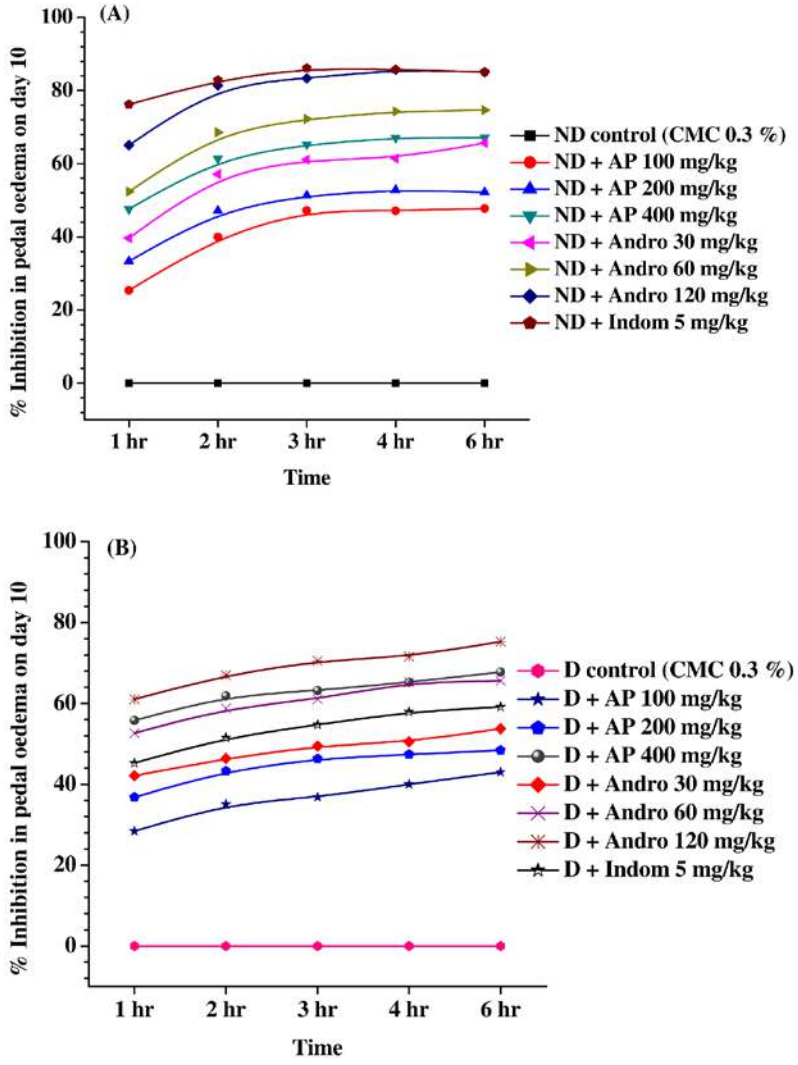

Figure 4.39: Effect of *Andrographis paniculata* extract (AP) and andrographolide on percentage inhibition of carrageenan-induced pedal oedema on day 10 in **(A)** nondiabetic and **(B)** diabetic rats. Andro= Andrographolide, Indom= Indomethacin. Note: Percentage inhibition for control rats was taken as 0.00 (relative value)

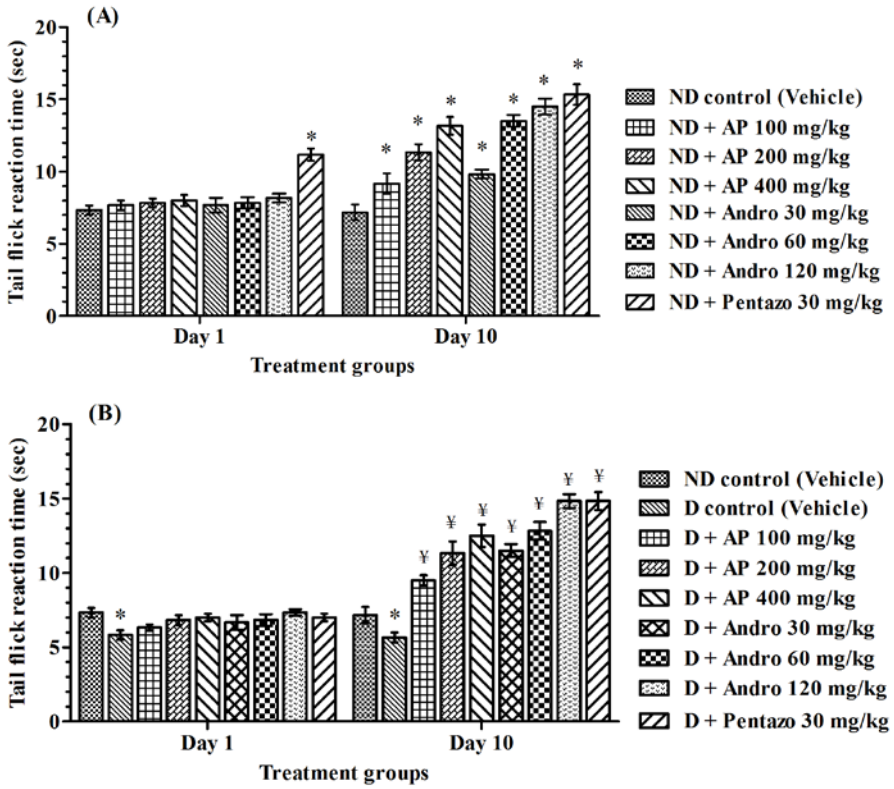

Figure 4.40: Effect of *Andrographis paniculata* extract (AP) and andrographolide on tail flick reaction time in **(A)** nondiabetic and **(B)** diabetic rats. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Pentazo= Pentazocine

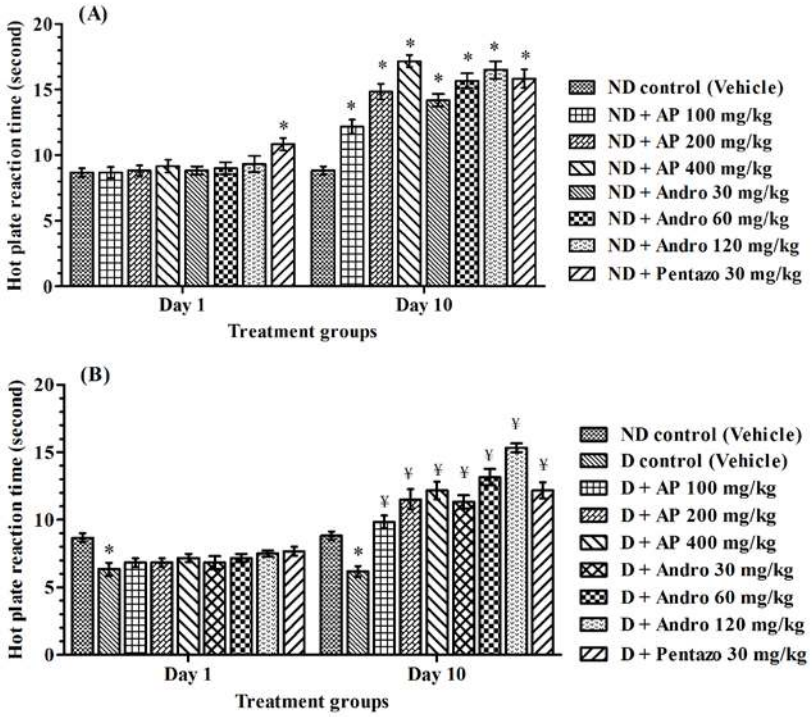

Figure 4.41: Effect of *Andrographis paniculata* extract (AP) and andrographolide on hot plate reaction time in **(A)** nondiabetic and **(B)** diabetic mice. *=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Pentazo= Pentazocine

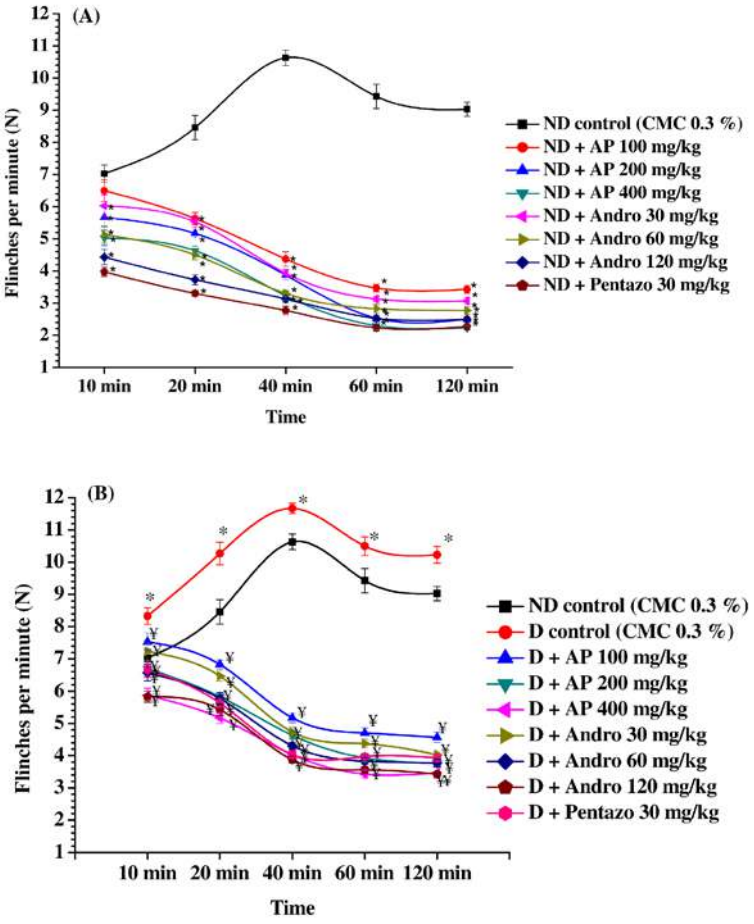

Figure 4.42: Effect of *Andrographis paniculata* extract (AP) and andrographolide on incidence of spontaneous flinch in **(A)** nondiabetic and **(B)** diabetic rats. *=$p<0.05$ vs. nondiabetic (ND) control; ¥=$p<0.05$ vs. diabetic (D) control (Two way ANOVA followed by Bonferroni post tests). Andro= Andrographolide, Pentazo= Pentazocine

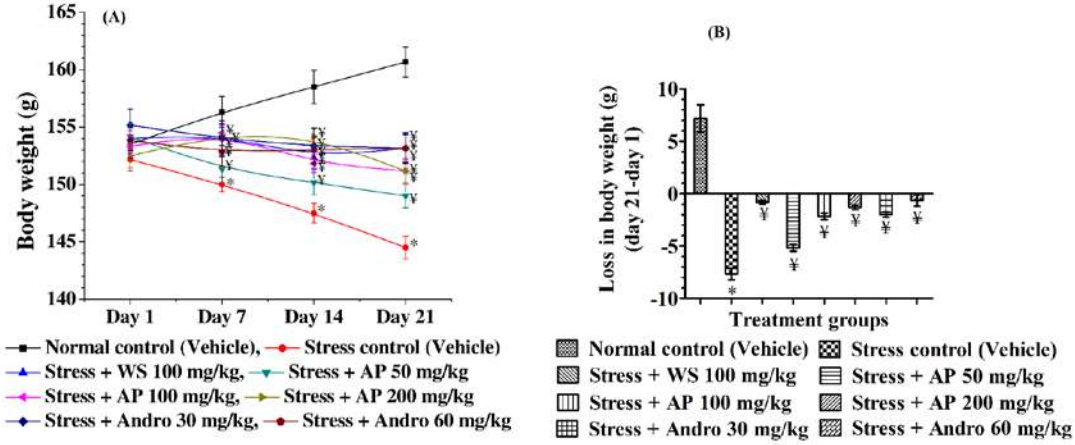

Figure 4.43: Effect of *Andrographis paniculata* extract (AP) and andrographolide treatments on **(A)** body weight, and **(B)** body weight changes observed between day 1 and 21. *=p<0.05 vs. normal control; ¥=p<0.05 vs. stress control. Andro= Andrographolide, WS= *Withania somnifera* extract

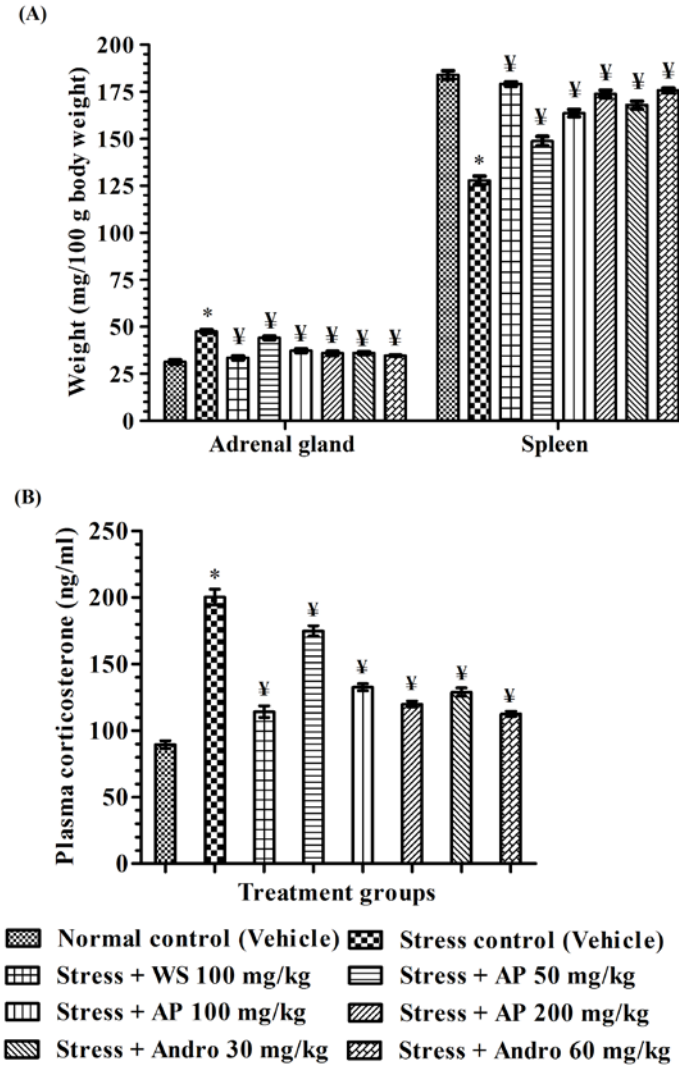

Figure 4.44: Effect of *Andrographis paniculata* extract (AP) and andrographolide on **(A)** weight of adrenal gland and spleen, and **(B)** plasma corticosterone level of chronically stressed rats. *=p<0.05 vs. normal control; ¥=p<0.05 vs. stressed control. Andro= Andrographolide, WS= *Withania somnifera* extract

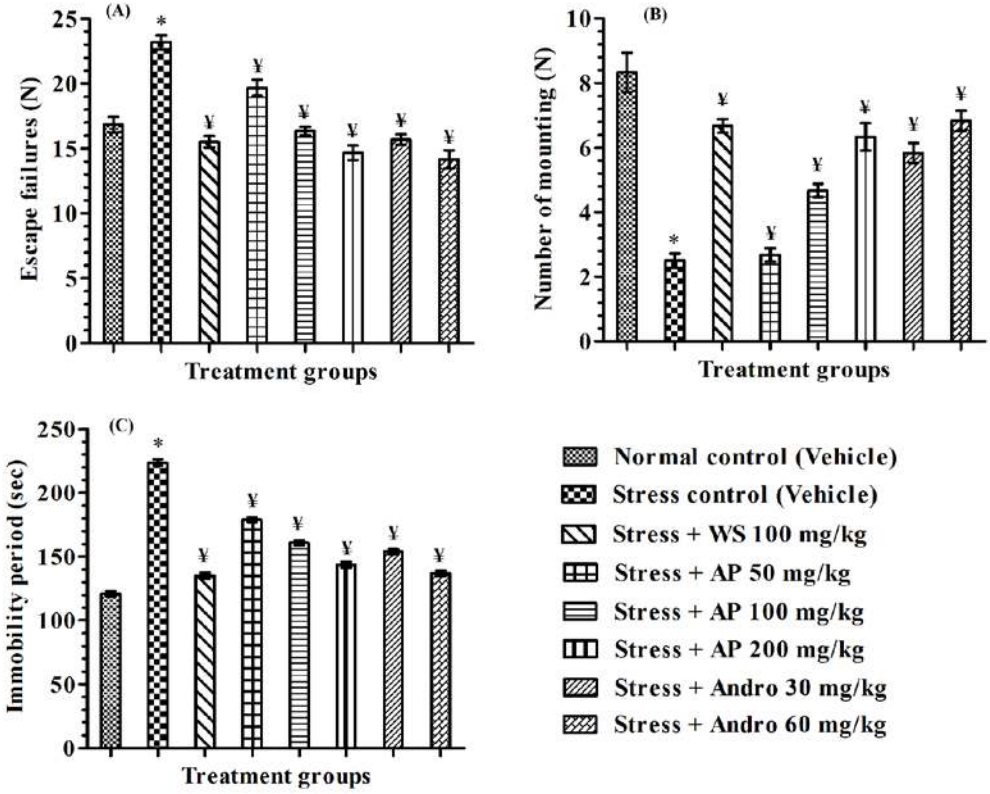

Figure 4.45: Effect of *Andrographis paniculata* extract (AP) and andrographolide on stressed rat **(A)** learned helpless test, **(B)** suppression in sexual behaviour test, and **(C)** behavioural despair test. *=p<0.05 vs. normal control; ¥=p<0.05 vs. stress control. Andro= Andrographolide, WS= *Withania somnifera* extract

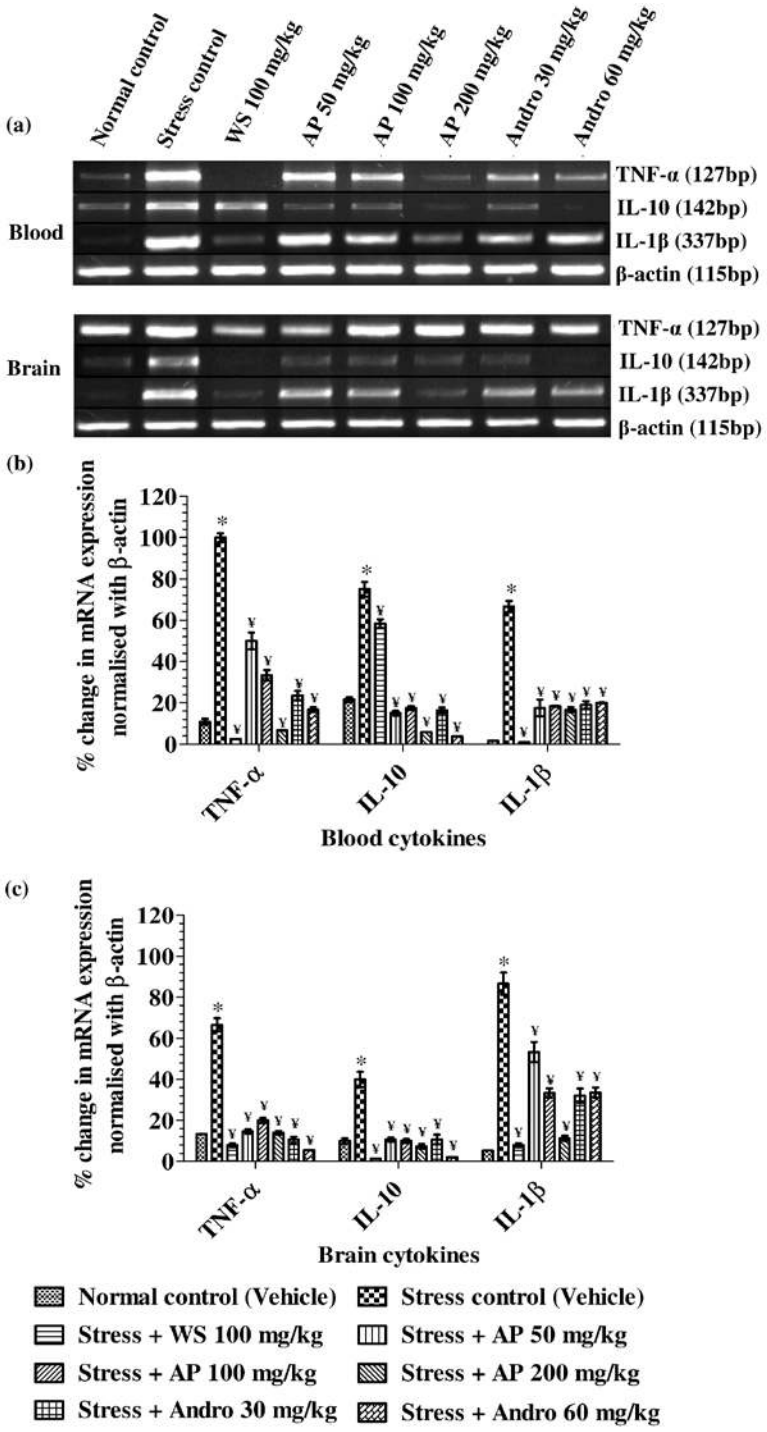

Figure 4.46: Effect of *Andrographis paniculata* extract (AP) and andrographolide on expressions of TNF-α, IL-10 and IL-1β in chronic stressed rats. **(A)** Gel picture showing expression of cytokines normalized with β-actin housekeeping gene in blood and brain, **(B)** percentage change in mRNA expression of cytokines in blood (WBC), and **(C)** in frontal cortex. *=$p<0.05$ vs. normal control, ¥=$p<0.05$ vs. stress control. Andro= Andrographolide, WS= *Withania somnifera* extract

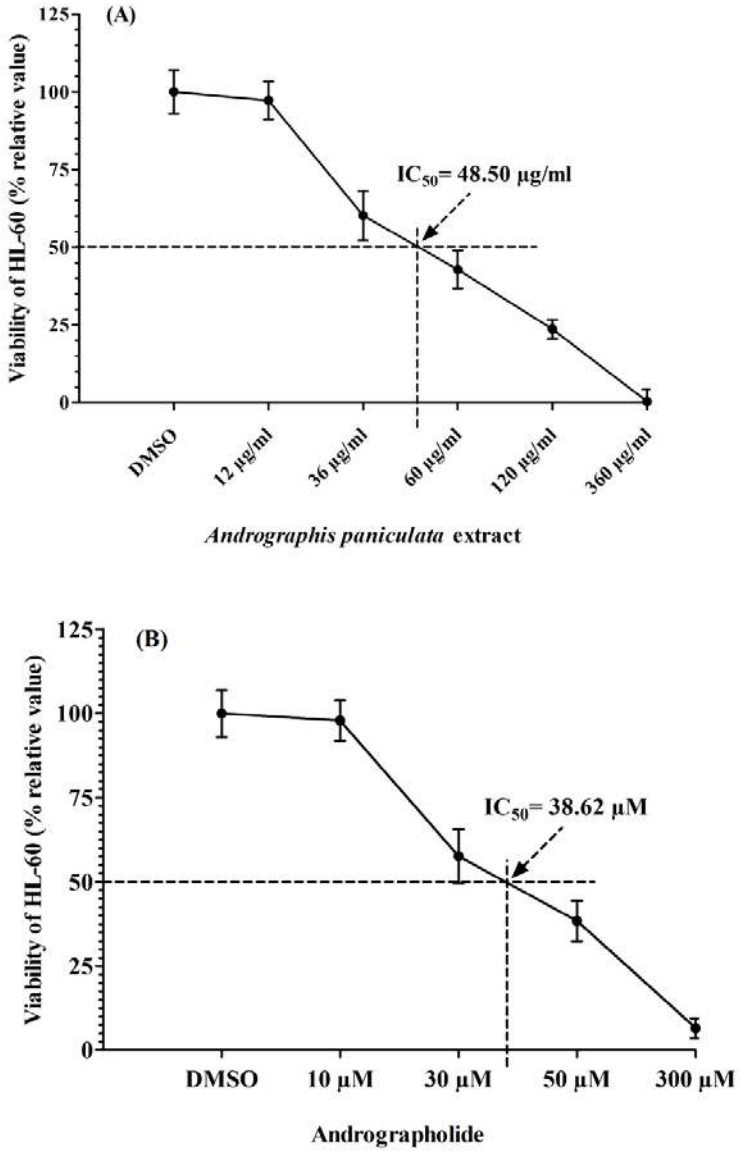

Figure 4.47: Viability of HL-60 cells (percentage relative value) in MTT assay with **(A)** *Andrographis paniculata* extract, and **(B)** andrographolide using HL-60 cell line

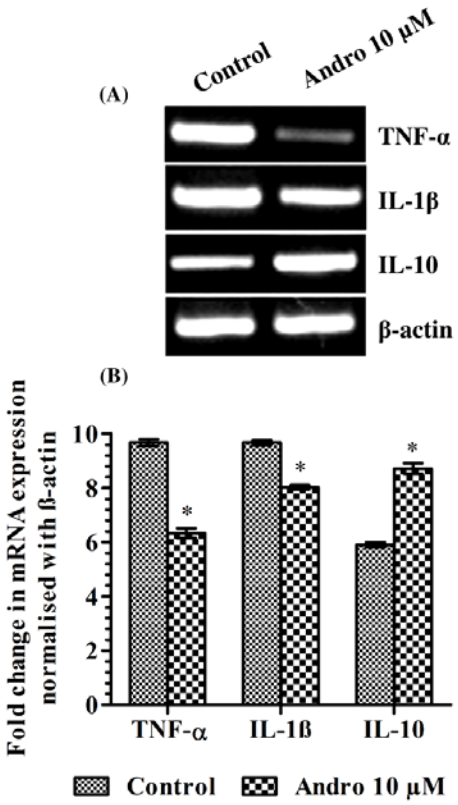

Figure 4.48: Effect of andrographolide on various cytokines expression viz. TNF-α, IL-1β and IL-10 in HL-60 cell-line. **(A)** Gel picture showing expression of cytokines, and **(B)** fold change in mRNA expression of cytokines normalised with β-actin housekeeping gene. *=$p<0.05$ vs. control (t-test). Andro= Andrographolide

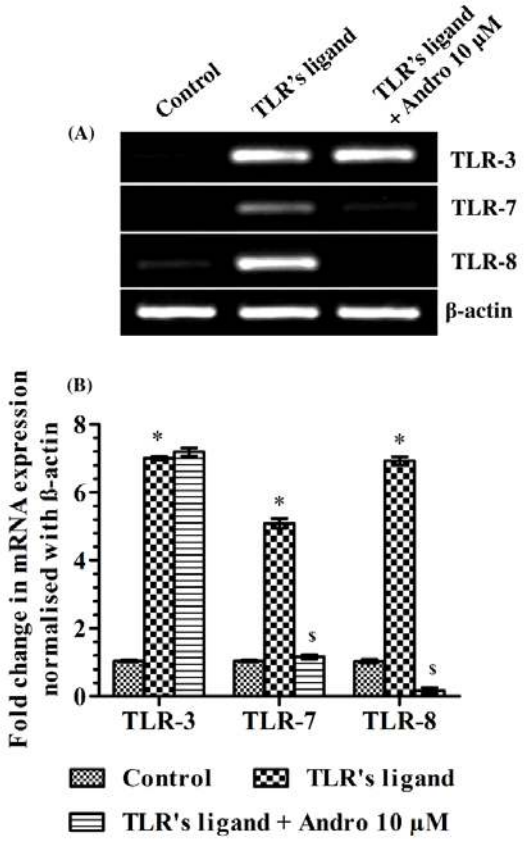

Figure 4.49: Effect of andrographolide (Andro) on various TLRs expression viz. TLR-3, TLR-7 and TLR-8 in HL-60 cell-line. **(A)** Gel picture showing expression of TLRs, and **(B)** fold change in mRNA expression of TLRs normalised with β-actin housekeeping gene. *=$p<0.05$ vs. control, $^{\$}$=$p<0.05$ vs. TLR's ligand. Andro= Andrographolide

Table 4.1: Effect of *Andrographis paniculata* extract (AP) on mice in ring test

Treatment groups	Immobility index		
	60 min	90 min	120 min
Day 1			
Control (0.3% CMC)	14.83 ± 0.37	16.44 ± 0.46	17.11 ± 0.37
AP (25 mg/kg)	14.66 ± 0.57	16.16 ± 0.67	16.83 ± 0.67
AP (50 mg/kg)	14.89 ± 0.48	16.38 ± 0.51	17.05 ± 0.51
AP (100 mg/kg)	15.00 ± 0.50	16.55 ± 0.47	17.22 ± 0.47
AP (200 mg/kg)	14.83 ± 0.55	16.61 ± 0.58	17.28 ± 0.58
AP (400 mg/kg)	14.94 ± 0.43	16.72 ± 0.52	17.39 ± 0.52
AP (600 mg/kg)	15.11 ± 0.38	16.78 ± 0.51	17.44 ± 0.51
AP (800 mg/kg)	15.17 ± 0.36	16.72 ± 0.46	17.33 ± 0.30
Day 5			
Control (0.3% CMC)	15.17 ± 0.48	16.83 ± 0.38	17.49 ± 0.49
AP (25 mg/kg)	14.83 ± 0.58	16.50 ± 0.58	17.17 ± 0.57
AP (50 mg/kg)	15.00 ± 0.57	16.67 ± 0.57	17.33 ± 0.57
AP (100 mg/kg)	15.17 ± 0.51	16.83 ± 0.52	17.44 ± 0.57
AP (200 mg/kg)	15.00 ± 0.46	16.67 ± 0.46	17.33 ± 0.46
AP (400 mg/kg)	15.05 ± 0.55	16.78 ± 0.55	17.44 ± 0.55
AP (600 mg/kg)	15.17 ± 0.39	16.83 ± 0.39	17.50 ± 0.55
AP (800 mg/kg)	15.28 ± 0.56	16.94 ± 0.47	17.61 ± 0.34
Day 7			
Control (Vehicle)	15.28 ± 0.36	17.00 ± 0.31	17.61 ± 0.36
AP (25 mg/kg)	15.00 ± 0.47	16.67 ± 0.47	17.33 ± 0.47
AP (50 mg/kg)	15.11 ± 0.46	17.00 ± 0.45	17.44 ± 0.47
AP (100 mg/kg)	15.16 ± 0.45	16.89 ± 0.46	17.55 ± 0.46
AP (200 mg/kg)	15.28 ± 0.43	16.95 ± 0.43	17.61 ± 0.43
AP (400 mg/kg)	15.33 ± 0.48	17.00 ± 0.49	17.67 ± 0.49
AP (600 mg/kg)	15.33 ± 0.38	17.00 ± 0.38	17.67 ± 0.39
AP (800 mg/kg)	15.44 ± 0.33	17.00 ± 0.28	17.56 ± 0.33
Day 10			
Control (Vehicle)	15.50 ± 0.33	17.16 ± 0.33	17.83 ± 0.33
AP (25 mg/kg)	15.11 ± 0.42	16.78 ± 0.42	17.50 ± 0.44
AP (50 mg/kg)	15.22 ± 0.48	16.89 ± 0.48	17.28 ± 0.38
AP (100 mg/kg)	15.28 ± 0.41	16.95 ± 0.41	17.61 ± 0.41
AP (200 mg/kg)	15.39 ± 0.40	17.06 ± 0.40	17.72 ± 0.40
AP (400 mg/kg)	15.50 ± 0.44	17.17 ± 0.44	17.83 ± 0.44
AP (600 mg/kg)	15.55 ± 0.31	17.45 ± 0.37	18.00 ± 0.37
AP (800 mg/kg)	15.61 ± 0.34	17.83 ± 0.25	18.50 ± 0.28

No significant effect on immobility index of mice (Two way ANOVA followed by Bonferroni post tests)

Table 4.2: Effect of *Andrographis paniculata* extract (AP) on mice in inverted screen test

Treatment groups	Screen index			
	Day 1	Day 5	Day 7	Day 10
Control (0.3% CMC)	0.66 ± 0.21	0.83 ± 0.30	0.67 ± 0.21	0.83 ± 0.30
AP (25 mg/kg)	0.66 ± 0.21	0.50 ± 0.22	0.67 ± 0.21	0.50 ± 0.22
AP (50 mg/kg)	0.83 ± 0.31	0.83 ± 0.30	0.83 ± 0.30	0.67 ± 0.21
AP (100 mg/kg)	0.66 ± 0.21	0.67 ± 0.21	0.67 ± 0.21	0.50 ± 0.22
AP (200 mg/kg)	0.50 ± 0.22	0.67 ± 0.21	0.83 ± 0.30	0.83 ± 0.30
AP (400 mg/kg)	0.66 ± 0.21	0.83 ± 0.30	0.67 ± 0.21	0.67 ± 0.21
AP (600 mg/kg)	0.50 ± 0.22	0.50 ± 0.22	0.50 ± 0.22	0.67 ± 0.33
AP (800 mg/kg)	0.83 ± 0.31	0.67 ± 0.21	0.83 ± 0.30	0.67 ± 0.21

No significant effect on screen index of mice (Two way ANOVA followed by Bonferroni post tests)

Table 4.3: Effect of *Andrographis paniculata* extract (AP) on maximal electroshock (MES)-induced seizures in rats

Treatment groups	Time (sec) in various phases of convulsion			
	Tonic flexion	Hind limb tonic extension (HLTE)	Clonus	Stupor
Control (0.3% CMC)	3.83 ± 0.31	13.67 ± 0.42	12.67 ± 0.88	163.17 ± 3.89
AP (50 mg/kg)	3.33 ± 0.49	13.17 ± 0.79	11.83 ± 1.08	160.33 ± 4.45
AP (100 mg/kg)	3.17 ± 0.31	12.33 ± 0.99	10.67 ± 0.61	154.33 ± 3.15
AP (200 mg/kg)	2.50 ± 0.22*	10.33 ± 0.95*	9.50 ± 0.56*	147.67 ± 3.38*
Phenytoin (30 mg/kg)	2.17 ± 0.17*	6.50 ± 0.43*	5.50 ± 0.50*	101.17 ± 3.70*

*=$p<0.05$ vs. control (0.3% CMC)

Table 4.4: Effect of *Andrographis paniculata* extract (AP) on pentylenetetrazole (80 mg/kg, i.p.)-induced convulsions in mice

Treatment groups	Myoclonic jerk latency (sec)	Clonic seizure (%)	Protection (%)
Control (0.3% CMC)	54.17 ± 3.41	100	0
AP (50 mg/kg)	59.67 ± 3.42	100	0
AP (100 mg/kg)	62.17 ± 3.70	100	0
AP (200 mg/kg)	66.83 ± 3.28*	83	33
Diazepam (10 mg/kg)	75.00 ± 4.44*	50	83

*=p<0.05 vs. control (0.3% CMC)

Table 4.5: Effect of *Andrographis paniculata* extract (AP) on body weight, fasting blood glucose and plasma insulin levels of the experimental groups observed 10 days after daily oral treatments

Treatment Group	Body weight change (g)	Fasting blood glucose (mg/dl)	Plasma insulin (μIU/ml)
Normal Control (0.3% CMC)	5.00±0.30	89.68±1.53	17.86±0.29
Diabetic Control (0.3% CMC)	-7.67±0.62*	290.43±1.45*	9.95±0.15*
Diabetic + AP 50 mg/kg	2.00±0.37¥	172.80±2.24¥	11.98±0.27¥
Diabetic + AP 100 mg/kg	2.50±0.50¥	146.18±1.78¥	12.88±0.21¥
Diabetic + AP 200 mg/kg	4.17±0.91¥	130.43±2.53¥	14.93±0.15¥
Diabetic + Glib 10 mg/kg	7.33±1.09¥	117.53±3.08¥	16.73±0.20¥

Glib= Glibenclamide. *=p<0.05 vs. normal control, ¥=p<0.05 vs. diabetic control

Table 4.6: Effect of *Andrographis paniculata* extract (AP) on oxidative status of liver, kidney, and pancreas of diabetic rats

Treatment Groups	LPO (nmol MDA/mg of protein)			SOD (Units/mg of protein)			CAT (µmole H_2O_2/min/mg of protein)		
	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas
Normal Control (0.3% CMC)	9.16 ±0.58	9.31 ±0.44	5.51 ±0.24	13.81± 0.67	18.03 ±0.87	4.70 ±0.29	18.61 ±0.68	14.02 ±0.59	12.54 ±0.39
Diabetic Control (0.3% CMC)	14.90 ±0.74*	20.65 ±0.91*	11.70 ±0.53*	5.91 ±0.32*	10.02 ±0.40*	1.67 ±0.16*	10.48 ±0.49*	7.92 ±0.34*	5.23 ±0.29*
Diabetic + AP 50 mg/kg	11.85 ±0.69¥	16.48 ±0.81¥	9.86 ±0.79¥	8.02 ±0.46¥	12.58 ±0.97¥	2.23 ±0.18¥	12.94 ±0.94¥	9.96 ±0.39¥	7.45 ±0.32¥
Diabetic + AP 100 mg/kg	9.57 ±0.57¥	13.38 ±0.66¥	7.52 ±0.58¥	8.77 ±0.51¥	14.81 ±1.02¥	3.12 ±0.31¥	14.25 ±0.98¥	11.58 ±0.58¥	9.51 ±0.59¥
Diabetic + AP 200 mg/kg	7.93 ±0.42¥	10.91 ±0.56¥	5.84 ±0.46¥	12.73 ±0.75¥	17.11 ±1.16¥	4.21 ±0.48¥	17.67 ±0.75¥	13.07 ±0.58¥	11.85 ±0.72¥
Diabetic + Glib 10 mg/kg	8.59 ±0.57¥	11.64 ±0.61¥	6.56 ±0.54¥	10.64 ±0.78¥	15.32 ±0.99¥	3.53 ±0.40¥	15.07 ±0.64¥	11.47 ±0.72¥	10.48 ±0.45¥

Glib= Glibenclamide. *=$p<0.05$ vs. normal control, ¥=$p<0.05$ vs. diabetic control

Table 4.7: Effect of *Andrographis paniculata* extract (AP) on body weight gain, food intake, and fasting plasma glucose and insulin level in high fat fed obese rats

Treatment group	Body weight gain (g)	Food intake (g/rat)	Fasting blood glucose (mg/dl)	Plasma insulin (µIU/ml)
NPD Control (0.3% CMC)	3.92±0.53	11.08±0.53	89.68±1.53	18.03±0.29
HFD Control (0.3% CMC)	45.83±3.21*	17.33±0.66*	290.43±1.45*	59.32±1.13*
HFD + AP 50 mg/kg	38.50±2.69¥	14.00±0.58¥	172.80±2.24¥	41.14±1.56 ¥
HFD + AP 100 mg/kg	30.00±3.45¥	13.33±0.67¥	146.18±1.78¥	35.72±0.79¥
HFD + AP 200 mg/kg	13.17±1.38¥	13.00±0.58¥	130.43±2.53¥	25.21±0.74¥
HFD + Atorv 10 mg/kg	7.67±0.71¥	12.17±0.48¥	117.53±3.08¥	21.54±0.73¥

Atorv = Atorvastatin. *=p<0.05 vs. normal control, ¥=p<0.05 vs. HFD control

Table 4.8: Effect of *Andrographis paniculata* extract (AP) on body weight gain and fasting plasma glucose and insulin level in fructose fed obese rats

Treatment group	Body weight gain (g)	Fasting blood glucose (mg/dl)	Plasma insulin (µIU/ml)
NPD Control (0.3% CMC)	5.33±0.58	90.97±1.52	18.02±0.28
FF Control (0.3% CMC)	30.67±2.07*	184.11±1.84 *	58.53±1.23*
FF + AP 50 mg/kg	22.83±3.04¥	165.16±2.34¥	40.62±1.32¥
FF + AP 100 mg/kg	13.17±1.89¥	138.66±2.38¥	30.53±0.82¥
FF + AP 200 mg/kg	9.00±1.32¥	107.80±2.10¥	21.54±0.83¥
FF + Atorv 10 mg/kg	4.67±0.76¥	125.48±2.44¥	25.20±0.71¥

Atorv = Atorvastatin. *=p<0.05 vs. normal control, ¥=p<0.05 vs. FF control

Table 4.9: Effect of *Andrographis paniculata* extract (AP) on body weight of nondiabetic rats

Treatment groups	Body weight (g)		
	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	156.83±1.01	164.00±1.18	7.17±0.48
Nondiabetic + AP 50 mg/kg	157.17±0.87	163.67±0.71	6.50±0.43
Nondiabetic + AP 100 mg/kg	156.83± 1.14	163.50±1.20	6.67±0.33
Nondiabetic + AP 200 mg/kg	157.67±0.56	163.83±0.91	6.17±0.70
Nondiabetic + Imipramine 15 mg/kg	156.50±1.06	160.83±0.95	4.33±0.33

*=p<0.05 vs. nondiabetic control

Table 4.10: Effect of *Andrographis paniculata* extract (AP) on body weight of diabetic rats

Treatment groups	Body weight (g)		
	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	156.83±1.01	164.00±1.18	7.17±0.48
Diabetic control (Vehicle)	149.00±0.89*	141.67±0.71*	-7.33±0.49*
Diabetic + AP 50 mg/kg	148.67±0.67	142.50±0.92	-6.17±0.48
Diabetic + AP 100 mg/kg	148.17±0.60	145.83±0.54¥	-2.33±0.42¥
Diabetic + AP 200 mg/kg	147.83±0.48	151.50±0.56¥	3.67±0.42¥
Diabetic + Imipramine 15 mg/kg	148.33±0.50	142.33±0.67	-6.00±0.37

*=p<0.05 vs. nondiabetic control; ¥=p<0.05 vs. diabetic control

Table 4.11: Effect of *Andrographis paniculata* extract (AP) on glucose level and insulin level in nondiabetic rats during learned helplessness test

Treatment groups	Glucose level (mg/dl)			Insulin level (µIU/ml)		
	Day 1	Day 10	Difference	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	86.67±0.71	91.00±0.82	4.33±0.33	17.67±0.49	19.50±0.50	1.83±0.31
Nondiabetic + AP 50 mg/kg	87.17±0.60	91.33±0.76	4.17±0.31	17.83±0.48	19.17±0.60	1.33±0.21
Nondiabetic + AP 100 mg/kg	86.83±0.60	90.83±0.60	4.00±0.45	17.67±0.33	19.33±0.49	1.67±0.21
Nondiabetic + AP 200 mg/kg	86.50±0.43	89.83±0.48	3.33±0.21	17.83±0.31	19.33±0.33	1.50±0.22
Nondiabetic + Imipramine 15 mg/kg	86.33±0.42	90.50±0.43	4.17±0.31	18.00±0.37	19.50±0.34	1.50±0.22

*=p<0.05 vs. nondiabetic control

Table 4.12: Effect of *Andrographis paniculata* extract (AP) on glucose level and insulin level in diabetic rats during learned helplessness test

Treatment groups	Glucose level (mg/dl)			Insulin level (µIU/ml)		
	Day 1	Day 10	Difference	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	86.67±0.71	91.00±0.82	4.33±0.33	17.67±0.49	19.50±0.50	1.83±0.31
Diabetic control (Vehicle)	269.67±7.00*	279.17±7.13*	9.50±0.22*	7.17±0.31*	5.50±0.34*	-1.67±0.33*
Diabetic + AP 50 mg/kg	270.67±6.15	196.17±1.58¥	-74.50±5.17¥	8.00±0.37	10.17±0.54¥	2.17±0.31¥
Diabetic + AP 100 mg/kg	270.17±6.97	168.17±3.96¥	-102.00±6.92¥	7.83±0.31	13.50±0.43¥	5.67±0.33¥
Diabetic + AP 200 mg/kg	269.17±5.26	125.50±2.08¥	-143.67±5.49¥	7.67±0.49	15.17±0.70¥	7.50±0.34¥
Diabetic + Imipramine 15 mg/kg	271.83±4.33	276.17±3.87	4.33±0.62	8.17±0.31	7.00±0.26	-1.50±0.22

*=p<0.05 vs. nondiabetic control; ¥=p<0.05 vs. diabetic control

Table 4.13: Effect of *Andrographis paniculata* extract (AP) on body weight of nondiabetic rats

Treatment groups	Body weight (g)		
	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	157.00±0.77	164.33±0.56	7.33±0.61
Nondiabetic + AP 50 mg/kg	157.17±0.87	163.33±1.09	6.17±0.31
Nondiabetic + AP 100 mg/kg	156.67±0.76	163.50±0.89	6.83±0.31
Nondiabetic + AP 200 mg/kg	157.83±0.60	164.50±0.56	6.67±0.56
Nondiabetic + Lorazepam 1 mg/kg	156.83±0.60	163.50±0.99	6.67±0.49

*=p<0.05 vs. nondiabetic control

Table 4.14: Effect of *Andrographis paniculata* extract (AP) on body weight of diabetic rats

Treatment groups	Body weight (g)		
	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	157.00±0.77	164.33±0.56	7.33±0.61
Diabetic control (Vehicle)	149.33±1.23*	140.50±1.20*	-8.83±0.30*
Diabetic + AP 50 mg/kg	148.33±0.88	144.67±0.55¥	-3.67±0.56¥
Diabetic + AP 100 mg/kg	148.17±0.60	146.50±0.50¥	-1.67±0.33¥
Diabetic + AP 200 mg/kg	148.33±0.67	152.83±0.65¥	4.50±0.43¥
Diabetic + Lorazepam 1 mg/kg	148.33±0.70	141.17±0.79	-7.67±0.67

*=p<0.05 vs. nondiabetic control; ¥=p<0.05 vs. diabetic control

Table 4.15: Effect of *Andrographis paniculata* extract (AP) on glucose level and insulin level of nondiabetic rats during elevated plus maze test

Treatment groups	Glucose level (mg/dl)			Insulin level (µIU/ml)		
	Day 1	Day 10	Difference	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	86.33±0.71	90.83±0.54	4.50±0.43	17.67±0.56	19.17±0.48	1.50±0.22
Nondiabetic + AP 50 mg/kg	86.83±0.54	91.50±0.43	4.67±0.33	17.83±0.70	19.60±0.61	1.83±0.48
Nondiabetic + AP 100 mg/kg	87.00±1.41	90.17±0.48	3.17±0.40	17.83±0.47	19.33±0.49	1.50±0.22
Nondiabetic + AP 200 mg/kg	87.17±0.60	90.00±0.37	2.83±0.31	17.67±0.49	19.33±0.42	1.67±0.21
Nondiabetic + Lorazepam 1 mg/kg	87.83±0.60	91.17±0.48	3.33±0.21	17.67±0.42	19.33±0.21	1.67±0.33

*=p<0.05 vs. nondiabetic control; ¥=p<0.05 vs. diabetic control

Table 4.16: Effect of *Andrographis paniculata* extract (AP) on glucose level and insulin level of diabetic rats during elevated plus maze test

Treatment groups	Glucose level (mg/dl)			Insulin level (µIU/ml)		
	Day 1	Day 10	Difference	Day 1	Day 10	Difference
Nondiabetic control (Vehicle)	86.33±0.71	90.83±0.54	4.50±0.43	17.67±0.56	19.17±0.48	1.50±0.22
Diabetic control (Vehicle)	270.67±5.46*	279.00±5.50*	8.33±0.33*	8.17±0.40*	6.83±0.48*	-1.33±0.21
Diabetic + AP 50 mg/kg	273.17±5.75	196.67±3.08¥	-76.50±4.70¥	8.33±0.33	10.67±0.21¥	2.33±0.21¥
Diabetic + AP 100 mg/kg	273.67±4.96	153.17±5.20¥	-120.50±2.58¥	8.17±0.31	14.17±0.48¥	6.00±0.68¥
Diabetic + AP 200 mg/kg	273.83±1.89	126.83±0.65¥	-147.00±2.35¥	8.33±0.21	17.17±0.48¥	8.83±0.40¥
Diabetic + Lorazepam 1 mg/kg	283.17±2.90	284.50±2.11	7.83±0.83	8.17±0.40	7.00±0.26	-1.17±0.48

*=p<0.05 vs. nondiabetic control; ¥=p<0.05 vs. diabetic control

Table 4.17: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight of nondiabetic rats during MWM test

Treatment groups	Body weight (g)		
	Day 1	Day 10	Difference (g)
ND Control (CMC 0.3%)	153.67±1.56	159.83±1.08	6.16±0.79
ND + AP 50 mg/kg	154.17±1.08	160.00±1.29	5.83±0.31
ND + AP 100 mg/kg	153.67±1.09	159.67±1.02	6.00±0.37
ND + AP 200 mg/kg	154.33±1.23	160.67±1.09	6.33±0.49
ND + Andro 15 mg/kg	153.50±1.26	159.67±1.31	6.17±0.48
ND + Andro 30 mg/kg	153.33±1.15	160.00±1.07	6.67±0.42
ND + Andro 60 mg/kg	154.83±1.25	161.33±0.99	6.50±0.62
ND + Piracetam 100 mg/kg	154.50±1.06	161.17±1.11	6.67±0.21

*=p<0.05 vs. nondiabetic (ND) control. Andro= Andrographolide

Table 4.18: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight of diabetic rats during MWM test

Treatment groups	Body weight (g)		
	Day 1	Day 10	Difference (g)
ND Control (CMC 0.3%)	153.67±1.56	159.83±1.08	6.16±0.79
D Control (CMC 0.3%)	145.17±0.48	136.33±0.80*	-8.83±0.48*
D + AP 50 mg/kg	143.67±1.43	143.00±1.83¥	-0.67±0.67¥
D + AP 100 mg/kg	143.83±1.11	147.33±1.17¥	3.50±0.43¥
D + AP 200 mg/kg	143.67±0.61	148.17±0.48¥	4.50±0.43¥
D + Andro 15 mg/kg	144.33±0.80	142.67±0.71¥	-1.67±0.61¥
D + Andro 30 mg/kg	143.50±0.67	146.83±0.79¥	3.33±0.42¥
D + Andro 60 mg/kg	143.67±0.71	147.83±0.65¥	4.17±0.31¥
D + Piracetam 100 mg/kg	143.83±0.79	137.50±0.92	-6.33±0.42

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

Table 4.19: Effect of *Andrographis paniculata* extract (AP) and andrographolide on blood glucose and insulin level of nondiabetic rats from MWM test

Treatment groups	Blood glucose (mg/dl)			Insulin level (µIU/ml)		
	Day 1	Day 10	Difference	Day 1	Day 10	Difference
ND Control (CMC 0.3%)	91.50±1.82	94.17±1.72	2.67±0.33	17.00±0.45	18.83±0.31	1.83±0.40
ND + AP 50 mg/kg	88.83±0.79	91.67±0.99	2.83±0.31	17.50±0.43	19.00±0.63	1.50±0.22
ND + AP 100 mg/kg	89.83±1.10	92.83±1.01	3.00±0.45	17.83±0.31	19.50±0.43	1.67±0.33
ND + AP 200 mg/kg	89.50±0.76	92.33±0.80	2.83±0.31	17.67±0.33	19.33±0.42	1.67±0.33
ND + Andro 15 mg/kg	89.67±0.67	92.17±0.70	2.50±0.22	17.33±0.49	19.00±0.37	1.67±0.42
ND + Andro 30 mg/kg	89.83±0.91	93.00±0.97	3.17±0.31	17.83±0.48	19.33±0.42	1.50±0.22
ND + Andro 60 mg/kg	90.17±0.87	93.00±1.03	2.83±0.31	18.00±0.37	19.67±0.56	1.67±0.21
ND + Piracetam 100 mg/kg	88.67±0.84	91.67±1.05	3.00±0.37	18.17±0.40	19.17±0.31	1.00±0.26

*=p<0.05 vs. nondiabetic (ND) control, Andro= Andrographolide

Table 4.20: Effect of *Andrographis paniculata* extract (AP) and andrographolide on blood glucose and insulin level of diabetic rats from MWM test

Treatment groups	Blood glucose (mg/dl)			Insulin level (µIU/ml)		
	Day 1	Day 10	Difference	Day 1	Day 10	Difference
ND Control (CMC 0.3%)	91.50±1.82	94.17±1.72	2.67±0.33	17.00±0.45	18.83±0.31	1.83±0.40
D Control (CMC 0.3%)	271.67±3.35*	280.83±3.25	9.17±0.54*	7.33±0.33*	5.67±0.33*	-1.67±0.21*
D + AP 50 mg/kg	273.83±3.50	201.00±3.13¥	-72.83±2.34¥	8.17±0.31	10.33±0.49¥	2.17±0.31¥
D + AP 100 mg/kg	272.67±3.05	147.17±3.96¥	-125.50±1.95¥	7.83±0.31	13.00±0.37¥	5.17±0.48¥
D + AP 200 mg/kg	272.50±2.81	119.67±2.91¥	-152.83±3.96¥	7.67±0.49	14.83±0.60¥	7.17±0.31¥
D + Andro 15 mg/kg	271.67±2.80	194.33±2.47¥	-77.33±2.28¥	8.00±0.37	10.83±0.48¥	2.83±0.40¥
D + Andro 30 mg/kg	273.17±2.97	131.50±2.14¥	-141.67±3.21¥	7.67±0.33	14.17±0.48¥	6.50±0.62¥
D + Andro 60 mg/kg	272.33±2.94	115.83±1.66¥	-156.50±3.43¥	7.50±0.43	15.33±0.33¥	7.83±0.48¥
D + Piracetam 100 mg/kg	271.50±2.31	279.67±2.09	8.17±0.48	8.33±0.33	6.83±0.31	-1.50±0.34

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control

Table 4.21: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of nondiabetic rats from spontaneous locomotor activity test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND Control (CMC 0.3%)	154.67±1.36	161.17±1.14	6.50±0.56	90.67±0.61	93.33±0.67	2.67±0.21
ND + AP 50 mg/kg	154.33±1.23	160.50±1.15	6.17±0.40	91.83±0.60	94.50±0.56	2.67±0.33
ND + AP 100 mg/kg	156.33±1.23	162.67±1.28	6.33±0.33	90.17±0.48	92.50±0.56	2.33±0.21
ND + AP 200 mg/kg	155.83±1.01	162.00± 1.10	6.17±0.31	89.83±0.60	91.50±0.43	1.67±0.21
ND + Andro 15 mg/kg	155.50±1.18	161.67±1.05	6.17±0.17	90.50±0.43	92.50±0.34	2.00±0.26
ND + Andro 30 mg/kg	153.33±1.52	159.67±1.54	6.33±0.21	89.33±0.76	90.83±0.79	1.50±0.22
ND + Andro 60 mg/kg	154.83±1.22	161.00±1.07	6.17±0.30	90.17±0.60	91.83±0.54	1.67±0.21
ND + Lorazepam 1 mg/kg	154.50±1.03	160.50±1.18	6.00±0.36	89.83±0.48	91.50±0.56	1.67±0.21

*=p<0.05 vs. nondiabetic (ND) control. Andro= Andrographolide

Table 4.22: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of diabetic rats from spontaneous locomotor activity test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND Control (CMC 0.3%)	154.67±1.36	161.17±1.14	6.50±0.56	90.67±0.61	93.33±0.67	2.67±0.21
D Control (CMC 0.3%)	147.50±0.76*	138.50±0.56*	-9.00±0.52*	274.00±4.76*	283.50±4.51*	9.50±0.43*
D + AP 50 mg/kg	148.17±0.79	144.50±0.81¥	-3.67±0.33¥	271.50±4.36	200.00±5.01¥	-71.50±1.57¥
D + AP 100 mg/kg	148.00±0.89	149.50±0.96¥	1.50±0.22¥	273.50±2.14	149.00±4.28¥	-124.50±3.37¥
D + AP 200 mg/kg	147.33±0.95	152.00±0.87¥	4.67±0.21¥	274.67±2.17	121.50±0.62¥	-153.17±2.39¥
D + Andro 15 mg/kg	146.67±0.56	143.67±0.61¥	-3.00±0.45¥	271.50±1.88	197.67±1.78¥	-73.83±3.03¥
D + Andro 30 mg/kg	146.17±0.75	148.00±0.63¥	1.83±0.31¥	274.17±2.04	146.83±1.85¥	-127.33±1.26¥
D + Andro 60 mg/kg	146.50±0.43	150.17±0.65¥	3.67±0.42¥	274.17±1.85	118.17±1.74¥	-156.00±1.98¥
D + Lorazepam 1 mg/kg	146.33±0.56	138.17±0.75	-8.17±0.31	274.67±1.76	283.00±1.98	7.67±0.80

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

Table 4.23: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of nondiabetic mice from 5-HTP-induced head twitches test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND Control (CMC 0.3%)	23.50±0.89	27.67±0.84	4.17±0.31	84.67±0.67	87.83±0.40	3.17±0.40
ND + AP 50 mg/kg	23.83±0.70	27.67±0.49	3.83±0.31	84.83±0.95	87.83±0.70	3.00±0.37
ND + AP 100 mg/kg	23.50±0.99	27.50±0.92	4.00±0.37	84.33±1.12	86.67±1.00	2.33±0.42
ND + AP 200 mg/kg	22.83±0.87	27.17±0.79	4.33±0.33	84.50±1.18	86.67±1.20	2.17±0.31
ND + Andro 15 mg/kg	23.67±0.61	27.67±0.56	4.00±0.26	85.17±0.70	88.17±0.60	3.17±0.31
ND + Andro 30 mg/kg	23.83±0.70	28.00±0.52	4.17±0.47	84.17±1.01	86.67±1.12	2.50±0.22
ND + Andro 60 mg/kg	22.83±0.70	27.00±0.68	4.17±0.31	84.17±0.60	87.00±0.26	2.83±0.60
ND + Imipramine 15 mg/kg	23.50±0.56	27.33±0.42	3.83±0.31	85.00±0.63	88.17±0.95	3.17±0.40

*=p<0.05 vs. nondiabetic (ND) control. Andro= Andrographolide

Table 4.24: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of diabetic mice from 5-HTP-induced head twitches test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	23.50±0.89	27.67±0.84	4.17±0.31	84.67±0.67	87.83±0.40	3.17±0.40
D control (CMC 0.3%)	17.00±0.45*	13.50±0.34*	-3.50±0.43*	265.50±1.63*	276.83±1.80*	11.33±0.80*
D + AP 50 mg/kg	17.50±0.50	15.83±0.31¥	-1.67±0.33¥	264.17±2.52	195.33±2.72¥	-65.50±4.02¥
D + AP 100 mg/kg	17.33±0.49	19.67±0.33¥	2.33±0.21¥	265.17±1.82	150.50±2.72¥	-117.50±4.63¥
D + AP 200 mg/kg	16.83±0.54	19.33±0.61¥	2.50±0.22¥	265.67±1.89	117.33±0.71¥	-148.33±1.99¥
D + Andro 15 mg/kg	17.00±0.52	14.83±0.40	-2.17±0.31¥	264.00±2.21	190.67±2.61¥	-73.33±3.60¥
D + Andro 30 mg/kg	17.33±0.42	19.50±0.22¥	2.17±0.31¥	263.83±1.85	143.83±2.18¥	-120.00±2.38¥
D + Andro 60 mg/kg	16.83±0.31	19.17±0.40¥	2.33±0.21¥	264.17±1.30	114.17±1.45¥	-150.00±2.58¥
D + Imipramine 15 mg/kg	17.17±0.17	14.00±0.37	-3.17±0.31	263.17±1.49	274.00±1.81	10.83±0.40

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

Table 4.25: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of nondiabetic mice from L-DOPA-induced hyperactivity test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	22.50±0.43	27.17±0.31	4.6±0.49	85.17±0.60	88.00±0.37	2.83±0.31
ND + AP 50 mg/kg	22.67±0.42	27.00±0.58	4.33±0.56	84.17±1.14	86.33±1.09	2.17±0.40
ND + AP 100 mg/kg	22.67±0.95	26.67±0.92	4.00±0.26	83.50±1.61	85.83±1.30	2.33±0.33
ND + AP 200 mg/kg	22.00±0.63	26.17±0.54	4.17±0.40	84.00±0.86	86.00±1.00	2.00±0.37
ND + Andro 15 mg/kg	22.33±0.49	26.67±0.61	4.33±0.33	84.50±0.89	86.67±0.88	2.17±0.31
ND + Andro 30 mg/kg	22.67±0.33	27.17±0.31	4.50±0.22	83.83±1.01	86.17±1.01	2.33±0.21
ND + Andro 60 mg/kg	22.17±0.60	27.00±0.68	4.83±0.98	83.50±0.89	85.33±0.80	1.83±0.60
ND + Imipramine 15 mg/kg	22.83±0.31	26.67±0.21	3.83±0.31	84.50±0.62	87.00±0.73	2.50±0.22

*=p<0.05 vs. nondiabetic (ND) control. Andro= Andrographolide

Table 4.26: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of diabetic mice from L-DOPA-induced hyperactivity test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	22.50±0.43	27.17±0.31	4.6±0.49	85.17±0.60	88.00±0.37	2.83±0.31
D control (CMC 0.3%)	17.17±0.48*	13.17±0.40*	-4.00±0.37*	264.67±1.38*	276.17±1.30*	11.50±0.76*
D + AP 50 mg/kg	17.33±0.42	16.33±0.76¥	-1.00±0.45¥	263.83±2.20	198.67±4.84¥	-65.17±4.00¥
D + AP 100 mg/kg	17.83±0.48	19.67±0.21¥	1.83±0.31¥	262.50±1.52	147.67±4.12¥	-114.83±4.59¥
D + AP 200 mg/kg	17.33±0.42	20.67±0.49¥	3.33±0.21¥	265.17±2.23	117.33±0.71¥	-147.83±2.77¥
D + Andro 15 mg/kg	17.00±0.37	16.00±0.37¥	-1.00±0.45¥	263.33±2.33	189.83±2.33¥	-73.50±3.99¥
D + Andro 30 mg/kg	17.50±0.22	20.33±0.49¥	2.83±0.40¥	262.83±1.83	142.83±1.33¥	-120.00±2.00¥
D + Andro 60 mg/kg	16.67±0.49	20.33±0.42¥	3.67±0.42¥	263.00±1.53	112.83±2.18¥	-150.17±2.37¥
D + Imipramine 15 mg/kg	17.33±0.33	13.83±0.31	-3.50±0.34	262.33±1.61	274.00±1.81	11.67±0.56

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

Table 4.27: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of nondiabetic mice from Apomorphine-induced hyperactivity

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND Control (CMC 0.3%)	21.83±0.54	26.33±0.56	4.50±0.22	84.33±0.67	87.33±0.56	3.00±0.52
ND + AP 50 mg/kg	22.83±0.48	27.00±0.37	4.17±0.31	83.67±0.95	86.67±0.92	3.00±0.26
ND + AP 100 mg/kg	21.83±0.60	26.16±0.60	4.33±0.33	83.33±1.50	86.17±1.30	2.83±0.48
ND + AP 200 mg/kg	22.33±0.56	26.83±0.70	4.50±0.56	84.83±0.79	87.00±1.00	2.17±0.31
ND + Andro 15 mg/kg	22.17±0.40	26.17±0.40	4.00±0.36	83.83±0.70	86.67±0.88	2.83±0.31
ND + Andro 30 mg/kg	22.83±0.48	26.50±0.34	3.67±0.21	83.50±0.89	85.83±0.91	2.33±0.21
ND + Andro 60 mg/kg	22.50±0.72	26.83±0.60	4.33±0.33	83.83±0.79	86.00±0.86	2.17±0.17
ND + Imipramine 15 mg/kg	22.83±0.31	27.00±0.36	4.17±0.48	84.67±0.56	87.00±0.63	2.33±0.21

*=p<0.05 vs. nondiabetic (ND) control. Andro= Andrographolide

Table 4.28: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of diabetic mice from Apomorphine-induced hyperactivity

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	21.83±0.54	26.33±0.56	4.50±0.22	84.33±0.67	87.33±0.56	3.00±0.52
D control (CMC 0.3%)	16.83±0.31	13.17±0.40*	-3.67±0.33*	264.17±2.20*	274.67±1.91*	10.50±0.43*
D + AP 50 mg/kg	16.67±0.33	16.17±0.79¥	-0.50±0.56¥	264.50±2.23	197.00±3.55¥	-67.50±3.43¥
D + AP 100 mg/kg	17.17±0.48	20.33±0.42¥	3.17±0.17¥	263.83±1.85	150.17±3.82¥	-113.67±3.82¥
D + AP 200 mg/kg	16.83±0.48	20.17±0.60¥	3.33±0.33¥	266.33±1.84	118.83±1.49¥	-147.50±3.23¥
D + Andro 15 mg/kg	16.67±0.21	15.83±0.31¥	-0.83±0.17¥	266.83±2.36	194.17±2.36¥	-72.67±3.91¥
D + Andro 30 mg/kg	17.00±0.36	19.83±0.17¥	2.83±0.31¥	263.50±1.82	142.50±2.83¥	-121.00±3.31¥
D + Andro 60 mg/kg	16.66±0.21	20.17±0.48¥	3.50±0.43¥	264.17±1.33	114.50±2.85¥	-149.67±2.46¥
D + Imipramine 15 mg/kg	16.83±0.31	13.67±0.33	-3.17±0.48	263.50±2.05	273.17±1.85	9.67±0.99

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide

Table 4.29: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of nondiabetic rats from cotton pellet test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	156.33±1.65	158.17±1.52	1.83±0.31	86.83±0.54	89.17±0.83	2.33±0.76
ND + AP 100 mg/kg	155.83±1.70	157.83±1.76	2.00±0.37	86.50±0.76	87.83±0.48	1.33±0.99
ND + AP 200 mg/kg	155.67±1.94	157.50±1.88	1.83±0.31	86.50±0.76	88.83±0.60	2.33±0.42
ND + AP 400 mg/kg	156.67±1.58	158.50±1.73	1.83±0.31	86.66±0.71	89.67±0.80	3.00±0.82
ND + Andro 30 mg/kg	156.17±1.66	158.17±1.45	2.00±0.37	87.33±0.67	88.67±0.71	1.33±1.02
ND + Andro 60 mg/kg	154.67±1.86	156.83±1.76	2.17±0.40	87.67±0.71	89.17±0.54	1.50±1.02
ND + Andro 120 mg/kg	157.67±0.56	158.67±0.76	1.00±0.45	86.67±0.42	89.33±0.49	2.67±0.76
ND + Indom 5 mg/kg	156.83±1.14	152.33±0.76*	-4.50±0.92*	87.50±0.42	88.83±0.48	1.33±0.76

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Indom= Indomethacin

Table 4.30: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of diabetic rats from cotton pellet test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	156.33±1.65	158.17±1.52	1.83±0.31	86.83±0.54	89.17±0.83	2.33±0.76
D control (CMC 0.3%)	148.83±0.70*	141.17±0.70*	-7.67±0.33*	272.50±5.62*	284.17±5.87*	11.67±2.03
D + AP 100 mg/kg	148.50±0.67	149.67±0.80¥	1.17±0.31¥	273.17±4.83	167.83±4.00¥	-105.33±4.83¥
D + AP 200 mg/kg	147.83±0.75	149.50±1.05¥	1.67±0.49¥	273.83±4.08	126.50±1.34¥	-147.33±4.05¥
D + AP 400 mg/kg	147.50±0.76	151.50±0.56¥	4.00±0.58¥	274.50±5.00	112.67±1.45¥	-161.83±5.22¥
D + Andro 30 mg/kg	147.83±0.79	149.50±0.89¥	1.67±0.21¥	271.83±4.30	168.33±4.39¥	-103.50±6.38¥
D + Andro 60 mg/kg	148.17±0.83	149.67±0.71¥	1.50±0.34¥	271.17±5.15	125.00±1.65¥	-146.17±5.87¥
D + Andro 120 mg/kg	147.33±1.00	150.33±1.09¥	3.00±1.24¥	271.83±5.89	118.00±2.92¥	-153.83±3.54¥
D + Indom 5 mg/kg	148.17±0.60	142.17±0.60	-6.00±0.97	273.33±5.68	279.33±6.21	6.00±1.87

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Indom= Indomethacin

Table 4.31: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of nondiabetic rats from tail flick test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	156.33±1.50	158.33±1.56	2.00±0.52	86.67±0.71	89.50±0.76	2.83±0.79
ND + AP 100 mg/kg	156.50±0.92	158.00±1.71	1.50±0.85	86.83±0.60	90.50±0.43	3.67±0.42
ND + AP 200 mg/kg	155.33±1.41	157.67±1.87	2.33±1.98	86.50±0.43	89.83±0.48	3.33±0.21
ND + AP 400 mg/kg	156.33±1.41	157.50±1.63	1.17±0.83	86.33±0.42	90.50±0.43	4.17±0.31
ND + Andro 30 mg/kg	157.50±1.34	158.33±1.61	0.83±1.66	89.17±0.48	89.83±0.70	0.67±0.76
ND + Andro 60 mg/kg	155.17±1.52	157.50±1.43	2.33±1.33	88.17±0.48	90.83±0.70	2.67±0.84
ND + Andro 120 mg/kg	158.17±0.54	160.67±0.76	2.50±0.34	86.83±0.48	89.33±0.49	2.50±0.56
ND + Pentazo 30 mg/kg	157.00±1.16	152.67±0.95*	-4.33±1.26	87.33±0.80	89.50±0.43	2.17±0.91

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Pentazo= Pentazocine

Table 4.32: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of diabetic rats from tail flick test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	156.33±1.50	158.33±1.56	2.00±0.52	86.67±0.71	89.50±0.76	2.83±0.79
D control (CMC 0.3%)	147.67±0.88*	140.33±0.61*	-7.33±0.80*	273.17±6.16*	280.67±7.20*	7.50±1.1*
D + AP 100 mg/kg	148.17±0.87	149.83±0.87¥	1.67±0.56¥	272.17±5.37	168.67±3.78¥	-103.50±4.93¥
D + AP 200 mg/kg	146.83±0.70	149.83±1.01¥	3.00±1.00¥	271.17±4.94	127.17±2.32¥	-144.00±4.99¥
D + AP 400 mg/kg	147.33±1.09	150.67±0.71¥	3.33±0.92¥	272.50±5.76	117.17±2.27¥	-155.33±5.73¥
D + Andro 30 mg/kg	147.33±0.67	149.83±0.87¥	2.50±0.34¥	269.50±5.45	170.67±4.24¥	-98.83±5.78¥
D + Andro 60 mg/kg	146.83±0.87	150.17±0.79¥	3.33±0.76¥	270.50±5.34	129.33±1.86¥	-141.17±6.38¥
D + Andro 120 mg/kg	146.17±1.11	150.33±0.99¥	4.17±1.68¥	272.50±6.30	122.17±2.28¥	-150.33±5.13¥
D + Pentazo 30 mg/kg	147.50±0.67	141.83±1.01	-5.67±1.36	273.83±5.86	275.50±5.60	1.67±0.40

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Pentazo= Pentazocine

Table 4.33: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of nondiabetic mice from hot plate test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	20.67±1.23	21.17±1.14	0.50±0.22	85.83±1.08	86.83±0.79	1.00±1.24
ND + AP 100 mg/kg	19.67±0.71	20.17±0.60	0.50±0.22	85.17±0.70	86.50±1.34	1.33±1.15
ND + AP 200 mg/kg	20.17±0.60	20.50±0.56	0.33±0.21	86.67±0.76	87.50±0.76	0.83±0.95
ND + AP 400 mg/kg	19.83±0.60	20.00±0.57	0.17±0.31	86.33±0.33	88.17±0.65	1.83±0.60
ND + Andro 30 mg/kg	19.83±0.48	20.16±0.47	0.33±0.21	87.67±0.67	89.83±0.70	2.17±1.14
ND + Andro 60 mg/kg	20.17±0.48	20.66±0.42	0.50±0.22	87.17±0.54	89.17±0.70	2.00±0.89
ND + Andro 120 mg/kg	19.67±0.50	20.17±0.54	0.50±0.22	85.83±0.48	87.67±0.33	1.83±0.40
ND + Pentazo 30 mg/kg	19.83±0.48	18.33±0.33*	-1.17±0.31	86.83±0.60	88.33±0.33	1.50±0.50

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Pentazo= Pentazocine

Table 4.34: Effect of *Andrographis paniculata* extract (AP) and andrographolide on body weight and blood glucose level of diabetic mice from hot plate test

Treatment groups	Body weight (g)			Blood glucose (mg/dl)		
	Day 1	Day 10	Difference (g)	Day 1	Day 10	Difference (mg/dl)
ND control (CMC 0.3%)	20.67±1.23	21.17±1.14	0.50±0.22	85.83±1.08	86.83±0.79	1.00±1.24
D control (CMC 0.3%)	17.33±0.49*	15.83±0.31*	-1.50±0.56	272.67±4.96*	285.17±6.20*	12.50±3.31
D + AP 100 mg/kg	17.00±0.63	18.50±0.56¥	1.50±0.43¥	271.50±4.28	165.83±3.62¥	-105.67±4.42¥
D + AP 200 mg/kg	16.67±0.50	18.67±0.33¥	2.00±0.37¥	271.33±4.88	123.67±3.24¥	-147.67±6.76¥
D + AP 400 mg/kg	16.83±0.40	18.67±0.42¥	1.83±0.31¥	271.33±5.59	112.33±2.17¥	-159.00±5.66¥
D + Andro 30 mg/kg	16.83±0.48	18.00±0.52¥	1.17±0.17¥	266.83±5.47	168.50±4.82¥	-98.33±3.33¥
D + Andro 60 mg/kg	17.17±0.48	18.50±0.34¥	1.33±0.21¥	267.67±3.14	125.83±1.89¥	-141.83±3.74¥
D + Andro 120 mg/kg	17.33±0.50	18.83±0.48¥	1.50±0.22¥	271.33±6.25	117.83±2.20¥	-153.50±6.61¥
D + Pentazo 30 mg/kg	17.17±0.30	14.83±0.48	-2.33±0.33	267.50±3.45	278.50±3.05	11.00±1.91

*=p<0.05 vs. nondiabetic (ND) control; ¥=p<0.05 vs. diabetic (D) control. Andro= Andrographolide, Pentazo= Pentazocine

Table 4.35: Effect of *Andrographis paniculata* extract (AP) and andrographolide on chronic stress-induced gastric ulceration of rats

Treatment groups	Number of ulcer (N)	Severity of ulcer (Score)	Ulcer incidence (%)
Normal control (Vehicle)	00	00	00
Stress control (Vehicle)	8.67±0.42*	22.17±0.48*	100
Stress + WS 100 mg/kg	2.67±0.21¥	4.67±0.42¥	21
Stress + AP 50 mg/kg	5.67±0.33	14.17±0.40	64
Stress + AP 100 mg/kg	4.50±0.43	9.17±0.48	41
Stress + AP 200 mg/kg	3.17±0.17¥	5.33±0.42¥	24
Stress + Andro 30 mg/kg	3.67±0.33	7.50±0.67	34
Stress + Andro 60 mg/kg	2.83±0.17¥	4.50±0.22¥	20

*=$p<0.05$ vs. normal control; ¥=$p<0.05$ vs. stressed control. Analysis was done by non-parametric ANOVA followed by Kruskal-Wallis (KW) test. Andro= Andrographolide, WS= *Withania somnifera* extract