CHAPTER-4

RESULTS

RESULTS

4.1. Analytical Characterization of *Curcuma longa* extracts, pure curcumin and turmeric oil

Curcuma longa extracts (CLE-1H: contains total curcuminoids 21.84% w/w by HPLC), (CLE-2B: contains total curcuminoids 18.83% w/w by HPLC), (CLE-3R: contains total curcuminoids 95.49% w/w by HPLC), (CLE-4M: contains total curcuminoids 16.6% w/w by HPLC) quantified to contain curcumin, demethoxycurcumin and bisdemethoxycurcumin (17.93 %, 3.39 %, 0.52 % w/w); (15.45 %, 2.92%, 0.46 % w/w); (77.94%, 15.03 %, 2.52 % w/w); (13.88%, 2.46 %, 0.26 % w/w), and pure curcumin are shown in **Figures 4.1A** and **4.1B** & **4.2A** and **4.2B** & **4.3A** respectively. GC chromatogram of turmeric oil is shown in **Figure 4.3B.**

4.2. Pilot Studies

4.2.1. Pilot experiments with 95.49% curcuminoids containing Curcuma longa extract (CLE-3R): Mean body weight of male and female mice treated with vehicle, or metformin, or CLE-3R are summarized in the **(Figure 4.4A & 4.4B)**. As expected, the animals of both the control groups continuously lost their body weights during the course of the experiments, whereas those of the 100 mg/kg metformin treated ones started gaining their body weights after its 5 daily doses. Changes in the mean body weights of the 5 mg/kg/day CLE-3R treated male or female groups were quite analogous to those of the corresponding metformin treated ones. Mean body weights of the male as well as female metformin treated groups were statistically significantly higher than the corresponding control ones on the 11^{th} and 12^{th} days of the experiments, and analogous were also the cases for the 5, 20 and 80 mg/kg CLE-3R treated male groups (**Table 4.1, 4.2, 4.3**). In females, mean body weights of all CLE-3R treated groups from day 7 onwards

were always significantly higher than those of the vehicle treated groups (**Table 4.4, 4.5, 4.6**). Daily oral doses of CLE-3R higher than 20 mg/kg/day were always more effective in counteracting intermittent foot shock triggered body weight losses than 100 mg/kg/day metformin. Mean basal core temperatures of different groups of male and female mice recorded during the course of the experiment are summarized in the (**Fig. 4.5A and 4.5B)**. During the course of the experiments basal core temperatures of both the vehicle treated control groups continued to increase gradually, whereas those of the 100 mg/kg/day metformin treated ones remained almost constant on all observational days. Metformin like preventive effect of CLE-3R on foot shock stress and daily handling and treatments induced slight elevation of basal core temperatures was apparent even after its lowest tested oral doses (5 mg/kg/day). Like in the case of metformin, mean basal rectal temperatures of all CLE-3R treated groups on day 1, as well as on day 12 (i.e. 24 hours after their last doses), of the experiments were in the same range observed for the vehicle treated control groups on the first treatment days. In both male and female vehicle treated control groups the magnitude of foot shock stress triggered transient hyperthermia gradually increased during the course of the experiments. On the first observational day, mean values of the all CLE-3R or metformin treated groups were not statistically significantly different from those of the corresponding control ones (**Figure 4.6A & 4.6B**). On the 5^{th} day of the experiments statistically significant treatment effects were observed in males only after the highest tested dose of CLE-3R (320 mg/kg), whereas in females the observed effects of metformin (100 mg/kg/day) as well as those of CLE-3R doses higher than 5 mg/kg/day were always statistically significant. Efficacies of metformin and all tested doses of CLE-3R increased somewhat on the 7^{th} and 10^{th} observational days. After 10 daily oral doses clear dose dependant efficacy of CLE-3R in suppressing foot shock stress triggered hyperthermia were

observed in both male and female mice. However, even after ten daily tested highest CLE-3R dose (320 mg/kg/day) stress induced hyperthermic responses were still observed in both male and female mice. Although stress induced hyperthermic responses in vehicle treated female mice were somewhat lower than that observed in male controls, there were no statistically significant differences between the mean values of the two groups on all test days. All tested doses of CLE-3R and metformin (100 mg/kg) significantly lowered the immobility period of mice after their 11 daily doses. It is apparent from the results summarized in (**Table 4.7 & 4.8**) that the efficacy of CLE-3R increased with its increasing daily doses, Statistically significant and metformin like effect of CLE-3R were observe in both males and females even after its lowest daily dose tested (5 mg/kg/day). Efficacies of 100 mg/kg daily doses of metformin in males and females were almost equal to those observed for 20 mg/kg/day CLE-3R doses. Results summarized in (**Table 4.9 & 4.10)** reveal that 24 hours after treatments with CLE-3R for 11 consecutive days it dose dependently shortens sleep induction period and prolongs duration of sleep induced by pentobarbital in both male and female mice. These statistically significant ($p < 0.05$) effects of the extract were qualitative analogous to that of metformin. Quantitatively, the efficacy of 20 mg/kg dose of CLE-3R was almost equal to that of similar treatments with 100 mg/kg/day metformin, and that the efficacy of both the test agents as central nervous system depressants, or as an inducers of pentobarbital metabolizing enzymes, are similar in both male and female mice. On the basis of the results, log dose response curve was plotted between dose and stress induced hyperthermia response shown that 5 mg/kg dose of CLE-3R was minimum effective whereas 20 mg/kg was maximum effective in all battery of animal models **(Figure 4.7A & 4.7B).** It was also found that 20 mg/kg dose of CLE-3R is qualitatively equal to 100 mg/kg metformin. Therefore

its intermediate dose 10 mg/kg of CLE-3R and 50 mg/kg dose of metformin were taken into consideration for further comparative pharmacological studies.

4.2.2.Pilot experiments with four different Curcuma longa extracts (CLE-1H, CLE-2B, CLE-3R and CLE-4M): Mean body weight of control mice was significantly reduced compared to day 1 due to daily handling and foot shock stress which was compensated by different *Curcuma longa* extracts (CLEs) and pure curcumin treatments and tends toward normal (**Figure 4.8A)**. Results revealed that CLE-3R was found to be more effective to compensate the stress induce body weight loss of mice. Mean basal rectal temperature of control mice was significantly increased due to foot shock stress but groups which were treated with *Curcuma longa* extract CLE-1H (21.9 mg/kg), CLE-2B (25.4 mg/kg) showed very little elevation in temperature, however CLE-3R (5 mg/kg), CLE-4M (28.7 mg/kg) and pure curcumin (5 mg/kg) did not produced such type of handling induced increment in basal temperature **(Figure 4.8B).** Stress induced hyperthermia significantly reduced by CLE-2B (25.4 mg/kg) CLE-3R (5 mg/kg), CLE-4M (28.7 mg/kg) and pure curcumin (5 mg/kg) treated male mice after their 10 daily doses **(Figure 4.9)**. On day 11 in tail suspension test CLE-3R (5 mg/kg), and pure curcumin (5 mg/kg) treated groups significantly reduced immobility time compare to control **(Figure 4.10A)**. In pentobarbital induced sedation test, no significant effect was found on onset and duration of sleep between control, different CLEs and pure curcumin except CLE-3R **(Figure 4.10B**).

4.2.3. Pilot experiments with turmeric oil (TO)

Mean body weights of the control and different doses of turmeric oil (1, 3, 10, 3, 100 mg/kg) treated groups are summarized in the (**Figure 4.11A)**. Mean body weights of the control animals recorded on the first observational day of a given experiment was not always significantly different from those of the group recorded on subsequent observational day. However, these

mean values of control groups decreased consistently during the 12 observational days. Results summarized in **Figure 4.11A** revealed that protective effects of turmeric oil against stress triggered body weight losses were observed only after its 7 or more daily oral doses higher than 10 mg/kg. However, mean body weight of 5mg/kg/day CLE-3R treated groups observed on the 12^{th} days of the experiments were quite analogous to that of the 100 mg/kg/day turmeric oil treated group. Mean basal rectal temperatures of different groups recorded during the course of the experiments are summarized in the **(Figure 4.11B)**. These mean values of the vehicle treated control groups increased slightly during the course of the experiments and remained almost constantly elevated on the 10^{th}, 11^{th}, and 12^{th} days of the experiments. Such elevations were not observed in CLE-3R treated groups and these values of all these groups on the last three observational days were statistically significantly lower than those of the vehicle tread control groups. However, such were not the observations made with the turmeric oil treated groups. Although on the last three observational days these mean values of the 3 mg/kg or higher daily oral TURMERIC OIL treated groups were numerically lower than those of the corresponding control group no statistically significant effect of the oil was observed during the course of the experiment. In stressed induced hyperthermia test control group animals shown elevation in hyperthermia on subsequent experimental days. However, 5 mg/kg CLE-3R and 100 mg/kg dose of TURMERIC OIL significantly compensate that elevation in hyperthermia after their 10 daily doses **(Figure 4.12)**. Results summarized in **Figure 4.13A** reveal that quantitatively the observed efficacies of eleven 5 mg/kg daily oral doses CLE-3R and 100 mg/kg dose of turmeric oil in tail suspension test are almost equal. Turmeric oil at its low doses (1, 3, 10, 30 mg/kg) not shown any significant effect in reducing the immobility time period. Results summarized in **Figure**

4.13B reveal that, no statistically significant effects of any of the turmeric oil doses were observed in this test.

4.3. Analgesic Activity

4.3.1. Body weights and core temperatures: Mean body weights of both the control groups decrease gradually during the course of the experiment, whereas their mean basal rectal temperatures increased gradually. Rates of these changes during the course of the experiments in both the control groups were quite similar. These stress triggered alterations caused by daily handling and occasional testing were not observed in the CLE-3R or metformin treated groups. Even 5 mg daily CLE-3R dose was highly effective in compensating such body weight loss and elevation in core temperature due to such thermal stress. These efficacies of 50 mg/kg/day metformin for antagonizing body weight loss and core temperature elevation were almost identical to those of 5 mg/kg/day and 20 mg/kg/day dose of CLE-3R respectively, and those of the 20 and 80 mg/kg/day CLE -3R against both the mild chronic stress triggered alterations were almost identical (**Figure 4.14 A& 4.14B**).

4.3.2. Hot plate test: Results summarized in **Figure 4.15A** reveal that one hour after their first oral doses neither metformin nor CLE-3R had any significant effects on the mean reaction time of male mice preselected for their sensitivity to pain responses in the hot plate test. Mean reaction time of the vehicle treated control group (CON + HPT) continued to decrease on the 5^{th} and subsequent observational days, whereas those of the CLE-3R treated ones steadily increased during the course of the experiment. Analogous observed effects of metformin treatments were qualitative similar to those of CLE-3R, but the observed effects of even the lowest tested daily CLE-3R dose (5 mg/kg/day) on the 5^{th} and subsequent observational days were somewhat higher than that of metformin dose (50 mg/kg/day) tested. It is apparent from the calculated values

summarized in **Figure 4.15B** that pain response sensitivity of the metformin treated group remained almost constant on all observational days whereas that of CLE-3R continued to increase dose dependently with increasing numbers of treatment days.

4.4. Antidepressant Activity

4.4.1. Forced swimming test: Results of forced swimming test summarized in **Figure 4.16** revealed that, CLE-3R (10 mg/kg) and metformin (50 mg/kg) treatments significantly decreased immobility period in stressed rats, and that such was not the case in non-stressed ones. Since the mean immobility period of stressed control group was significantly higher than that of non-stressed one, these results suggest that the observed antidepressants like effects of CLE-3R and metformin in this test is mainly due to its protective effect against stress triggered exaggerated depressive state of the animals.

4.4.2. Blood glucose, insulin and corticosterone levels: Results summarized in **Table 4.11** revealed that, mean plasma glucose and corticosterone levels of the stressed group were significantly higher than those of the non-stressed control one, and that mean plasma insulin levels of the stressed control group was lower than the non-stressed control. Elevated plasma corticosterone levels observed in CLE-3R or metformin treated stressed group were significantly lower than that in the stressed control group. However, unlike metformin, CLE-3R had no statistically significant effects on plasma glucose level in foot shock stressed animals. Antihyperglycemic effect of metformin was observed only in stressed animals, but this effect of metformin was not accompanied by significant alterations in insulin levels of stressed animals. In non-stressed animals neither metformin nor CLE-3R had any statistically significant effects on plasma glucose, insulin or corticosterone levels.

4.4.3. Organ weight: Results summarized in **Table 4.12** revealed that adrenal gland weight was higher in stressed control group as respect to corresponding nonstressed control group. However, spleen and heart weight was found less in stressed control group. Although, such alteration in weight of spleen and heart were significant compensated by CLE-3R (10 mg/kg) and metformin (50 mg/kg) treated stressed group. However, no significant change was observed in liver weight of stressed and non-stressed animals.

4.5. Anti-inflammatory Activity

4.5.1. Body weight and basal rectal temperature: In this exploratory experiment significant effect was found between the saline and formalin injected control groups. Although formalin injected control group shown little decrement in body weight but such effect was not found in CLE-3R and metformin treated animals (**Figure 4.17**). Basal rectal temperature of formalin injected animals shown significant elevation as compare to saline injected control group. CLE-3R and metformin treated animals maintain their basal rectal temperatures at physiological level during entire course of experiment **(Figure 4.18)**.

4.5.2. Formalin test in rats: CLE-3R (10 mg/kg) and metformin (50 mg/kg) treatments in rats after 7 daily treatments showed significant ($p<0.05$) decrease in number of spontaneous flinch per minute compared to control rats at different time points **(Figure 4.19A).** However, CLE-3R and metformin treated rats also inhibited the paw edema volume even after their 7 daily doses **(Figure 4.19B)**. It was observed that drug treated animals decreases formalin induce paw edema continuously after their repeated daily dose. On hot plate test CLE-3R and metformin both shown increased reaction time, although formalin injected rats decreased reaction time **(Table 4.13)**

4.6. Food and water intake behaviour

4.6.1. Food, water intake and body weight: In this study control group fed with 20% fructose water shown elevation in food and water intake from their first day administration of it. Although, sudden rise in daily food and water intake after fructose intervention get decreases in all fructose fed groups during experiment. Pooled data of animals, maintain on normal pellet diet and normal drinking water shown normal increment in daily food and water intake before intervention of fructose (**4.20A & 4.21A**). However CLE-3R and metformin treated groups were counteracted such elevation of daily food and water intake in fructose fed animals as shown in (**Figure 4.20B & 4.21B**). Results summarized in (**Figure 4.22A & 4.22B)** revealed that the body weight gains of the control rats observed significantly ($p<0.05$) higher than those of the CLE-3R and metformin treated groups. But such significant difference was observed on 25th day of experiment.

4.6.2. Blood glucose, insulin, plasma level: Results are summarized in **Figure 4.23A** revealed that no significant changes were obtained in plasma glucose, insulin and corticosterone level of normal pellet diet and normal drinking water taken control and treated animals. However, CLE-3R (10 mg/kg) and metformin (50 mg/kg) both significantly reduces the elevated level of glucose, insulin and corticosterone as compared to fructose fed control animals **(Figure 4.23B).**

4.6.3. Total cholesterol, triglycerides, HDL and LDL level: Moreover, the elevated plasma levels of total cholesterol, triglycerides and LDL, and lower plasma levels of HDL observed in fructose fed rats were significantly antagonized by CLE-3R (10 mg/kg) and metformin (50 mg/kg) treatments **(Figure 4.24A & 4.24B)**. All these observed effects of CLE-3R treatment were qualitatively analogous to that of the metformin. HDL and LDL ratio was less (0.30±0.03) in fructose water fed control group however this level was significantly increased by CLE 10

mg/kg (0.52±0.06) and metformin 50 mg/kg (0.46±0.04) treated fructose consuming rats **(Table 4.14).** Although, no significant effect in ratio of HDL and LDL was found between normal water consuming control and treatment groups.

4.6.4. Organ weight: Results summarized in **Table no 4.15** revealed significant organ weight gains in fructose consuming control group as compare to normal water consuming control group. However, such abnormal organ weight gain was significantly antagonized by CLE-3R and metformin treated groups.

4.7. Other pharmacological studies

4.7.1. Body weight, basal rectal temperature and stress induced hyperthermia: Mean body weights of the stressed diabetic and stressed nondiabetic control groups decreased continuously during the course of the experiments, whereas body weight of non-stressed nondiabetic control group continued to increase during subsequent days of entire experiment. Such intermittent foot shock stress-triggered body weight losses in the stressed diabetic groups were compensated after seven daily oral doses 10 mg/kg of CLE-3R and 50 mg/kg of metformin. Rate of body weight changes of CLE-3R 10 mg/kg treated animals observed during the course of the experiment were quite analogous to that of the metformin 50 mg/kg treated group **Figure 4.25A**. Results summarised in **Figure 4.25B** reveal that mean basal rectal temperatures of the stressed control groups on the fifth and subsequent observational days were slightly higher than that of the groups recorded on the first day of the experiment. This value of the non-stressed nondiabetic control group remained almost constant on all observational days. Although, CLE-3R (10 mg /kg) and metformin (50 mg/kg) treatments attenuates basal rectal temperature as compare to stressed diabetic control animals after their ten daily repeated doses. In foot shock stress induced hyperthermia rate of increment of transient hyperthermic response of the stressed diabetic control

and stressed nondiabetic group remained almost constant on all observational days. However, statistically significant suppressing effects of 10 mg/kg daily oral doses of CLE-3R and 50 mg/kg daily oral doses of metformin were observed on 10^{th} day of observation **(Figure 4.26).**

4.7.2. Spontaneous Locomotor activity: The locomotor activity of the stressed diabetic and stressed nondiabetic control groups on the fifth and subsequent observational days was slightly higher than that of the groups recorded on the first day of the experiment. However, observed effect on last minute during their stay in the activity cage was slightly lower than the effects recorded on first min after exposure (**Figure 4.27A & 4.27B**). Observed locomotor activity was found more with stressed diabetic rats than stressed nondiabetic rats. This value of the non-stressed nondiabetic control group remained almost unchanged on all observational days. However, 10 mg/kg CLE-3R and 50 mg/kg metformin treated animals attenuates increased locomotor behaviour in diabetic animals as compared to diabetic control group. Such protective effect was found by CLE-3R and metformin even after their five repeated daily doses.

4.7.3. Elevated plus maze test: CLE-3R (10 mg/kg) and metformin (50 mg/kg) treated stressed diabetic group shown statistically significant ($p<0.05$) increase in mean numbers of entries and mean time spent in the open arm of elevated plus maze. These observations reconfirm that ten daily oral doses of CLE-3R (10 mg/kg) is high enough to observe its significant anxiolytic like activities in rat models and analogous effect was found with 50 mg/kg oral dose of metformin (**Figure 4.28A & 4.28B**). Vehicle treated stressed diabetic and stressed non diabetic rats shown significant decrease in mean numbers of entries and mean time spent in the open arm compared to nondiabetic control.

4.7.4. Marble burying test: Results of the marble burying test conducted one hour after eleven daily oral treatments revealed that the mean numbers of the marbles buried by the animals of the

stressed diabetic control group was significantly higher than that of the non-stressed non diabetic control group (**Figure 4.29**). On contrary, number of marbles buried by CLE-3R (10 mg/kg) treated diabetic rats was significantly lower than the vehicle (0.3% CMC) treated stressed diabetic and stressed nondiabetic control group. Moreover, no statistically significant effect was observed with metformin (50 mg/kg) treated diabetic group when compared with stressed diabetic and stressed nondiabetic groups.

4.7.5. Forced swimming test: Results of forced swimming test are summarized in **Table 4.16**. Results revealed that daily oral treatments with 10 mg/kg of CLE-3R significantly ($p<0.05$) reduced the immobility period as compared to the stressed diabetic and stressed nondiabetic control group. Treatment with 50 mg/kg of metformin also reduced the immobility time significantly in diabetic animals. However, mean immobility period of the stressed diabetic control group was significantly higher than than that of the non-stressed non diabetic control group.

4.7.6. Adrenal gland and spleen weights: Significant ($p<0.05$) adrenal hypertrophy and spleen hypotrophy were apparent in stressed diabetic and stressed nondiabetic rats. CLE-3R (10 mg/kg), and metformin 50 mg/kg treatments successfully reversed such stress and diabetes induced abnormality in weights of spleen and adrenal gland maintain at their normal physiological range **(Figure 4.30A & Figure 4.30B)**. Liver weight of diabetic animals was observed to be decreased in stressed diabetic rats, although CLE-3R and metformin counteracted such hypotrophy in liver **(Figure 4.30C).**

4.7.7. Plasma glucose, insulin and corticosterone: In comparison to vehicle treated non-stressed and non diabetic animals, elevated plasma corticosterone and glucose level was observed in stressed diabetic and stressed nondiabetic control rats. Treatments with CLE-3R (10 mg/kg) and

metformin (50mg/kg) treated diabetic rats significantly ($p<0.05$) suppressed the elevation of plasma glucose and corticosterone level in stressed diabetic animals and tends toward their normal values **(Figure 4.31A & 4.31C)**. Decreased level of insulin in stressed diabetic animals was significantly elevated by CLE-3R and metformin treatment **(Figure 4.31B)**.

4.7.8. Gastric ulceration: Results summarized in **Table 4.17** revealed that vehicle treated stressed diabetic and stressed nondiabetic animals had developed gastric ulcers, and that mean number of ulcers index in stressed rats were significantly ($p<0.05$) lower in CLE-3R (10 mg/kg) and metformin (50 mg/kg) treated groups. Such quantified effect of CLE-3R and metformin against stress-induced ulcers was qualitatively analogous to each other **(Figure 4.32).**

4.8. Biochemical estimations

4.8.1. Plasma levels of glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GTP): Diabetic control rats treated with vehicle (0.3% CMC) have shown significant ($p<0.05$) increase in GOT and GTP enzymes as compared to nondiabetic control rats. However, eleven daily treatments with low oral doses (10 mg/kg) of CLE-3R and (50 mg/kg) metformin significantly compensated the elevated levels of these enzymes in stressed diabetic rats (**Figure 4.33A & 4.33B**).

4.8.2. Anti-oxidant activity: In comparison to the vehicle (0.3% CMC) treated non-stressed nondiabetic control group, lipid peroxidation (LPO) activity in stressed nondiabetic or diabetic animals were significantly higher in blood and brain of rats (**Figure 4.34A & Figure 4.34B**). Such elevations of LPO activity was found significantly less in CLE-3R (10 mg/kg) and metformin (50 mg/kg) treated animals. Effects observed with CLE-3R in reducing LPO activity was almost analogous to that of the metformin treated animals. Similarly, the level of anti-oxidant superoxide dismutase (SOD) and catalase (CAT) enzymes in STZ-induced diabetic

animals were significantly lower than those of the vehicle (0.3% CMC) treated nondiabetic controls. Such reductions in the level of both the anti-oxidative enzymes were reversed by repeated daily treatments with CLE-3R and metformin in blood as well as brain tissue sample of diabetic rats.

4.8.3. Glyoxalase I and Paraoxonase 1 (PON1) enzyme activity in liver: In STZ induced diabetic control rats the level of Glyoxalase-I enzyme significantly decreased as compared to stressed non-diabetic control groups. However, repeated daily oral treatments with CLE-3R (10 mg/kg) and metformin (50 mg/kg) groups significantly increased the level of Glyoxalase-1 enzyme as compared to stress diabetic control group (**Figure 4.35**). Analogous effect was observed with Paraoxonase 1 activity, whereas the level of Paraoxonase 1 enzyme reduced in diabetic control rats. However, CLE-3R and metformin significantly inhibited such reduction in the level of Paraoxonase 1 enzyme in liver tissue sample (**Figure 4.36**).

4.8.4. Monoamines level in hippocampus: CLE-3R (10 mg/kg) and metformin (50 mg/kg) treated diabetic rats demonstrated significant increased in all the three monoamines (NA, DA, 5-HT) level in hippocampus. Although, hippocampus levels of monoamines were observed significantly lower in stressed diabetic and stressed nondiabetic control rats as compare to non-stressed nondiabetic control rats **(Figure 4.37A, 4.37B & 4.37C).**

4.8.5. Monoamine oxidase activity in hippocampus: Results of the MAO-A and MAO-B assays performed with mitochondrial preparations of hippocampus sections from stressed diabetic and stressed nondiabetic rat are summarized in **Figures 4.38A & 4.38B**. In stressed diabetic control group, mean enzymatic activities of both MAO-A and MAO-B were significantly higher than the nondiabetic control groups. However, daily treatments with low oral doses of CLE-3R (10 mg/kg) significantly decreased the elevated levels of these two enzymes. Although, metformin at

dose 50 mg/kg significantly reduced the elevated level of both MAO-A and MAO-B in diabetic rats, but observed effect was not equivalent to that of 10 mg/kg dose of CLE-3R.

4.8.6. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity: The results are summarized in **Figures 4.39A, 4.39B & 4.39C** showed that, CLE-3R (10 mg/kg) and metformin (50 mg/kg) decreased AChE and BChE activity in blood, prefrontal cortex and hippocampus as compared to stressed diabetic and stressed non diabetic rats. The diabetic rats showed significant increase in AChE and BChE enzyme activity in blood and both regions of brain as compared to nondiabetic control rats.

4.8.7. Inducible nitric oxide synthase (iNOS), nitric oxide (NO) and expression of nuclear factor kappa beta (NFκB): Stressed diabetic control group treated with vehicle (0.3% CMC) have shown significant increased in iNOS and NO activities in blood (**Figures 4.40A**) and in frontal cortex (**Figures 4.40B**) and hippocampus (**Figures 4.40C**). However, daily treatments with 10 mg/kg of CLE-3R and 50 mg/kg of metformin treatments significantly counteracted the elevated levels of both iNOS and NO as compared to stressed diabetic control group. Results revealed that expression of NFκB in blood (**Figures 4.40A**) and in two different brain regions viz. frontal cortex (**Figures 4.40B**) and hippocampus (**Figures 4.40C**) of stressed diabetic and stressed nondiabetic rats significantly higher than non stressed non diabetic animals. However, mean expression of NFκB was significantly reduced by daily treatments with CLE-3R (10 mg/kg) and metformin (50 mg/kg) in blood as well as brain of rats.

FIGURES AND TABLES

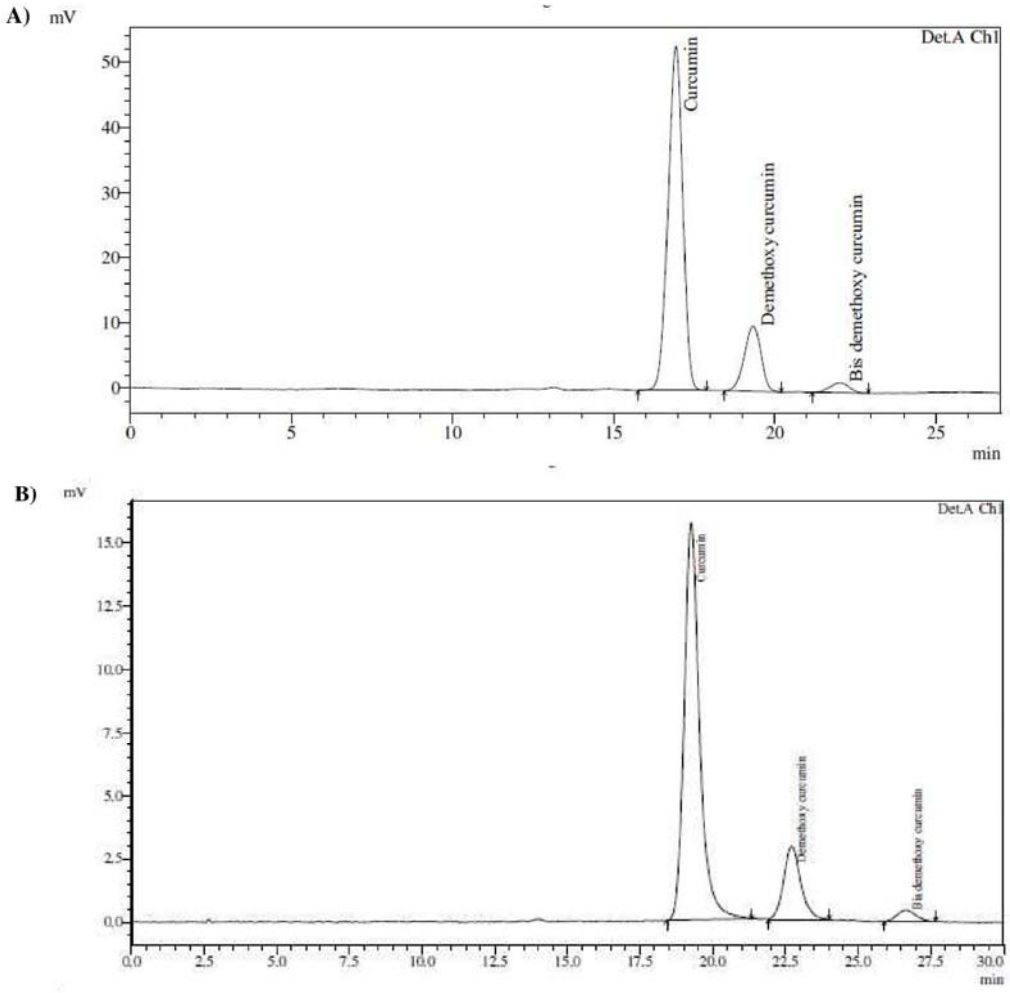

Figure 4.1: HPLC Chromatogram of **A)** *Curcuma longa* extract 1H sample **B)** *Curcuma longa* extract 2B sample.

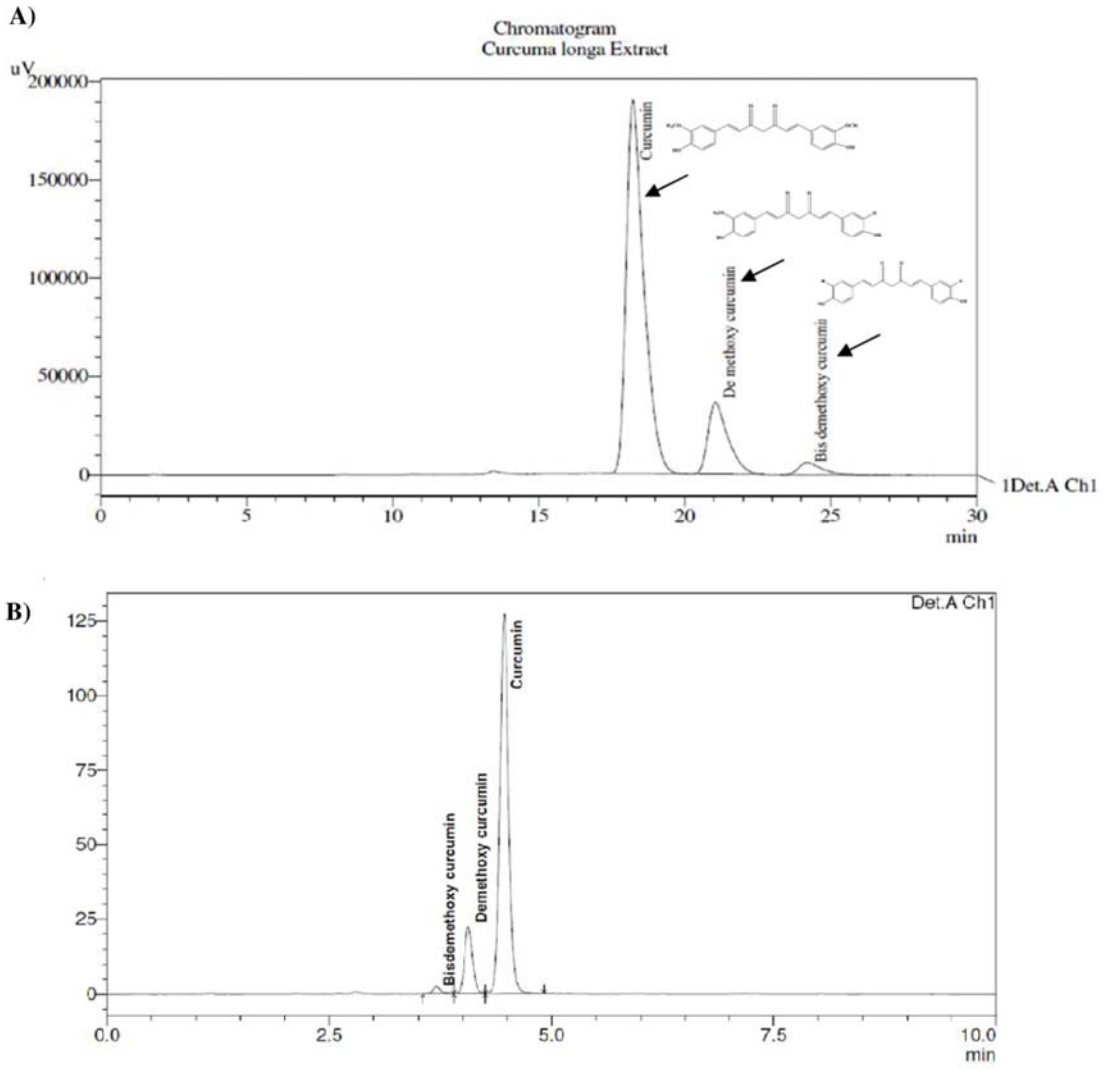

Figure 4.2: HPLC Chromatogram of **A)** *Curcuma longa* extract 3R sample **B)** *Curcuma longa* extract 4M sample

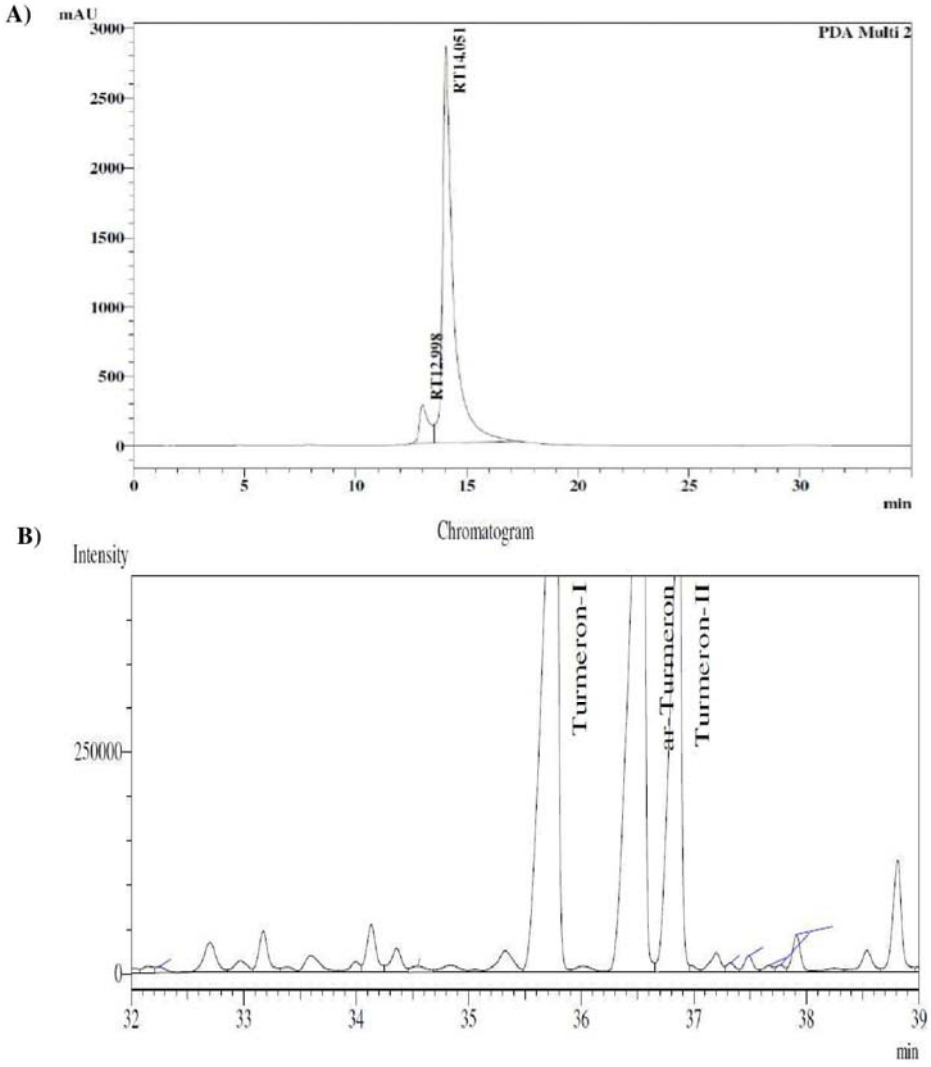

Figure 4.3: HPLC Chromatogram of **A)** pure curcumin **B)** turmeric oil.

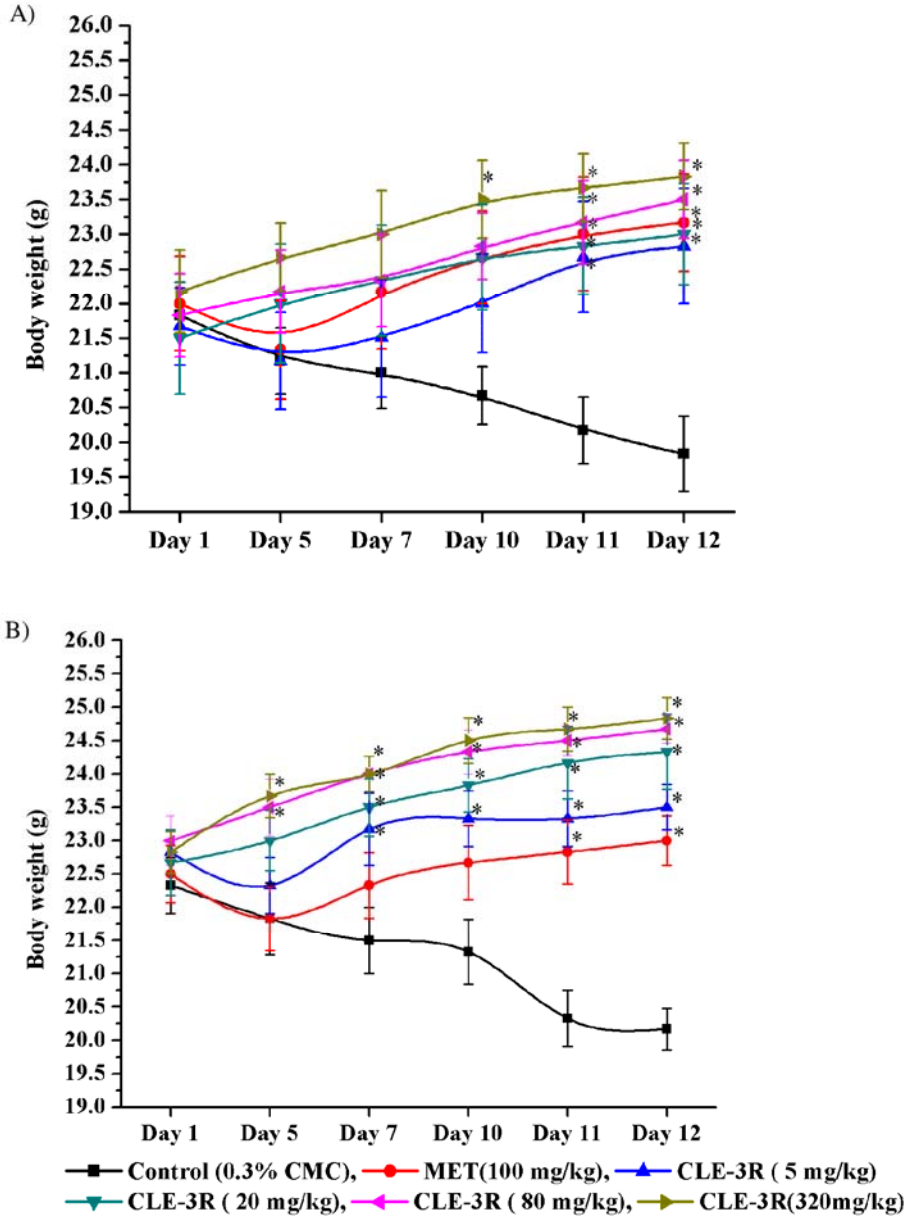

Figure 4.4: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight of stressed **A)** male mice **B)** female mice. Values are mean±SEM, n=6. *=p<0.05 vs. control group (Two way ANOVA followed by Bonferroni post hoc test). MET=metformin. CMC=Carboxymethyl cellulose.

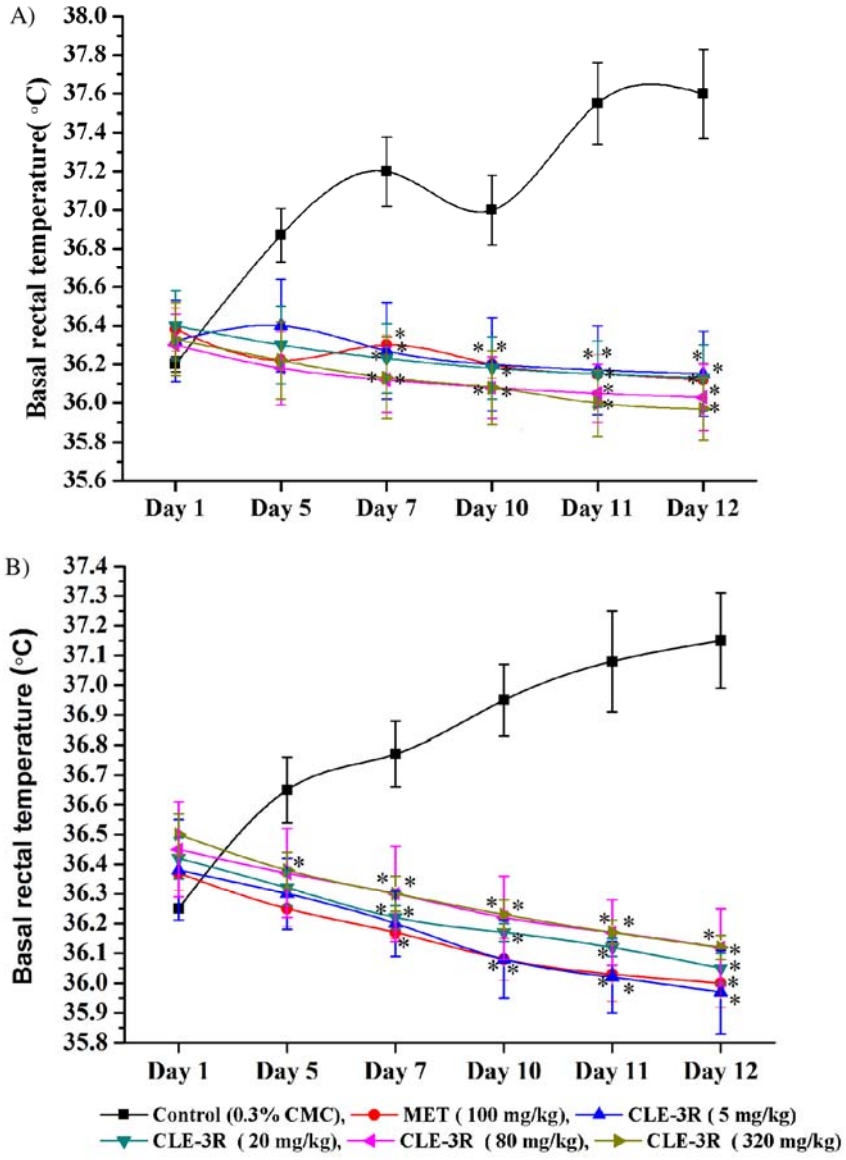

Figure 4.5: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on basal rectal temperature of stressed **A)** male mice **B)** female mice. Values are mean±SEM, n=6. *=$p<0.05$ vs. control group (Two way ANOVA followed by Bonferroni post hoc test).

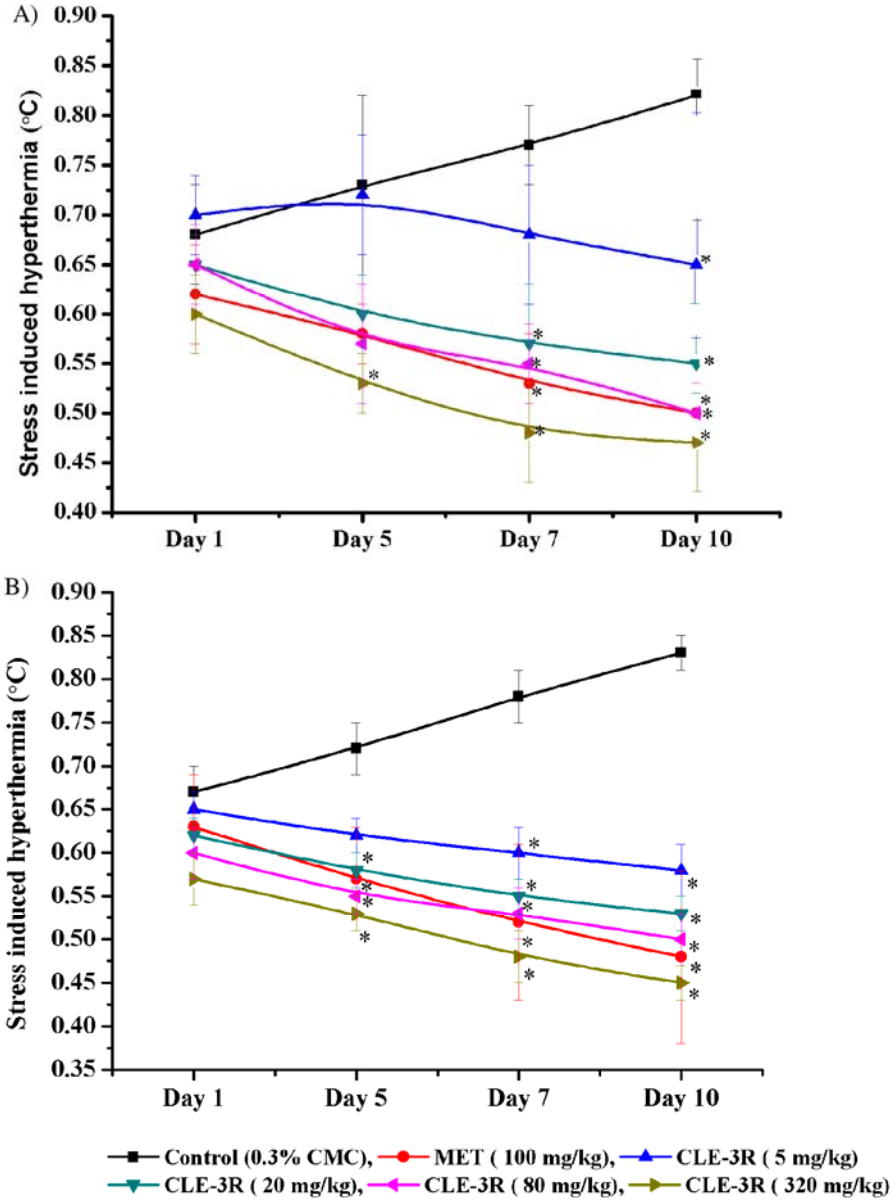

Figure 4.6: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on stress induced hyperthermia of stressed **A)** male mice **B)** female mice. Values are mean±SEM, n=6. *=p<0.05 vs. control group (Two way ANOVA followed by Bonferroni post hoc test).

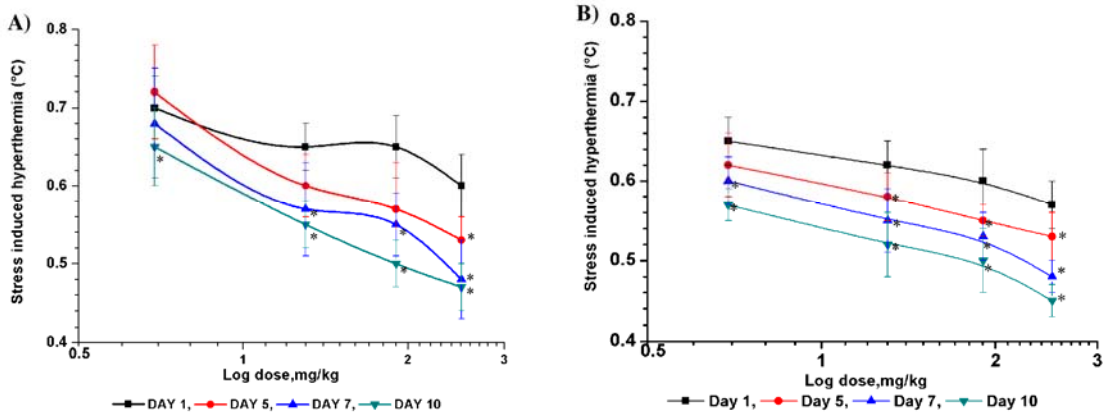

Figure 4.7: Log dose response curve of **A)** male mice **B)** female mice. Values are mean±SEM, n=6. *=p<0.05 vs. control group (Two way ANOVA followed by Bonferroni post hoc test).

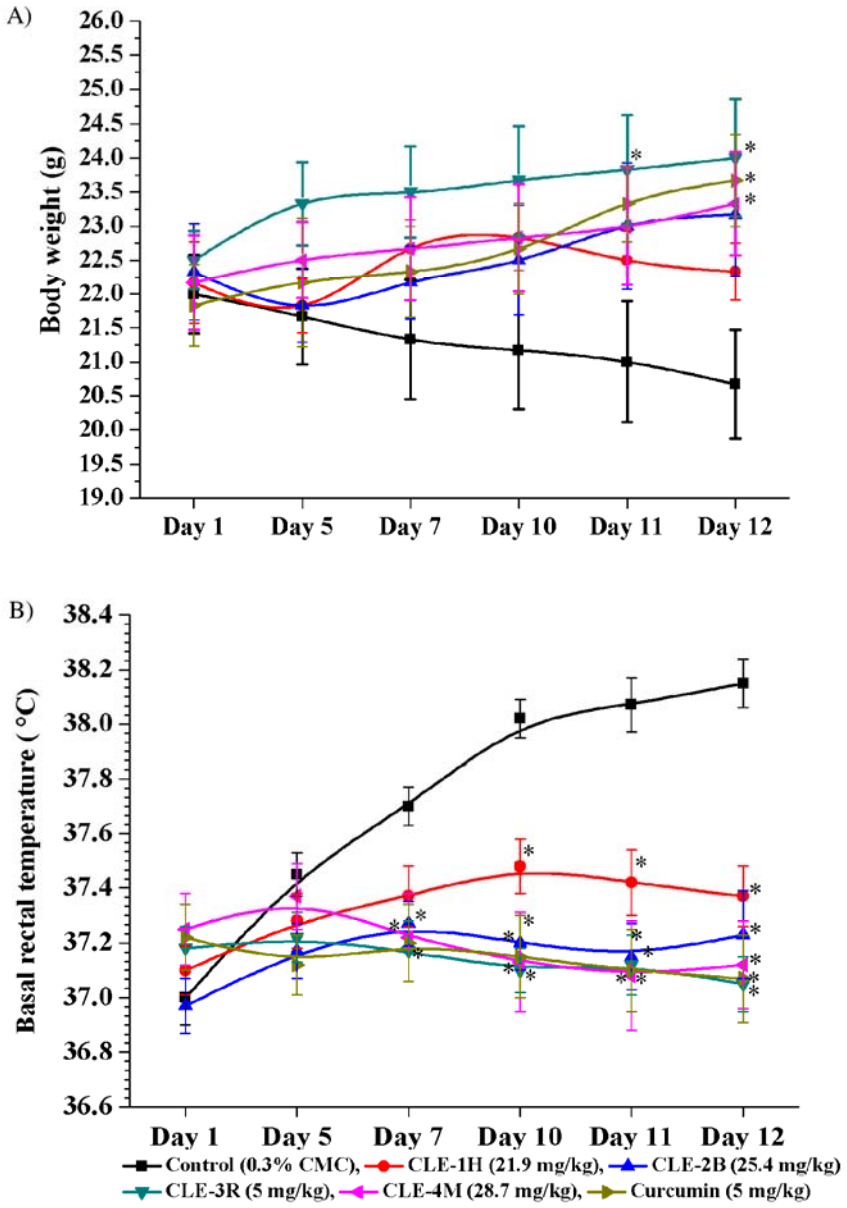

Figure 4.8: Effect of different *Curcuma longa* extracts (CLEs) on **A)** body weight and **B)** basal rectal temperature of male mice. Values are mean±SEM, n=6. *=$p<0.05$ vs. control group (Two way ANOVA followed by Bonferroni post hoc test).

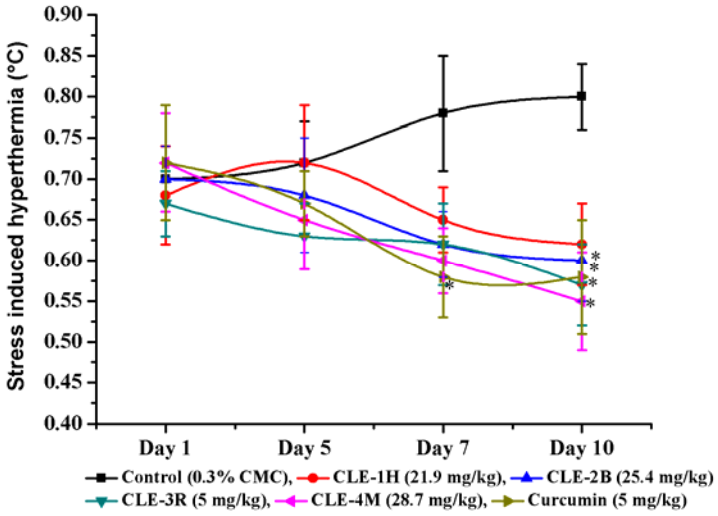

Figure 4.9: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on stress induced hyperthermia in male mice. Values are mean±SEM, n=6. *=p<0.05 vs. control group (Two way ANOVA followed by Bonferroni post hoc test).

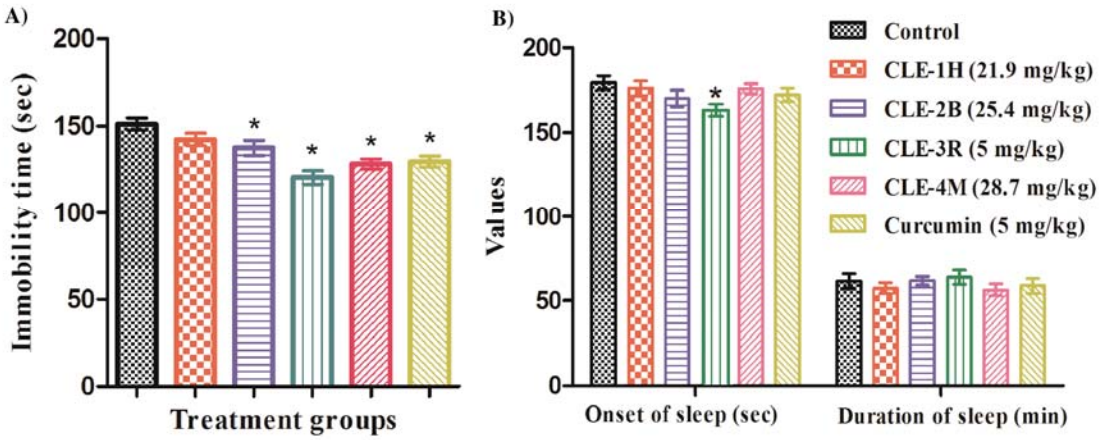

Figure 4.10: Effect of different *Curcuma longa* extracts (CLE) on **A)** immobility time and **B)** onset and duration of sleep in stressed male mice. Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

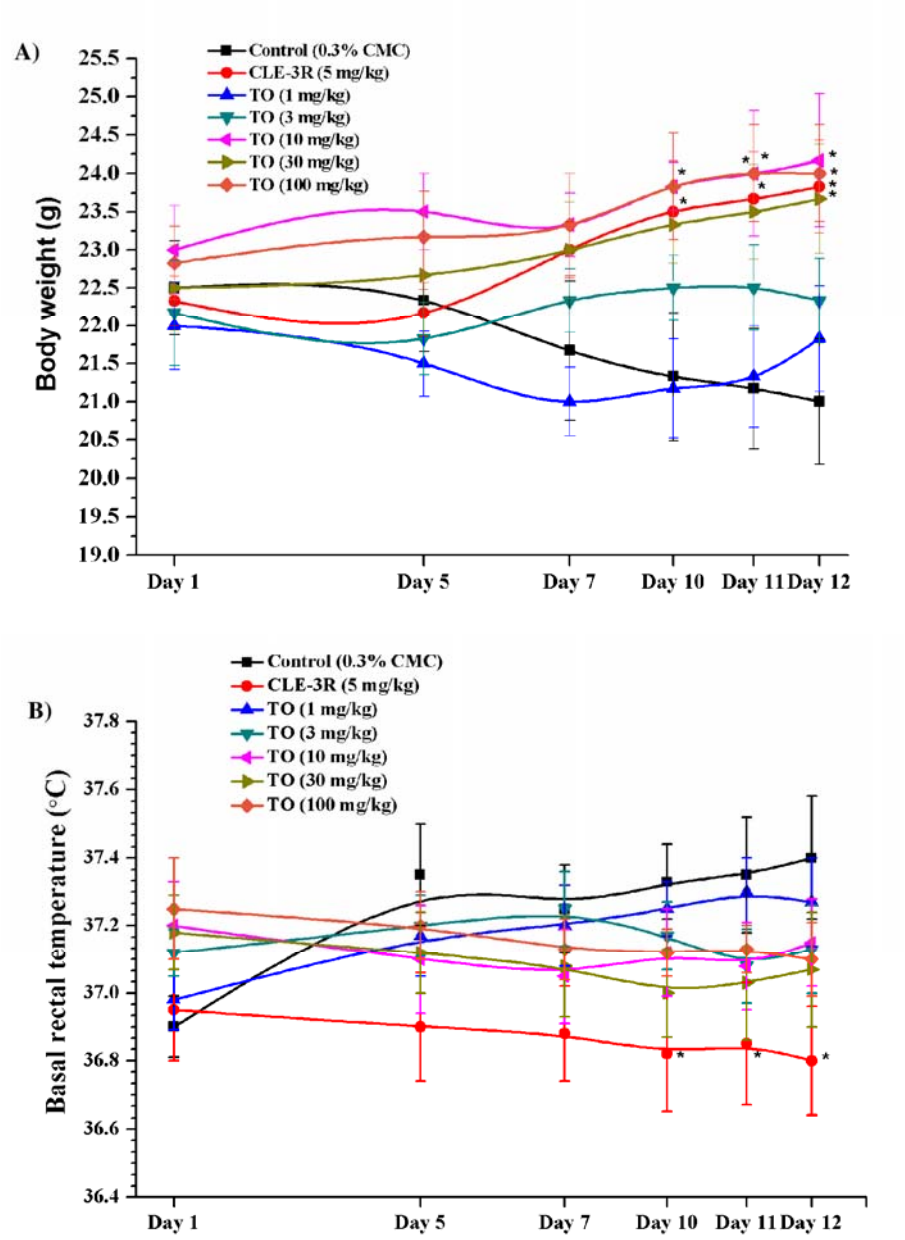

Figure 4.11: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and turmeric oil on **A)** body weight and **B)** basal rectal temperature of stressed male mice. Values are mean±SEM, n=6. *=$p<0.05$ vs. control group (Two way ANOVA followed by Bonferroni post hoc test). TO=Turmeric oil.

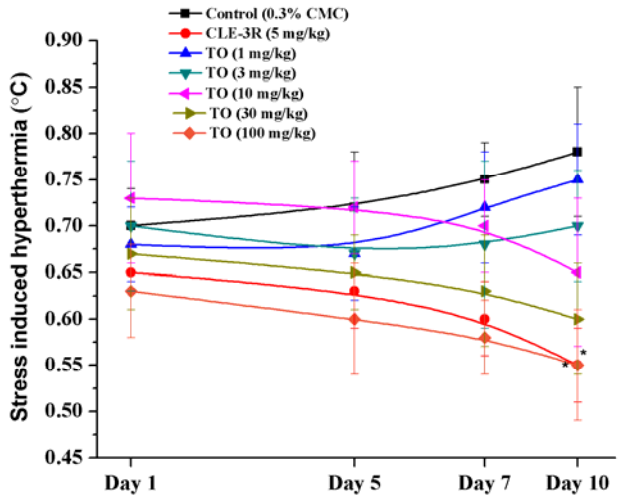

Figure 4.12: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and turmeric oil on stressed induced hyperthermia of stressed male mice. Values are mean±SEM, n=6. *=p<0.05 vs. control group (Two way ANOVA followed by Bonferroni post hoc test).

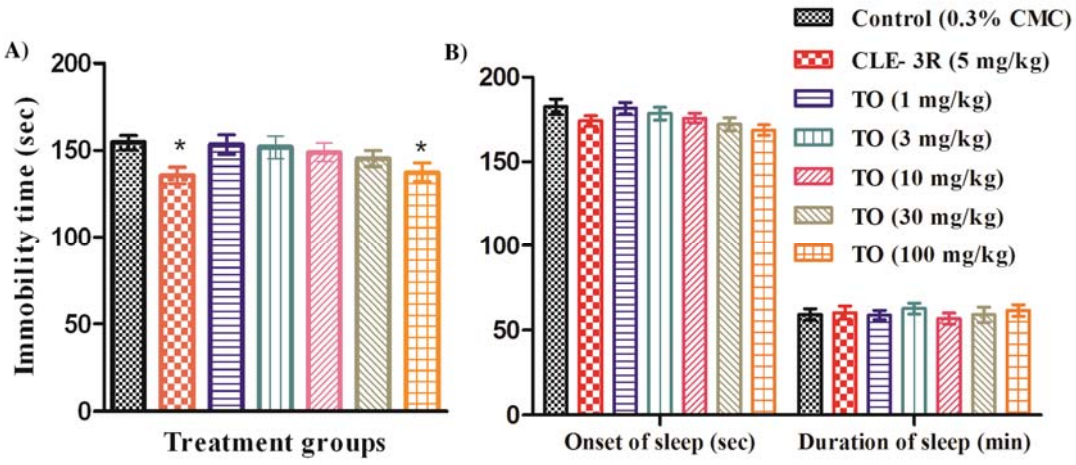

Figure 4.13: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and turmeric oil on **A)** immobility time and **B)** onset and duration of stressed male mice. Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

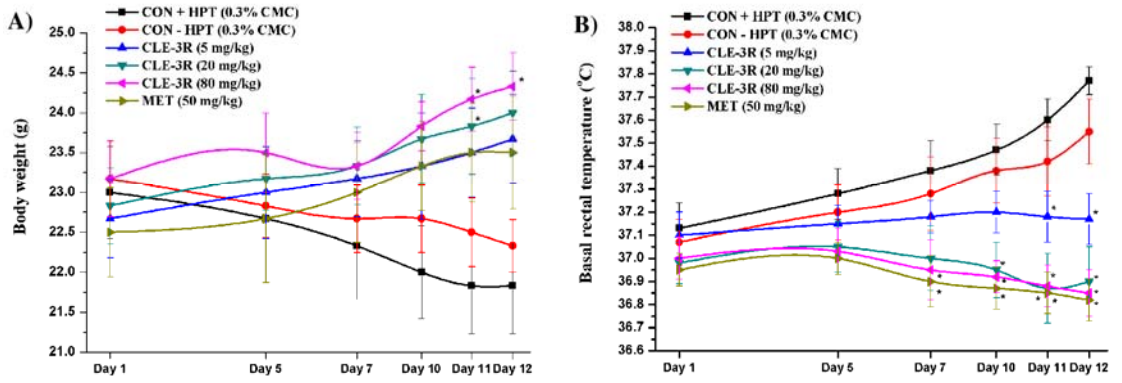

Figure 4.14: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on **A)** body weight and **B)** basal rectal temperature of stressed male mice. Values are mean±SEM, n=6. *=p<0.05 vs. CON+HPT group (Two way ANOVA followed by Bonferroni post hoc test).CON=control, HPT=Hot plate test.

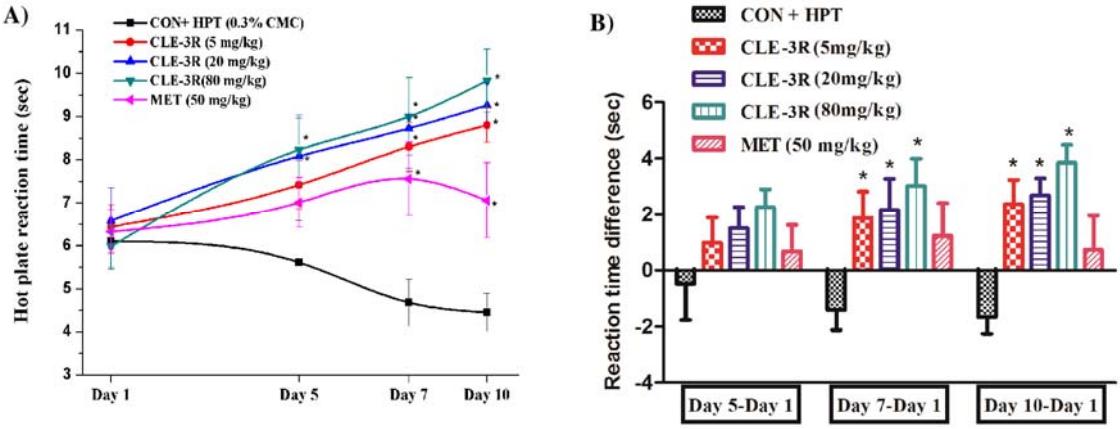

Figure 4.15: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on **A)** hot plate reaction tim**e B)** reaction time difference. Values are mean±SEM, n=6. *=p<0.05 vs. CON+HPT group (Two way ANOVA followed by Bonferroni post hoc test). CON=control, HPT=Hot plate test.

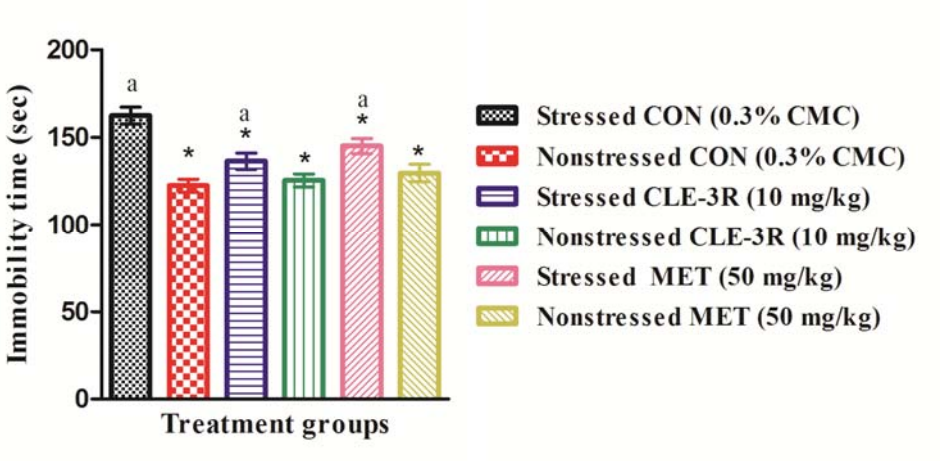

Figure 4.16: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin treated stressed and non-stressed male rats in forced swim test. Values are mean±SEM, n=6. *=$p<0.05$ vs. stressed control (One way ANOVA followed by Student-Newman-Keuls multiple comparison test). [a] =$p<0.05$ vs. non stressed control (t-test).

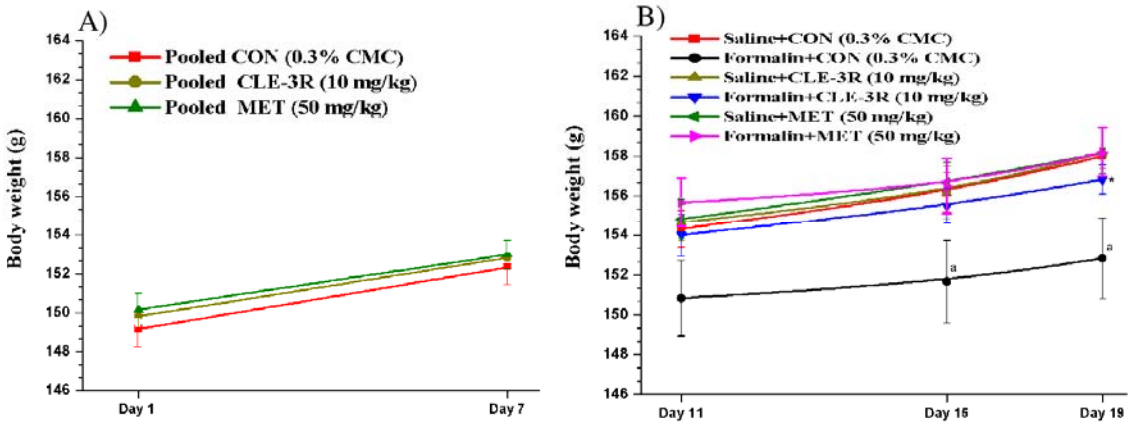

Figure 4.17: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight of male rats **A)** pooled data of all corresponding groups **B)** data from all groups. Values are mean±SEM, n=6. [a] =p<0.05 vs. corresponding saline group.

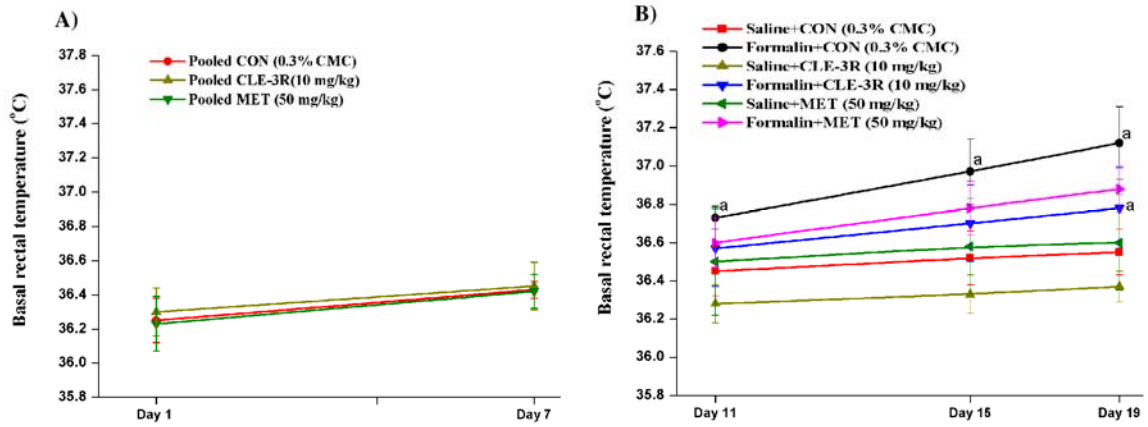

Figure 4.18: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on basal rectal temperature of male rats **A)** pooled data of corresponding saline group **B)** data from all groups. Values are mean±SEM, n=6. [a] =p<0.05 vs. corresponding saline group.

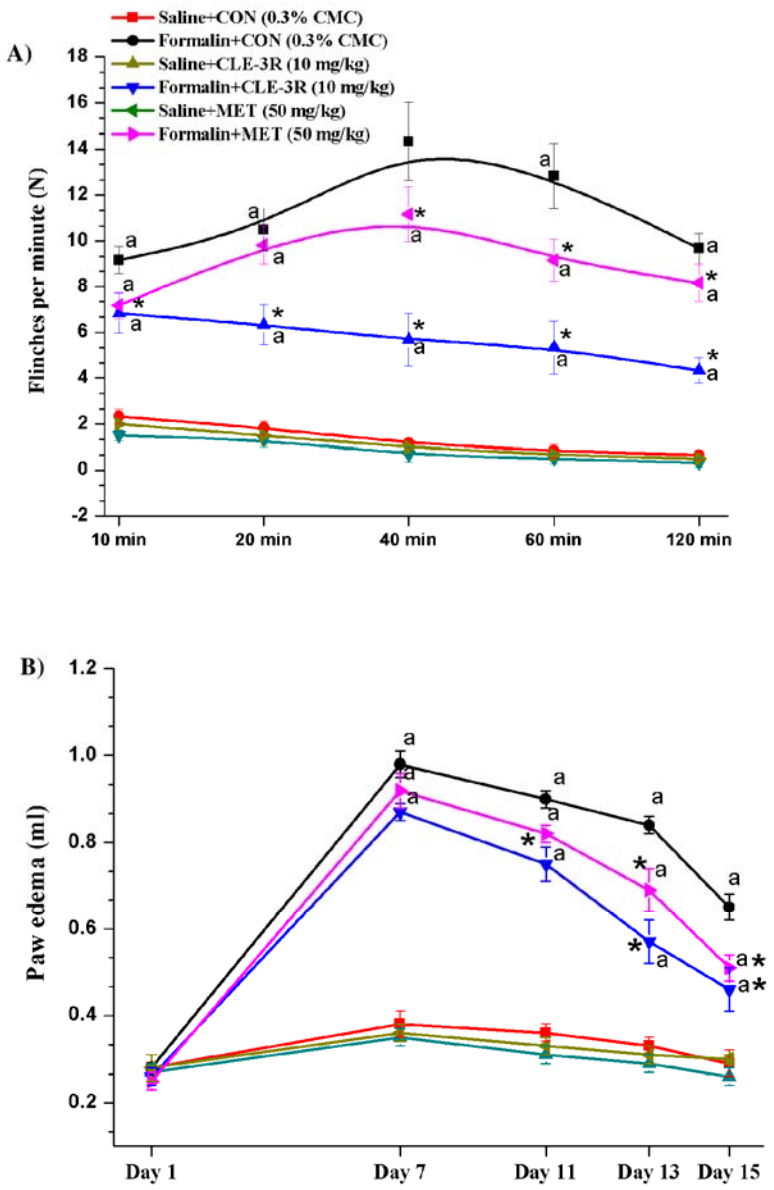

Figure 4.19: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on **A)** flinches per minute and **B)** paw edema in saline and formalin injected rats. Values are mean±SEM, n=6. *=$p<0.05$ vs. formalin control group (Two way ANOVA followed by Bonferroni post hoc test). [a]=$p<0.05$ vs. corresponding saline group (t-test).

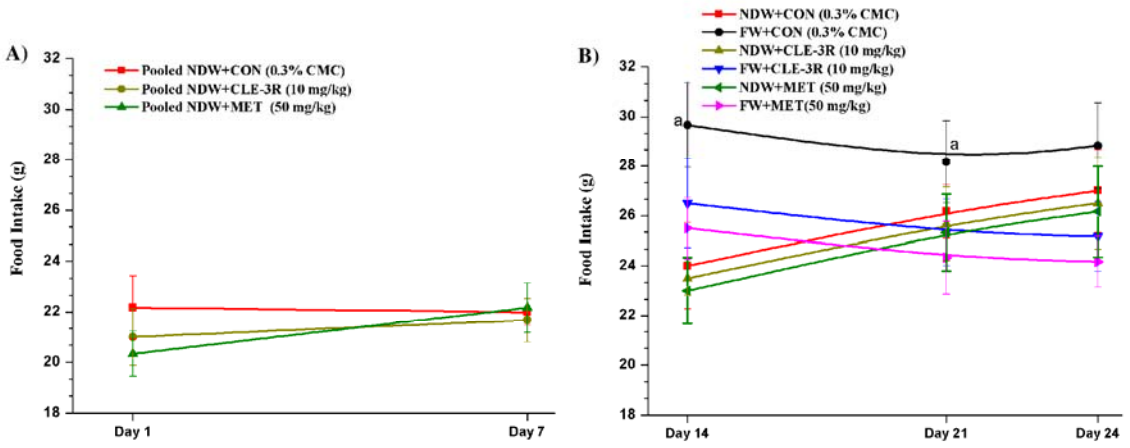

Figure 4.20: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on food intake of male rats **A)** pooled data of corresponding NDW group and **B)** data from all groups. Values are mean±SEM, n=6. [a] =p<0.05 vs. corresponding NDW group. NDW=Normal drinking water, FW=20% Fructose water.

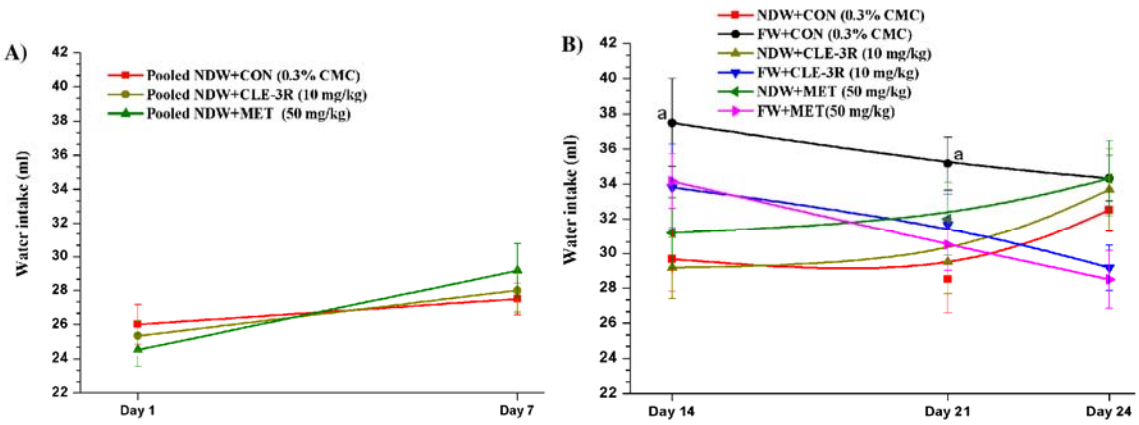

Figure 4.21: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on water intake of male rats **A)** pooled data of corresponding NDW group and **B)** data from all groups. Values are mean±SEM, n=6. [a] =p<0.05 vs. corresponding NDW group. NDW=Normal drinking water, FW=20% Fructose water.

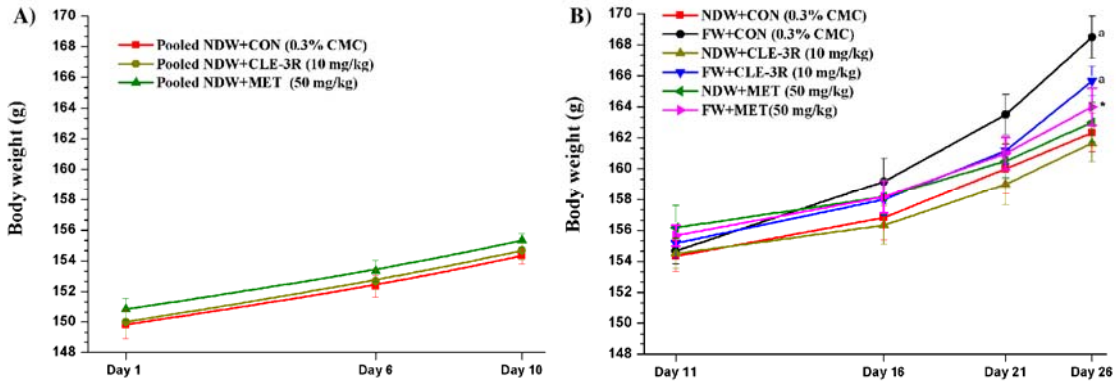

Figure 4.22: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight of male rats **A)** pooled data of NDW and **B)** data from all groups. Values are mean±SEM, n=6.* =p<0.05 vs. FW control group. [a] =p<0.05 vs. corresponding NDW group. NDW=Normal drinking water, FW=20% Fructose water.

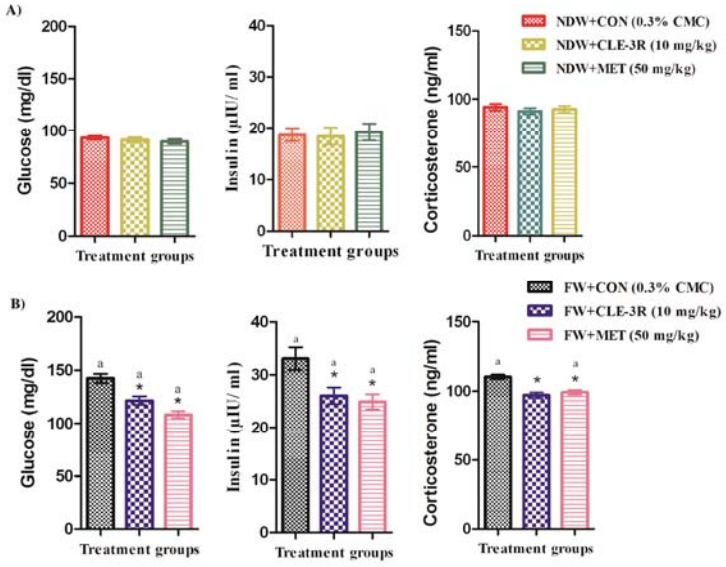

Figure 4.23: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on plasma glucose, insulin, and corticosterone levels of **A)** normal drinking water and **B)** fructose water consuming male rats. Values are mean±SEM, n=6. *=p<0.05 vs. FW control group. [a] =p<0.05 vs. corresponding NDW group. NDW=Normal drinking water, FW=20% Fructose water.

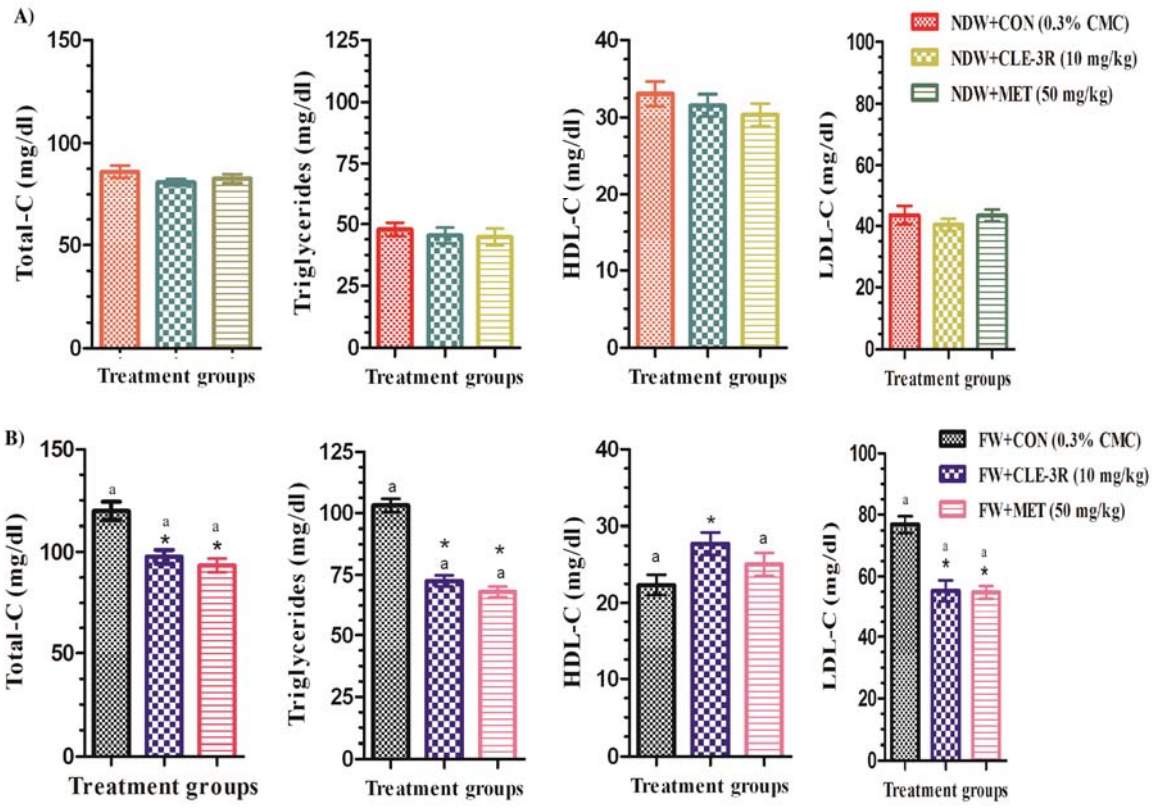

Figure 4.24: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on total cholesterol, triglyceride, HDL and LDL levels of **A)** normal drinking water and **B)** fructose drinking water consuming male rats Values are mean±SEM, n=6. *=$p<0.05$ vs. FW control group. [a] =$p<0.05$ vs. corresponding NDW group. HDL= High density lipoprotein, LDL= Low density lipoprotein, NDW=Normal drinking water, FW=20% Fructose water.

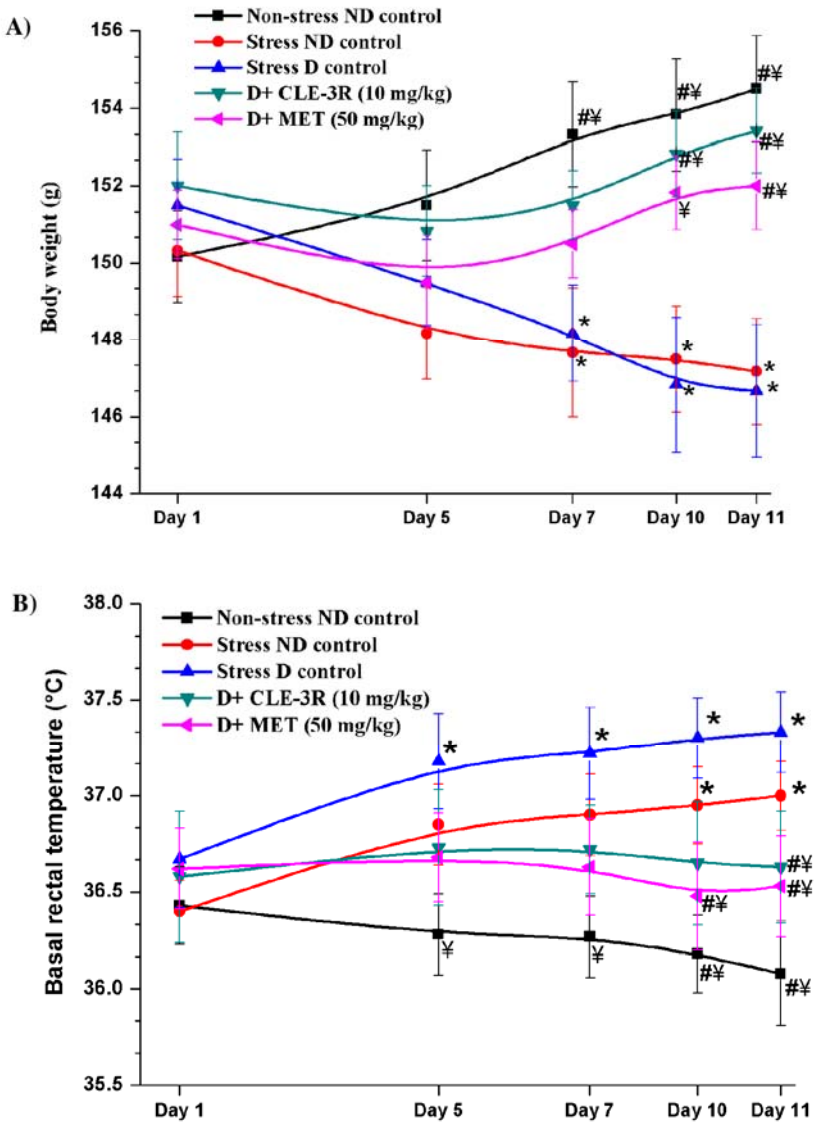

Figure 4.25: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on **A)** body weight and **B)** basal rectal temperature of normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control (Two way ANOVA followed by Bonferroni post hoc test).

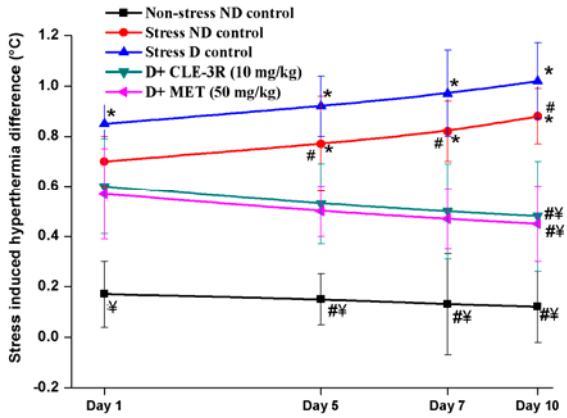

Figure 4.26: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on stress induced hyperthermia of normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control.

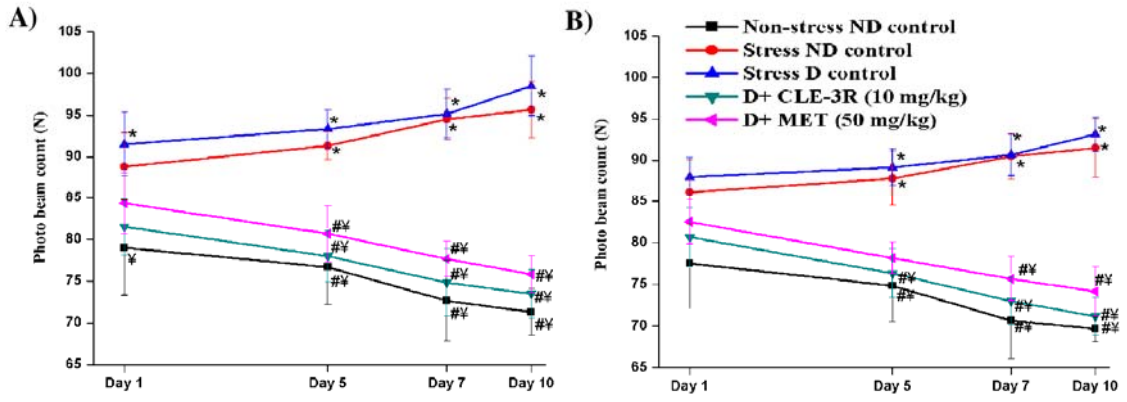

Figure 4.27: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on spontaneous locomotor activity **A)** photo beam count for the first 60 sec and **B)** photo beam count for the last 60 sec of total 10 min period in actophotometer of normal and diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control.

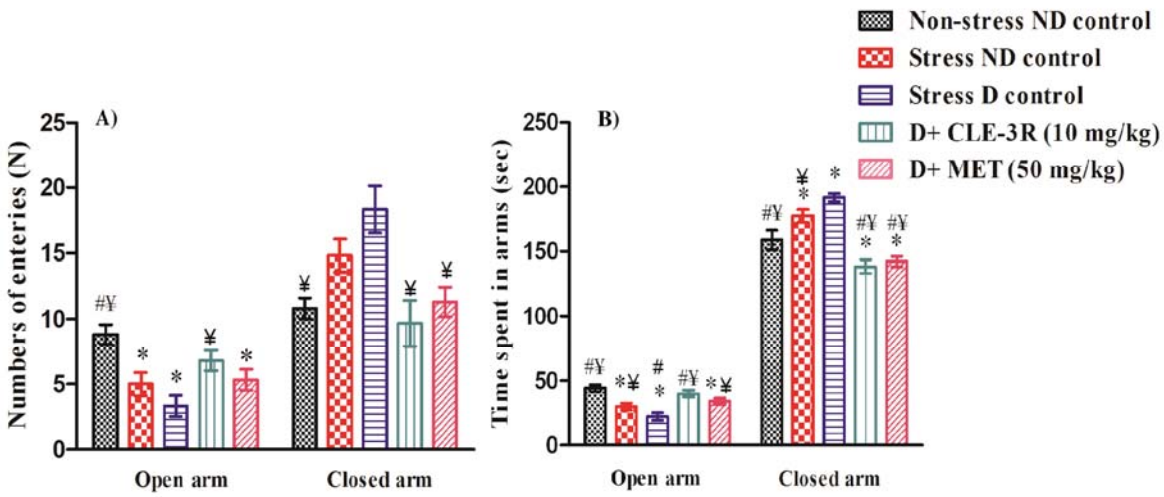

Figure 4.28: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on **A)** numbers of entries and **B)** time spent in open and closed arms on elevated plus maze in normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

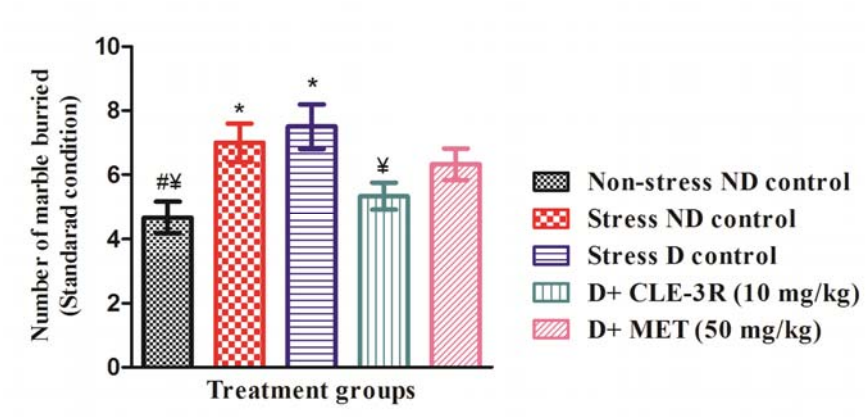

Figure 4.29: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on marble burying behavior in normal and diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control.

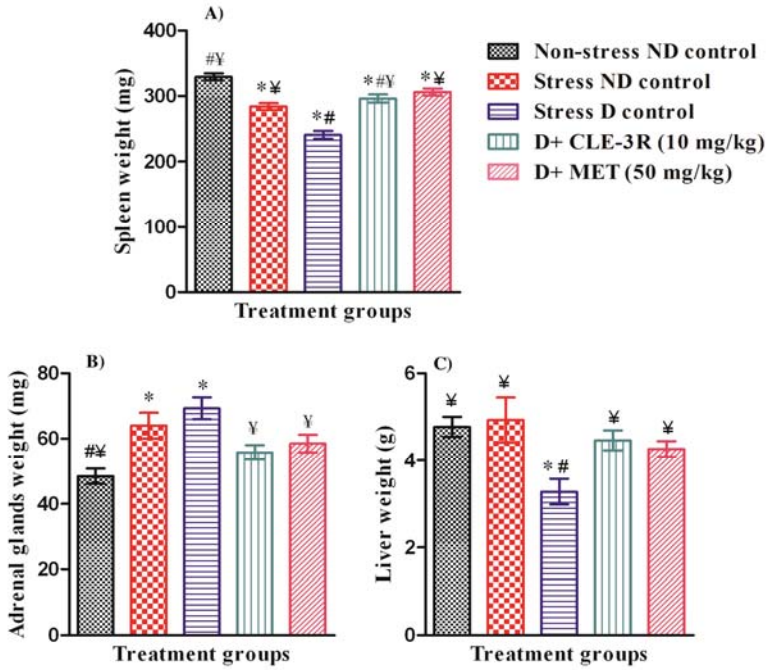

Figure 4.30: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on the weights of **A)** spleen **B)** adrenal glands and **C)** liver of normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; #=$p<0.05$ vs. Stress non-diabetic (ND) control; ¥=$p<0.05$ vs. Stress diabetic (D) control.

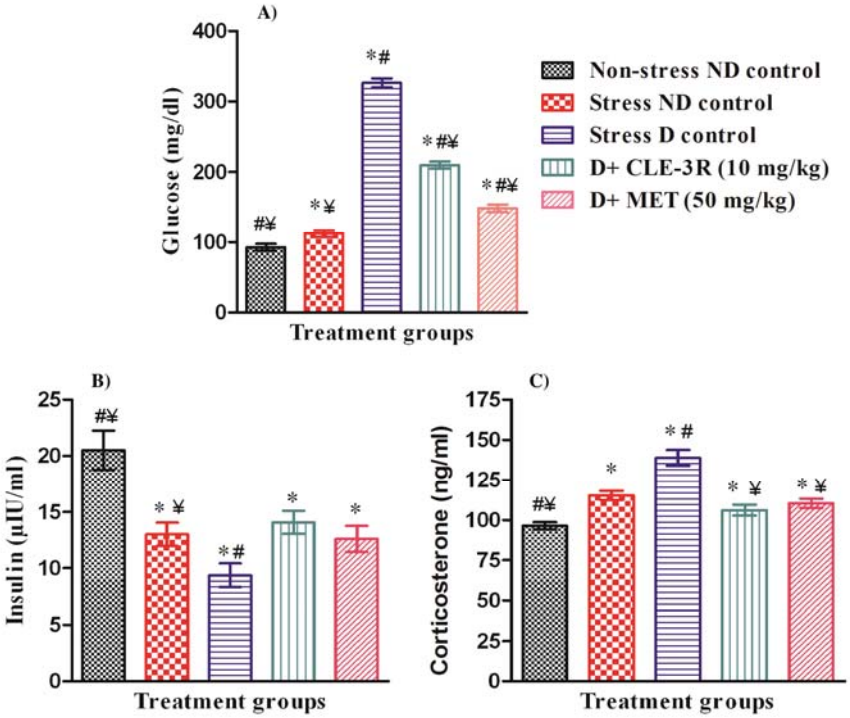

Figure 4.31: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on **A)** plasma glucose **B)** insulin and **C)** corticosterone level of normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; #=$p<0.05$ vs. Stress non-diabetic (ND) control; ¥=$p<0.05$ vs. Stress diabetic (D) control.

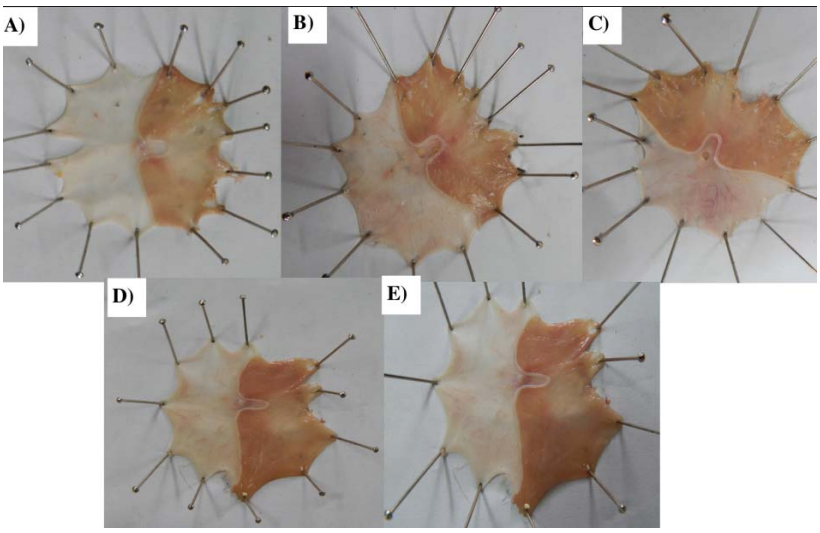

Figure 4.32: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on stomach ulceration index of **A)** non-stressed non diabetic **B)** stressed non diabetic **C)** stressed diabetic **D)** diabetic+ CLE-3R **E)** diabetic+ MET.

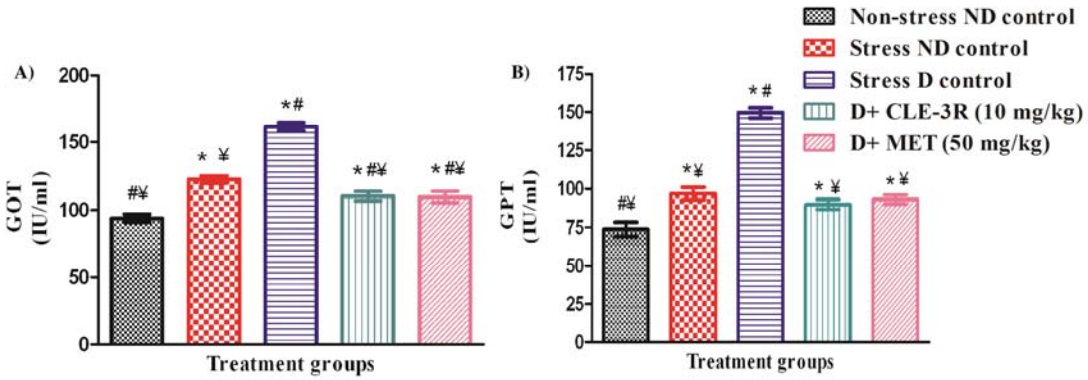

Figure 4.33: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on levels of **A)** glutamate oxaloacetate transaminase (GOT) and **B)** glutamate pyruvate transaminase (GTP) in normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; $^{\#}$=$p<0.05$ vs. Stress non-diabetic (ND) control; $^{¥}$=$p<0.05$ vs. Stress diabetic (D) control.

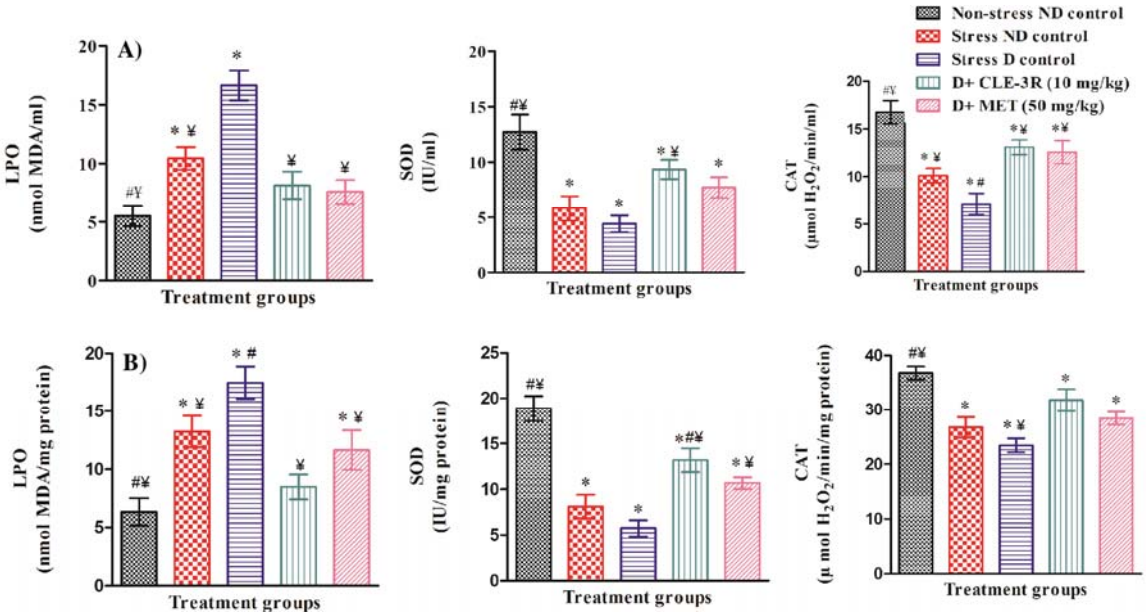

Figure 4.34: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on **A)** blood and **B)** brain antioxidant status in normal and diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control.

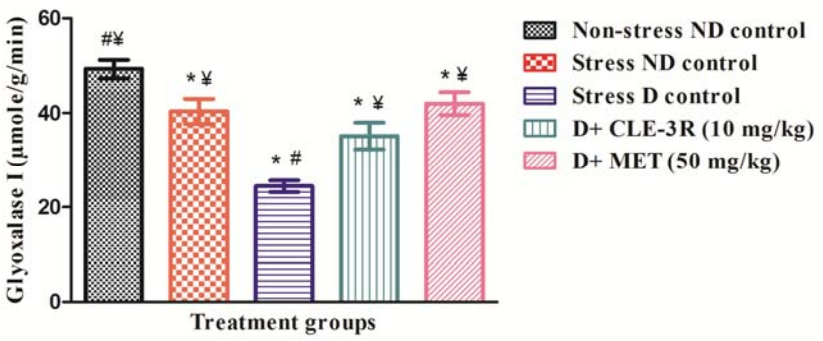

Figure 4.35: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on Glyoxalase-I enzyme activity in normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control.

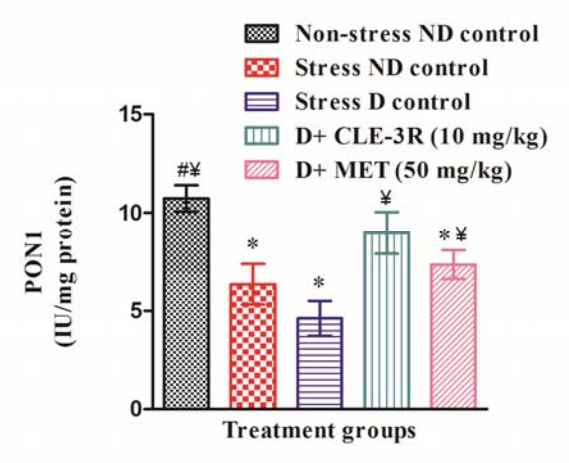

Figure 4.36: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on Paraoxonase 1 activity in normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control.

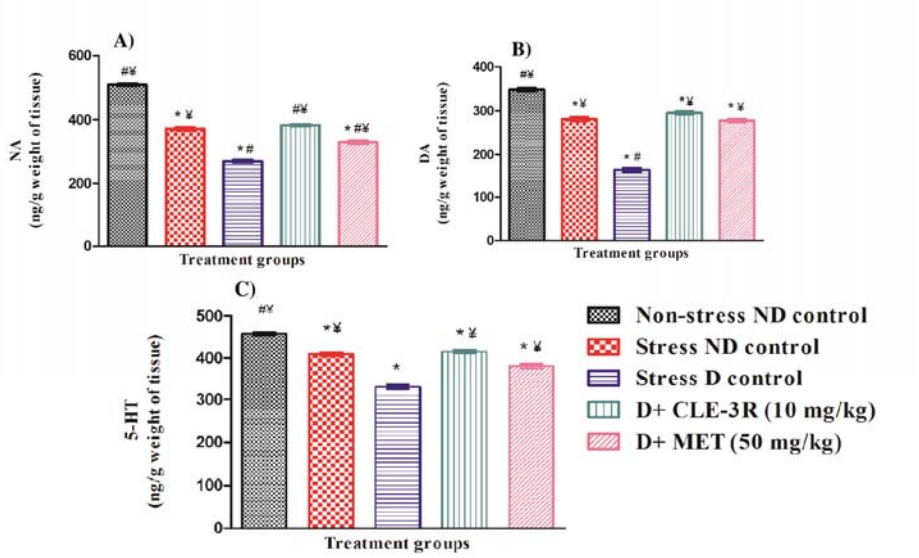

Figure 4.37: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on monoamines **A)** Noradrenaline (NA), **B)** Dopamine (DA) and **C)** 5-Hydroxytryptamine (5-HT) levels in brain samples of normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=p<0.05 vs. Non-stress non-diabetic (ND) control; #=p<0.05 vs. Stress non-diabetic (ND) control; ¥=p<0.05 vs. Stress diabetic (D) control.

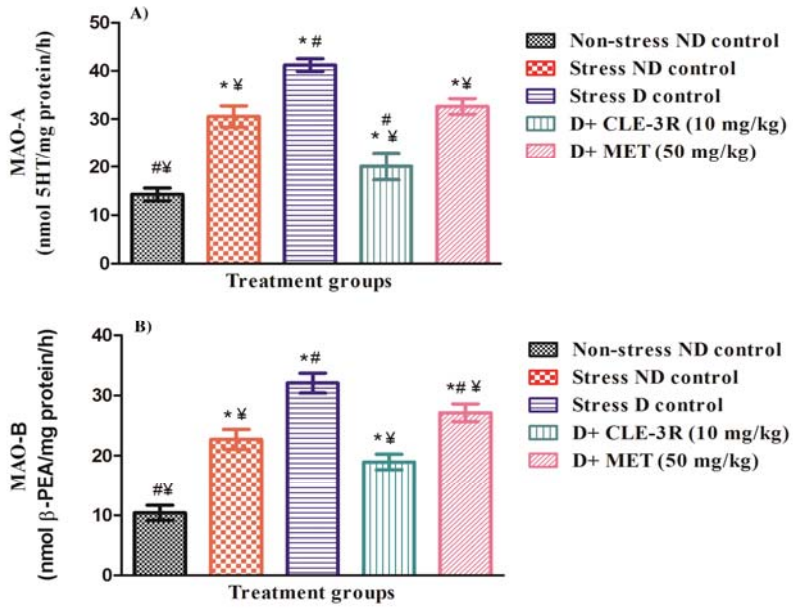

Figure 4.38: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on monoamine oxidase (MAO) enzyme level **A)** MAO-A and **B)** MAO-B activity in brain samples of normal and diabetic rats. Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; $^{\#}$=$p<0.05$ vs. Stress non-diabetic (ND) control; $^{¥}$=$p<0.05$ vs. Stress diabetic (D) control.

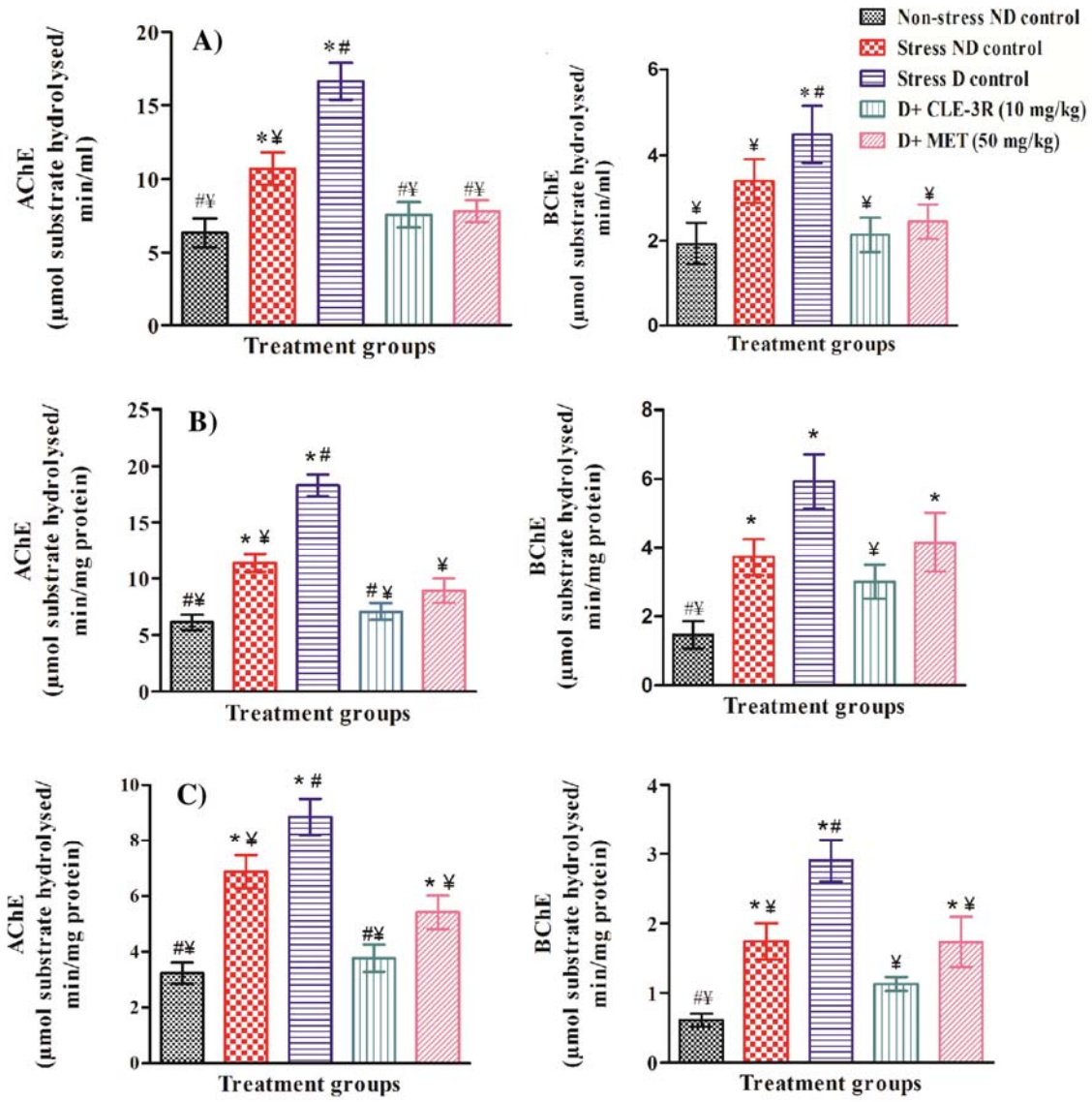

Figure 4.39: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity in **A)** blood **B)** brain frontal cortex and **C)** hippocampus of normal and stressed diabetic rats. Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; $^{\#}$=$p<0.05$ vs. Stress non-diabetic (ND) control; $^{¥}$=$p<0.05$ vs. Stress diabetic (D) control.

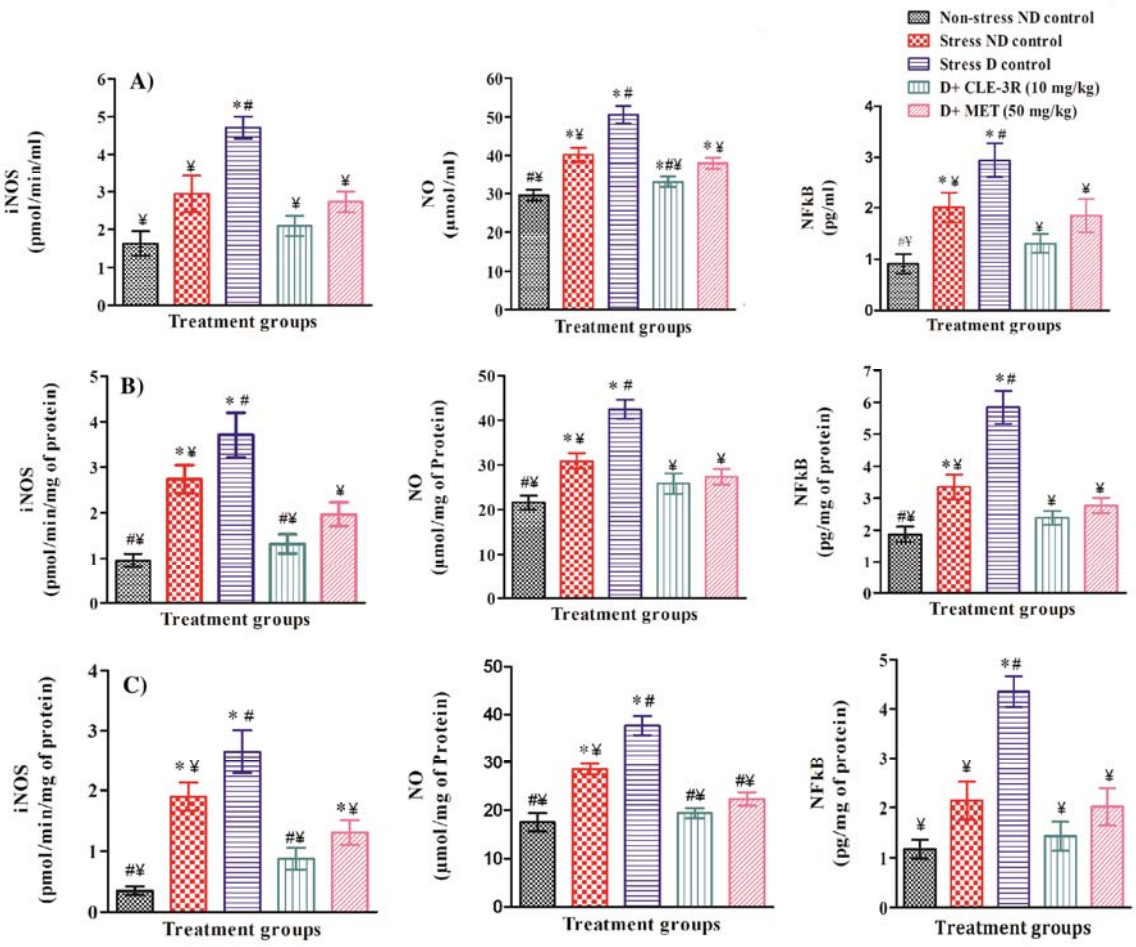

Figure 4.40: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on iNOS, NO and NF-κB level in **A)** blood **B)** brain frontal cortex and **C)** hippocampus of normal and diabetic rats. Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; #=$p<0.05$ vs. Stress non-diabetic (ND) control; ¥=$p<0.05$ vs. Stress diabetic (D) control.

Table 4.1: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight difference of male mice on day 5.

Treatment groups	Body weight		
	Day 1	Day 5	Difference
Control (0.3% CMC)	21.83±0.60	21.17±0.48	-0.67±0.21
MET (100 mg/kg)	22.00±0.68	21.33±0.71	-0.67±0.21*
CLE-3R (5 mg/kg)	21.67±0.56	21.17±0.70	-0.50±0.22*
CLE-3R (20 mg/kg)	21.50±0.81	22.00±0.86	0.50±0.22*
CLE-3R (80 mg/kg)	21.83±0.60	22.17±0.60	0.33±0.21*
CLE-3R (320 mg/kg)	22.16±0.60	22.66±0.49	0.50±0.22*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.2: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight difference of male mice on day 7.

Treatment groups	Body weight		
	Day 1	Day 7	Difference
Control (0.3% CMC)	21.83±0.60	21.00±0.52	-0.83 ±0.17
MET (100 mg/kg)	22.00±0.68	22.17±0.83	0.17±0.31*
CLE-3R (5 mg/kg)	21.67±0.56	21.50±0.85	-0.17±0.40
CLE-3R (20 mg/kg)	21.50±0.81	22.33±0.80	0.83±0.17*
CLE-3R (80 mg/kg)	21.83±0.60	22.33±0.67	0.50±0.22*
CLE-3R (320 mg/kg)	22.17±0.60	23.00±0.63	0.83±0.17*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.3: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight difference of male mice on day 10.

Treatment groups	Body weight		
	Day 1	Day 10	Difference
Control (0.3% CMC)	21.83±0.60	20.67±0.42	-1.17±0.31*
MET (100 mg/kg)	22.00±0.68	22.67±0.67	0.67±0.21*
CLE-3R (5 mg/kg)	21.67±0.56	22.33±0.71	0.67±0.33*
CLE-3R (20 mg/kg)	21.50±0.81	22.67±0.76	1.17±0.17*
CLE-3R (80 mg/kg)	21.83±0.60	22.83±0.48	1.00±0.26*
CLE-3R (320 mg/kg)	22.17±0.60	23.50±0.56	1.33±0.21*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.4: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight difference of female mice on day 5.

Treatment groups	Body weight		
	Day 1	Day 5	Difference
Control (0.3% CMC)	22.33±0.42	21.83±0.54	-0.50±0.22
MET (100 mg/kg)	22.50±0.43	21.83±0.48	-0.67±0.21
CLE-3R (5 mg/kg)	22.83±0.31	22.33±0.42	-0.50±0.22
CLE-3R (20 mg/kg)	22.67±0.49	23.00±0.45	0.33±0.33*
CLE-3R (80 mg/kg)	23.00±0.37	23.50±0.43	0.50±0.22*
CLE-3R (320 mg/kg)	22.83±0.31	23.67±0.33	0.83±0.16*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.5: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight difference of female mice on day 7.

Treatment groups	Body weight		
	Day 1	Day 7	Difference
Control (0.3% CMC)	22.33±0.42	21.50±0.50	-0.83±0.17
MET (100 mg/kg)	22.5±0.43	22.33±0.49	-0.17±0.40*
CLE-3R (5 mg/kg)	22.83±0.31	23.17±0.54	0.33±0.33*
CLE-3R (20 mg/kg)	22.67±0.49	23.50±0.43	0.83±0.16*
CLE-3R (80 mg/kg)	23.00±0.37	24.00±0.26	1.00±0.25*
CLE-3R (320 mg/kg)	22.83±0.31	24.00±0.26	1.16±0.16*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.6: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on body weight difference of female mice on day 10.

Treatment groups	Body weight		
	Day 1	Day 10	Difference
Control (0.3% CMC)	22.33±0.42	21.33±0.49	-1.00±0.26
MET (100 mg/kg)	22.50±0.43	22.67±0.56	0.17±0.31*
CLE-3R (5 mg/kg)	22.83±0.31	23.33±0.42	0.50±0.22*
CLE-3R (20 mg/kg)	22.67±0.49	23.83±0.40	1.16±0.30*
CLE-3R (80 mg/kg)	23.00±0.37	24.33±0.33	1.33±0.21*
CLE-3R (320 mg/kg)	22.83±0.31	24.50±0.34	1.66±0.21*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.7: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on tail suspension test in male mice.

Treatment groups	Immobility period (sec)
Control (0.3% CMC)	148.33±1.26
MET (100 mg/kg)	126.17±0.99*
CLE-3R (5 mg/kg)	134.33±1.20*
CLE-3R (20 mg/kg)	125.50±1.16*
CLE-3R (80 mg/kg)	112.83±0.83*
CLE-3R (320 mg/kg)	94.83±1.10*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.8: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on tail suspension test in female mice.

Treatment groups	Immobility period (sec)
Control (0.3% CMC)	151.83±1.10
MET (100 mg/kg)	127.83±1.43*
CLE-3R (5 mg/kg)	137.17±1.60*
CLE-3R (20 mg/kg)	126.33±1.61*
CLE-3R (80 mg/kg)	116.67±0.76*
CLE-3R (320 mg/kg)	95.33±1.11*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.9: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on pentobarbital induced sleep test in male mice.

Treatment groups	Onset of sleep (sec)	Duration of sleep (min)
Control (0.3% CMC)	164.67±0.88	64.67±1.67
MET (100 mg/kg)	151.83±0.83*	70.67±1.05*
CLE-3R (5 mg/kg)	160.83±0.70*	66.17±0.67
CLE-3R (20 mg/kg)	149.17±1.01*	73.83±1.01*
CLE-3R (80 mg/kg)	137.17±1.01*	76.33±1.48*
CLE-3R (320 mg/kg)	131.67±1.15*	79.33±1.05*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.10: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on pentobarbital induced sleep test in female mice.

Treatment groups	Onset of sleep (sec)	Duration of sleep (min)
Control (0.3% CMC)	165.33±0.88	66.17±1.65
MET (100 mg/kg)	152.17±0.87*	71.33±0.95*
CLE-3R (5 mg/kg)	162.50±1.41*	67.17±1.65
CLE-3R (20 mg/kg)	150.00±0.93*	72.17±0.83*
CLE-3R (80 mg/kg)	138.67±0.76*	77.83±1.83*
CLE-3R (320 mg/kg)	133.83±0.95*	80.67±1.01*

Values are mean±SEM, n=6. *=p<0.05 vs. control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.11: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on glucose, insulin and corticosterone level in non-stressed and stressed male rats.

Treatment groups	Glucose (mg/dl)	Insulin (µIU/ml)	Corticosterone (ng/ml)
Non-stressed CON (0.3% CMC)	93.68±1.36	18.01±0.86	93.18±1.46
Non-stressed CLE-3R (10 mg/kg)	92.97±1.09	17.08±0.69	90.05±2.14
Non-stressed MET (50 mg/kg)	93.03±1.15	17.87±1.04	92.51±1.40
Stressed CON (0.3% CMC)	108.39±3.01 [a]	12.84±0.67 [a]	117.35±2.34 [a]
Stressed CLE-3R(10 mg/kg)	102.08±2.05 [a]	15.76±0.89	104.50±3.17* [a]
Stressed MET(50 mg/kg)	97.85±2.43*	14.37±0.91 [a]	109.66±1.66* [a]

Values are mean±SEM, n=6. *=$p<0.05$ vs. stressed control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test). [a]=$p<0.05$ vs. corresponding non- stressed group (t-test).

Table 4.12: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on organ weight of stressed male rats.

Treatment groups	Absolute organ weight			
	Adrenal glands (mg)	Spleen (mg)	Heart (mg)	Liver (g)
Non-stressed CON (0.3% CMC)	46.67±1.56	339.00±3.16	502.67±3.11	4.32±0.17
Non-stressed CLE-3R (10 mg/kg)	44.17±2.07	345.17±2.46	507.33±1.31	4.34±0.12
Non-stressed MET (50 mg/kg)	48.17±1.66	342.50±4.41	513.00±1.79	4.75±0.22
Stressed CON (0.3% CMC)	54.00±2.31 [a]	282.50±3.45 [a]	482.33±0.71 [a]	4.44±0.20
Stressed CLE-3R(10 mg/kg)	50.50±2.19	315.83±3.74* [a]	501.00±3.10*	4.38±0.12
Stressed MET(50 mg/kg)	52.50±1.71	326.50±3.39* [a]	514.17±1.74*	4.80±0.11

Values are mean±SEM, n=6. *=$p<0.05$ vs. stressed control group (One way ANOVA followed by Student-Newman-Keuls multiple comparison test). [a]=$p<0.05$ vs. corresponding non-stressed group (t-test).

Table 4.13: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on hot plate reaction time of saline and formalin injected male rats**.**

Treatment groups	Hot plate reaction time (sec)
Saline CON (0.3%CMC)	6.33±0.93
Saline CLE (10 mg/kg)	10.83±0.83
Saline MET (50 mg/kg)	7.89±0.74
Formalin CON (0.3%CMC)	3.62±0.41 [a]
Formalin CLE (10 mg/kg)	8.83±1.04*
Formalin MET (50 mg/kg)	5.72±0.89*

Values are mean±SEM, n=6. *=p<0.05 vs. Formalin control difference (One way ANOVA followed by Student-Newman-Keuls multiple comparison test). [a] =p<0.05 vs. corresponding saline group (t-test).

Table 4.14: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on ratio of HDL and LDL of normal drinking water and fructose water consuming male rats.

Treatment groups	Ratio of HDL/LDL (mg/dL)
NDW+CON (0.3% CMC)	0.78±0.08
NDW+ CLE-3R(10 mg/kg)	0.79±0.06
NDW+MET(50 mg/kg)	0.71±0.04
FW+CON(0.3% CMC)	0.30±0.03 [a]
FW+ CLE-3R (10 mg/kg)	0.52±0.06 *[a]
FW+MET(50 mg/kg)	0.46±0.04 *[a]

Values are mean±SEM, n=6. *=p<0.05 vs. FW control (One way ANOVA followed by Student-Newman-Keuls multiple comparison test). [a]=p<0.05 vs. corresponding NDW group (t-test).NDW=Normal drinking water, FW=Fructose water.

Table 4.15: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on organ weight of normal drinking water and fructose water consuming male rats.

Treatment groups	Adrenal glands (mg)	Spleen (mg)	Heart (mg)	Liver (g)	Kidney (mg)
NDW+CON (0.3% CMC)	50.83±2.01	363.17±4.40	561.50±5.35	5.43±0.27	1314.00±9.30
NDW+ CLE-3R (10 mg/kg)	52.33±2.08	367.17±4.60	566.50±5.60	5.59±0.23	1298.33±9.13
NDW+MET (50 mg/kg)	53.17±2.57	365.33±5.19	563.33±4.08	5.80±0.22	1314.50±7.71
FW+CON(0.3% CMC)	66.00±3.04 [a]	399.67±4.63 [a]	594.00±4.49 [a]	6.54±0.22[a]	1368.67±8.51 [a]
FW+ CLE-3R (10 mg/kg)	56.33±1.91*	381.17±3.56 [a]*	574.50±5.23*	5.97±0.12	1310.67±6.99*
FW+MET (50 mg/kg)	59.33±1.78	378.33±4.17*	570.50±4.88*	6.11±0.18	1338.17±7.59*

Values are mean±SEM, n=6. *=$p<0.05$ vs. FW control (One way ANOVA followed by Student-Newman-Keuls multiple comparison test). [a]=$p<0.05$ vs. corresponding NDW group (t-test). NDW=Normal drinking water, FW=Fructose water.

Table 4.16: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on immobility time period of normal and stressed diabetic rats in forced swim test.

Treatment group	Immobility period (sec)
Non-stress ND control	122±4.13[#¥]
Stress ND control	155±4.45*[¥]
Stress D control	170±4.60*[#]
D+ CLE-3R (10 mg/kg)	136± 3.95[¥#*]
D+ MET (50 mg/kg)	145±4.82[¥*]

Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; [#]=$p<0.05$ vs. Stress non-diabetic (ND) control; [¥]=$p<0.05$ vs. Stress diabetic (D) control (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 4.17: Effect of 95.4% curcuminoids containing *Curcuma longa* extract (CLE-3R) and metformin on stomach ulceration index in normal and stressed diabetic rats.

Treatment groups	**Mean Ulcer Index**
Non-stress ND control	0.00±0.00$^{\#¥}$
Stress ND control	0.67±0.24*$^{¥}$
Stress D control	0.92±0.20*$^{\#}$
D+ CLE-3R (10 mg/kg)	0.17±0.11$^{¥}$
D+ MET (50 mg/kg)	0.17±0.11$^{¥}$

Values are mean ± SEM (n=6). *=$p<0.05$ vs. Non-stress non-diabetic (ND) control; $^{\#}$=$p<0.05$ vs. Stress non-diabetic (ND) control; $^{¥}$=$p<0.05$ vs. Stress diabetic (D) control (One way ANOVA followed by Student-Newman-Keuls multiple comparison test).