CHAPTER 7

SUMMARY, CONCLUSION AND RECOMMENDATIONS

During 1936, Hans Selye defined, stress as the “non-specific response of the body to any demand for change” [1]. This definition of selye on stress is widely accepted, and still this concept is the basis of evaluation of stress and antistress agents. Stress is an integral part of living organisms, which is counteracted by homeostatic mechanism of the body to keep internal environment within normal physiological limits. However if stress is extreme, unusual or chronic, the normal homeostatic mechanism will be disturbed leading to well known stress related diseases such as peptic ulcer, hypertension, depression, stroke, cancer, diabetes etc. [18].

Treatment of stress includes use of medicine from different traditional as well as modern system. Benzodiazepine are synthetic drugs, having significant antistress activity in acute stress models, but they are not proved effective in chronic stress models [191]. They also possess more adverse effects. Some medicinal plants have shown state of nonspecific resistance in the organism, with less adverse effects. Polyherbal formulations like Geriforte have been developed for antistress action. But still the need of effective antistress agents to combat noxious stressful situations of life is high.

This prompted to carry research on Vitis vinifera and Cichorium intybus, and to prove it scientifically for their use as antistress agent. Since both the plants have not explored as antistress agent to a significant extent, and on the background of available information of plant chemotaxonomic condition with traditional ethno medical information the present work was planned with the following aim:

Evaluation of antistress activity of Vitis vinifera and Cichorium intybus in albino rats and albino mice.

- Collection of plant material and its authentication.
The selected plants namely, fruits of *Vitis vinifera* (common grapes) and roots of *Cichorium intybus* (chicory) were collected, identified, confirmed and authenticated by Dr. Athaulla khan, Botanist. The voucher specimen (MMU/RMGM/VV-CI/2008-09) was maintained.

- Extraction of *Vitis vinifera* and *Cichorium intybus*.

*Vitis vinifera* seed extract was found to be brown in color, with percentage yield of 12% w/w. Where as *Cichorium intybus* root extract was found to be yellowish brown in color, with percentage yield of 14% w/w.

- Preliminary qualitative studies of extracts.

Qualitative studies have shown the presence of carbohydrates, proteins and phenolic compounds in *Vitis vinifera* seed extract. Even tests for Flavonoids were found to be positive for extract, indicating its presence in the *Vitis vinifera* seed. Test for fixed oils was found to be positive in seeds extract of *Vitis vinifera*. Qualitative studies on *Cichorium intybus* root extract exhibited presence of carbohydrates, proteins and phenolic compounds. Tests for Phytosterols and Triterpenoids were found to be positive revealing the presence of steroids and triterpenes.

- Acute toxicity study

During acute toxicity studies of both plant extracts, mice showed normal behavioral, neurological and autonomic response. Mice were neither depressed nor excited throughout study. Mice did not show any change in alertness, visual placing, grooming, reactivity, spontaneous activity, touch response, pain response, posture, limb tone, grip strength and abdominal tone. Pupil size, corneal and pinna reflex were normal. There were no signs of central nervous system or autonomic stimulation, such as vocalization, restlessness, aggression, fearfulness, tremors, convolution, straub tail phenomenon, writhing, piloerection, salivation and urination. There was no significant change in body weight as compared to control group. During acute toxicity studies of both plant extracts, there was no noticeable toxicity and no deaths till the dose tested (up to the dose of 5000 mg per kg body weight of animal).
Vitis vinifera and Cichorium intybus extracts have shown wide margin of safety up to the dose of 5000 mg per kg body weight of animal.

Different experimental models and their results are summarized as follows.

1. Effect of extracts of Vitis vinifera seeds and Cichorium intybus roots on swimming endurance of mice.

This investigation was done as described by the previous investigators [145, 179], using six groups of albino mice and swimming survival time was recorded. In this method, the antistress effect of extracts was evaluated by determining the improvement in swimming endurance period and over all performance of the animals, when subjected to swim in restricted space like water vessel [145]. This test is widely used to evaluate antistress activity of drugs, since swim endurance reflects physical endurance [144]. Pretreatment with extracts of Vitis vinifera and Cichorium intybus have very significantly (p<0.01) increased the survival time or swimming endurance of mice at the tested doses of 200 and 400 mg per kg body weight, when compared to the untreated mice. Vitis vinifera at 400 mg per kg body wt of animal showed maximum increase in swimming survival time (highly significant with p<0.001).

The swimming endurance of mice was increased by Vitis vinifera and Cichorium intybus extracts, thereby showing antistress activity of this plant extracts.

2. Effect of extracts of Vitis vinifera seeds and Cichorium intybus roots on drug induced narcosis in mice.

This investigation was done as described by the previous investigators [146], using seven groups of albino mice and sleeping time (narcosis time) was recorded. In this method, the antistress effect of extracts was evaluated by determining their (Adaptogen) ability to synergize with CNS depressant such as barbiturates, and it could be the mechanism of antistress activity [146].
Pretreatment with extracts of *Vitis vinifera* and *Cichorium intybus* have very significantly (p<0.01) increased the narcosis time or prolonged the duration of loss of righting reflex in mice. This effect of extracts was observed at the tested doses of 200 and 400 mg per kg body weight, when compared to the control group of mice. *Vitis vinifera* at 400 mg per kg showed maximum increase in sleep time (very significant with p<0.01). It was Comparable to the values of standard drug treated group, but statistically non significant.

*Vitis vinifera* and *Cichorium intybus* extracts have the capacity to depress the CNS along with other CNS depressants and this could be the mechanism of antistress activity.

3. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on cold and restraint stress induced brain lipid peroxidation.

This investigation was done as described by the previous investigators [19, 38, 149 and 172], using seven groups of albino rats and brain lipid peroxidation was determined. In this method, the antistress effect of extracts was evaluated by determining their adaptogenic ability of protection against stress induced brain lipid peroxidation and oxidative damage of tissue [149]. This could be the mechanism of antistress activity.

In this model, chronic exposure of rats to cold and restraint stress leads to oxidative damage and this result into lipid peroxidation [149]. Lipid peroxidation can be determined by measuring malondialdehyde formed as an end product of lipid peroxidation. MDA can be measured by a well accepted thiobarbituric acid assay. Here formed Chromogenic dithiobarbituric acid adduct, absorbs strongly at 532 nm [153]. Pretreatment with extracts of *Vitis vinifera* and *Cichorium intybus* at the tested doses were found to be extremely significant (p<0.0001), for preventing rise in brain lipid peroxidation.
Pretreatment of albino rats with *Vitis vinifera* seeds and *Cichorium intybus* roots extracts have prevented rise of lipid peroxidation in brain on chronic stress and this could be the mechanism of antistress activity.

4. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on swimming stress induced gastric ulceration in albino rats.

This investigation was done as described by the previous investigators [19, 174 and 175]; using seven groups of albino rats and gastric ulcers formed were scored. In this method, the antistress effect of extracts was evaluated by determining their adaptogenic ability of protection against gastric ulceration in albino rats, induced by Chronic forced swimming stress.

In this model behavioral despair (emotional stress) and vigorous muscular activity (physiological stress) of animals are responsible for the pathogenesis of gastric ulcers [187]. In this model rats were exposed to stressful condition repeatedly, as chronic stress is a potent inducer of stress ulcers [148].

Both the extracts of *Vitis vinifera* and *Cichorium intybus* on pretreatment have shown very significant (p<0.01) protective effect for stress induced ulcers in albino rats at the tested doses. Both the extracts of *Vitis vinifera* and *Cichorium intybus* 500 mg per kg body weight of animal, exhibited significant protection, which was found to be near to the value of standard Omeprazole treated group, but statistically non significant. Even histopathological studies confirmed the protective effect of *Vitis vinifera* and *Cichorium intybus* against stress induced ulcers.

*Vitis vinifera* and *Cichorium intybus* on pretreatment have shown significant protective effect for stress induced ulcers in albino rats, through nonspecific rise in resistance. This study demonstrates the presence of antistress activity in both *Vitis vinifera* and *Cichorium intybus*.

5. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on adrenocortical activity in stress induced rats.

This investigation was done as described by the previous investigators [19, 157, and 174], using nine groups of albino rats. Adrenal glands weights with
plasma corticosterone level were determined. In this method, the antistress effect of extracts was evaluated by determining their adaptogenic effect on adrenocortical activity in albino rats, during cold water swimming stress. This could be the mechanism of antistress activity. During stress, hypothalamic-pituitary-adrenocortical axis activation leads to increase in plasma corticosterone and alteration in weights of adrenal gland [157, 174 and 187]. This has been utilized as evaluation parameter of stress. It has been well established phenomenon that cold swimming stress increases plasma corticosterone level [19, 157, 174 and 187]. Due to cold stress, temperature regulatory centre in the hypothalamus is activated, resulting in activation of HPA axis with increase in plasma corticosterone level [19]. Acute stress raises plasma ACTH and corticosterone level within 5 min and 30-45 min respectively [148].

Both the extracts of *Vitis vinifera* and *Cichorium intybus* on pretreatment have very significantly (p<0.01) prevented elevation in plasma corticosterone level and adrenal gland weight at the dose of 250 mg per kg body weight of animal. Both the extracts of *Vitis vinifera* and *Cichorium intybus* at 500 mg per kg body weight of animal, exhibited extremely significant (p<0.0001) protection, which was found to be near to the value of standard treated group, but statistically non significant.

The protective effect of *Vitis vinifera* and *Cichorium intybus* extracts on plasma corticosterone level and weight of adrenal gland during stress, justifies their use as antistress agents. The observed antistress effect of *Vitis vinifera* and *Cichorium intybus* extracts was found to be through hypothalamic pituitary adrenal axis of the animal.

6. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on liver glycogen of albino rats during weight loaded forced swimming stress.

This investigation was done as described by the previous investigators [145, 158], using nine groups of albino rats. Duration of immobility (behavioral despair) with Liver glycogen level was determined. During stress, depletion of liver glycogen has been well established [158]. During swimming induced stress energetic
demand occurs [159], and this energy is provided from breakdown of stored glycogen in liver [161]. So assessment of liver glycogen depletion is a biomarker of stress.

Behavioral despair represents an experimental model of endogenous depression in animal [145]. Depression occurs, if stress is continued for prolonged period of time [145, 152]. This method is based on observation of passiveness and immobility of rats after a period of vigorous activity, when they are subjected for forced swimming [145, 162]. Forced swimming stress model is widely used to induce depression in animal.

Both the extracts of *Vitis vinifera* and *Cichorium intybus* at the tested doses prevented decrease in liver glycogen with extremely significance (p<0.0001). It was found to be near to the value of standard treated group, but statistically non significant. Even both the extracts of *Vitis vinifera* and *Cichorium intybus* at the tested doses exhibited decrease in immobility period with extremely significant (p<0.0001). The antistress activity of *Vitis vinifera* and *Cichorium intybus* extracts seems to be mediated by attenuating energy depletion. It might have resulted in augmentation of the endurance capacity of the animal. This is also confirmed with the reduced behavioral despair observation of animal during investigation.

Pretreatment with *Vitis vinifera* and *Cichorium intybus* have prevented stress induced depletion of liver glycogen and also behavioral despair, which could be due to rise of nonspecific resistance.

7. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots as Immunomodulatory during stress and drug induced myelosuppression in albino rats.

This investigation was done as described by the previous investigators [163,176]; using eleven groups of albino rats and haematological parameter were evaluated. That is total leucocytes count, differential leucocytes count, RBC count, haemoglobin content & platelet count. In this method, the antistress effect of extracts was evaluated by determining their adaptogenic effect or their
protective effect on stress induced immunosuppression and drug induced myelosuppression in albino rats. Physical or psychological stress influences function and efficiency of the immune system, resulting in immunosuppression [163]. During chronic stress, corticosterone level remains high, resulting in reduction of white blood cell numbers in circulating blood [166]. It has been found that stress in rodents have induced decrease of leucocytes number in blood with decrease in lymphocyte and monocyte percentage, whereas increase in neutrophils percentage [165].

Pretreatment with *Vitis vinifera* and *Cichorium intybus* extracts have prevented immunosuppression in albino rats due to stress, thereby showing significant protective effect against restraint stress induced immunosuppression. Protection of immunosuppression due to stress was high by *Cichorium intybus*. Pretreatment with, *Vitis vinifera* and *Cichorium intybus* extracts have prevented myelosuppression in albino rats due to cyclophosphamide (myelosuppressive drug), thereby showing significant protective effect against myelosuppression. This may be due to it’s mechanism of antistress action, which involves effect on the neuroendocrine-immunologic axis that constitutes the stress system [40]. HPA axis and sympatho adrenal system are the primary site of action of adaptogens, whereas components of immune system are their secondary site of action [4, 40].

Pretreatment with *Vitis vinifera* and *Cichorium intybus* have prevented immunosuppression in albino rats due to stress as well as by myelosuppressive drug. This immunomodulation of *Vitis vinifera* and *Cichorium intybus* could be the mechanism of rise in nonspecific resistance.

**Different experimental models in which antistress activity of Vitis Vinifera and Cichorium intybus established are as follows:**

1. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on swimming endurance of mice.

2. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on drug induced narcosis in mice.
3. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on cold and restraint stress induced brain lipid peroxidation.

4. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on swimming stress induced gastric ulceration.

5. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on adrenocortical activity in stress induced rats.

6. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on Liver glycogen of rats during weight loaded forced swimming stress.

7. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots as the Immunomodulatory during stress and drug induced myelosuppression in albino rats.

*Vitis vinifera* and *Cichorium intybus* extracts have shown extremely significant (p<0.0001) antistress activity in the following experimental models:

1. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on cold and restraint stress induced brain lipid peroxidation.

2. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on adrenocortical activity in stress induced rats.

3. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on Liver glycogen of albino rats during weight loaded forced swimming stress.

*Vitis vinifera* at higher dose showed maximum antistress activity in the following experimental models:

1. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on swimming endurance of mice.

2. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on drug induced narcosis in mice.
3. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on cold and restraint stress induced brain lipid peroxidation.

4. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on swimming stress induced gastric ulceration in albino rats.

5. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on adrenocortical activity in stress induced rats.

6. Effect extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots on Liver glycogen of albino rats, during weight loaded forced swimming stress.

*Cichorium intybus* at higher dose showed maximum antistress activity in the following experimental model:

1. Effect of extracts of *Vitis vinifera* seeds and *Cichorium intybus* roots as the Immunomodulatory during stress and drug induced myelosuppression in albino rats.

All the above observed activities may be due to the presence of phytocstituents present in the seeds of *Vitis vinifera* and roots of *Cichorium intybus*. Polyphenols in the form of procyanidin present in *Vitis vinifera* are responsible for its antistress activity. Polyphenols are established antistress phytoconstituent [75]. Phytosterols, triterpenoids, flavonoids, sesquiterpene lactones, coumarins (including cichoriin), caffeic acid derivatives, Inulin and tannins present in *Cichorium intybus* are responsible for its antistress activity. Panossian and other researchers reported that, active constituents of plant responsible for adaptogenic activity are phenolic, phytosterols of triterpenes [4, 39-40]. Triterpenes involve with HPA-axis mediated regulation of immune and neuroendocrine system [4]. Both the plant extracts were found to resist the adverse consequences of different variety of stress by increasing body’s nonspecific resistance through following different mechanism of action.

- By re-establishing the functioning of HPA-axis: They might have improved the sensitivity of receptors involved in negative feedback mechanism. So it prevented failure of negative feedback mechanism. This is in agreement with the established mechanism of all antistress agents [39].
- By prevention of stress induced increase in nitric oxide and the associated decline in ATP production. Consequently rise of energy (ATP) might have increased
performance and endurance of animals. This is also confirmed with the reduced behavioral despair observation of animal during investigation. This is in agreement with established concept [45].

- By preventing lipid peroxidation in the brain, which ultimately resulted in augmentation of the endurance capacity and improvement in overall performance of the organism.

This investigation may be concluded that *Vitis vinifera* seeds and *Cichorium intybus* roots extracts possess potential antistress activity.

The present work justifies use of *Vitis vinifera* and *Cichorium intybus* extracts as antistress agents to combat noxious stress situation of life and to avoid stress related diseases. This investigation contributed to the emergence of *Vitis vinifera* and *Cichorium intybus* as new antistress medicinal plants in the limited field of antistress therapy.