Results and Discussion

5. RESULTS AND DISCUSSION

Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and / or from a decrease in antioxidant defense potential (Gumieniczek *et al.*, 2002). Recently, great attention has been focused on the role of the antioxidative defense system in oxidative stress. The endogenous antioxidants in medicinal herbs may play an important role in antioxidative defense against oxidative damage, possibly protecting the biological functions of cells (Gunther, 2004).

Triticum aestivum, commonly called as wheat grass, can be effectively used for skin diseases and ulcerated wounds. The chlorophyll contained in wheat grass juice is very beneficial for the body. It purifies the blood due to its vitamin and mineral contents. This property is used by the body to cleanse and rebuild itself (http://www.wheatgrass.professional.info/letters/letter_may04).

The study of model invertebrates like Drosophila has a long history of yielding valuable insights into both fundamental and pathobiology. Drosophila has become one of the most tractable multicellular organisms to study the developmental biology and genetic analysis (Wilson *et al.*, 2005).

In this study, the antioxidant activity of *Triticum aestivum* was investigated using Drosophila as a model system under conditions of carbon tetrachloride (CCl_4) and hydrogen peroxide (H_2O_2) induced oxidative stress. Two different concentrations of each oxidant were used for the study. CCl_4 was used at the doses of 130mM and 195 mM and H_2O_2 was used at the doses of 20mM and 30mM.

These doses were selected based on the results of a pilot study, wherein varying doses of CCl_4 (50 to 200mM) and H_2O_2 (5 to 30mM) were studied. These two standard compounds were chosen to check if there existed a difference in the stress induced by a direct-acting oxidant (H_2O_2) and the one that requires metabolic activation (CCl_4).

The oxidant and / or the leaf extract treatment was given for seven days by mixing the agents in the diets fed to Drosophila of the different treatment groups. After the seventh day of exposure to the oxidant and / or leaf extract, the activities of enzymic (SOD, CAT and POX) and the levels of non-enzymic antioxidants (vitamins C, E and reduced glutathione) were recorded.

The effect of the aqueous extract of *Triticum aestivum* on the oxidative stress condition was evaluated using the enzymic and non-enzymic antioxidant parameters as given below.

SUPEROXIDE DISMUTASE

SOD is the major attractive metalloprotein in the antioxidant family. The activity of SOD in the control and H_2O_2 / CCl_4 exposed flies co-treated with *Triticum aestivum* leaf extract is shown in Table 1.

The activity of SOD was found to be decreased upon the exposure to oxidants (H_2O_2 and CCl_4). The activity was decreased to a great extent when exposed to H_2O_2 than to CCl_4. As the concentration of the oxidants were increased, greater was the reduction in the activity of SOD. The higher dose of H_2O_2 reduced the activity of SOD to the lowest level. This effect was same in the males and females.

TABLE I

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF SUPEROXIDE DISMUTASE IN THE CONTROL AND TREATED *Drosophila melanogaster*

SEX	GROUP	SUPEROXIDE DISMUTASE (Units / 30 mg of flies)				
		WITHOUT OXIDANT	WITH H_2O_2		WITH CCl_4	
			LOW DOSE	HIGH DOSE	LOW DOSE	HIGH DOSE
Male	No extract	43.49 ± 0.76	28.03 ± 0.56	24.5 ± 0.96	29.05 ± 2.00	28.12 ± 0.80
	Triticum aestivum	37.73 ± 0.80	37.50 ± 0.30	28.4 ± 0.00	40.55 ± 0.92	38.40 ± 0.10
Female	No extract	27.30 ± 0.53	19.10 ± 0.20	18.3 ± 0.38	23.43 ± 0.45	19.50 ± 0.87
	Triticum aestivum	24.50 ± 0.96	24.33 ± 0.67	23.2 ± 0.10	24.23 ± 0.76	21.13 ± 1.30

The values are means ± SD of triplicates

1 Unit = Amount of enzyme that causes 50% reduction in NBT oxidation

There was a slight decline in the SOD activity in the plant extract treated group compared to the untreated control. Co-administration of the plant extract increased the activity of SOD in the groups exposed to CCl_4 and H_2O_2. The activity of SOD was reverted to near normal in the males exposed to lower dose of CCl_4 co-treated with the plant extract. There was a marked increase of activity in the groups exposed to higher doses of CCl_4 and H_2O_2 but the values were not restored to normal levels.

The males showed slightly higher activities of SOD compared to the females in all the groups including the controls.

SOD activity is reflective of the extent of oxidative stress in biological systems, as evidenced by several studies reported in the literature.

The study conducted by Venukumar and Latha (2002) showed that the decline in SOD level in CCl_4-administered rats were brought to near normal level by the administration of methanolic extracts of rhizome of *Curculiga orchioides*.

Singanan *et al.* (2007) observed that the activity of SOD was found to be decreased in ethanol intoxicated rats. The therapeutic treatment with *Aegle marmelos* herbal drug significantly improved the level of SOD in liver, indicating the hepatoprotection by elevating free radical scavenging activity.

The activation of SOD was found to be lower in diabetic control rats. The treatment with the ethanolic extract of *Aloe vera* brought about a significant increase in the activity of SOD in the diabetic rats (Rajasekaran *et al.*, 2005). A similar effect has been reported with *Helicteres isora* bark

extracts increasing the SOD activity in the cardiac tissues of diabetic rats (Kumar *et al.*, 2008).

The SOD activity decreased markedly in the liver and kidney homogenates of the rats administered with CCl_4 (Shi *et al.*, 2006). The authors suggested that the mistletoe alkali could be a potential herbal medicine for improving SOD activity in liver and kidney, as their scavenging activity steadily increased with the increase of drug concentration.

Sethi *et al.* (2004) reported significantly increased activity of SOD and GSH in the liver following the treatment with aqueous extract of *Ocimum sanctum*, which decreased the damage produced by oxidative stress in diabetic rats.

Li *et al.* (2008) studied the antioxidant effect of green tea catechins and broccoli, wherein the SOD activity increased significantly in the Drosophila fed with green tea catechins or broccoli extract diet compared with those fed with a control 5% fat diet.

The results of the present study show that SOD responds to oxidative stress induced by both H_2O_2 and CCl_4 in Drosophila. Oxidative stress significantly decreased SOD activity, which effect was efficiently counteracted by the co-administration of *Triticum aestivum* leaf extract.

CATALASE

The defensive antioxidant enzyme next to SOD is catalase. It traps the harmful H_2O_2 and converts it into water and oxygen.

TABLE II

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF CATALASE IN THE CONTROL AND TREATED *Drosophila melanogaster*

SEX	GROUP	CATALASE (Units / 30mg of flies)				
		WITHOUT OXIDANT	WITH H_2O_2		WITH CCl_4	
			LOW DOSE	HIGH DOSE	LOW DOSE	HIGH DOSE
Male	No extract	171.0 ± 1.00	39.17 ± 1.11	31.93 ± 1.00	56.37 ± 0.90	39.10 ± 0.53
	Triticum aestivum	143.1 ± 0.66	62.10 ± 1.94	44.86 ± 0.10	72.30 ± 0.53	56.30 ± 0.40
Female	No extract	104.5 ± 1.15	31.80 ± 0.50	24.38 ± 0.61	44.49 ± 0.96	34.89 ± 0.00
	Triticum aestivum	98.0 ± 1.00	44.86 ± 0.05	39.30 ± 0.45	62.11 ± 1.92	52.09 ± 0.25

The values are means ± SD of triplicates

1 Unit = Amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units

Table 2 presents the results obtained for catalase activity in the Drosophila exposed to oxidative stress in the presence and the absence of *Triticum aestivum* leaf extract.

The activity of catalase was found to be decreased in the H_2O_2 and CCl_4 treated groups. Higher dose of the oxidants reduced the catalase activity to a greater extent, with the severe depletion in the female Drosophila treated with the higher dose of H_2O_2

There was a slight decline in the catalase activity in the plant extract treated group compared to the untreated control group. The table shows that the treatment of aqueous extract leads to significant enhancement in the activity of catalase in all the groups where there was a decrease in the catalase activity under the exposure of oxidants. Though there was a marked increase in the activity, complete recovery was not obtained in any case.

The catalase activity in the male flies was in general lower than the female flies.

Catalase is an important member of the endogenous antioxidant system and the literature is rich with reports showing modulation in the activity under various stress conditions.

Shahjahan *et al.* (2004) demonstrated the hepatoprotective and antioxidant activities of *Solanum trilobatum* extract in the experimental rat model. This extract recovered the decline in the activity of catalase in CCl_4 administered rats, revealing that oxidative stress elicited by CCl_4 intoxication has been nullified due to the antioxidant effect of the extract of *Solanum trilobatum*.

A significant decrease in the activity of the enzymic antioxidants (SOD and CAT) was noted after a single administration of paracetamol. Upon administration of chloroform and methanolic extract of *Ichnocarpus frutescens* the activities of enzymic antioxidants were significantly reversed to near normal levels. The administration of these extracts prevented the accumulation of excessive free radicals and protected the liver from paracetamol intoxication (Dash *et al.*, 2007).

Dhanalakshmi *et al.* (2006) observed a significant decrease in CAT in the adrenal tissue of cold stressed rats. This decline in CAT was significantly prevented by the pretreatment with Triphala.

CAT decomposes H_2O_2 and protects tissue from highly reactive hydroxyl radicals. Administration of methanolic extract of *Berberis tinctoria* increased the activity of CAT in paracetamol induced liver damage in rats to prevent the accumulation of excessive free radicals and protected the liver from paracetamol intoxication (Murugesh *et al.*, 2005).

Catalase activity was significantly decreased in diabetic rats as compared to normal control animals. The levels were significantly increased by an ethanolic extract of *Embelia ribes* (Bhandari *et al.*, 2007).

The results of the present study show that catalase is a crucial determinant for evaluating the antioxidant level in the experimental studies. The decrease in the catalase activity by the oxidative stress induced was recovered by the administration of aqueous extract of *Triticum aestivum* leaves, indicating its antioxidant capacity.

PEROXIDASE

The peroxidase activity was estimated by the method of Reddy *et al.* (1995). Table 3 shows the activity of peroxidase in the oxidant treated flies in the presence and absence of *Triticum aestivum* leaf extract.

Treatment with H_2O_2 and CCl_4 caused a marked decrease in the activity of peroxidase. There was a greater reduction in the activity of peroxidase in the females exposed to H_2O_2 compared to CCl_4 treated group.

The plant extract treated group showed a slight decrease in the peroxidase activity when compared to the control group unexposed to any oxidants. The co-treatment of the aqueous extract of *Triticum aestivum* increased the peroidase activity in the H_2O_2 and CCl_4 treated groups. The activity was increased to a great extent that the males eposed to the lower dose of CCl_4 recovered to near normal levels after the co-administration of plant extract. A similar trend was seen in the males exposed to the lower dose of H_2O_2. Peroxidase activity was also found to be lower in the females than in the males.

Peroxidase activity has also been followed as an index indicative of the extent of oxidative stress by many researchers.

The decrease in the glutathione peroxidase in hyperammonemic rats, was increased to normal by the addition of an ethanolic extract of *Pongamia pinnata* (Essa and Subramanium, 2007). Bhandarkar and Khan (2003) have reported that the administration of *Lawsonia alba* extract significantly elevated the hepatic GPx activity during CCl_4 stress in rats.

TABLE III

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF PEROXIDASE IN THE CONTROL AND TREATED *Drosophila melanogaster*

SEX	GROUP	PEROXIDASE (Units / 30mg of flies)				
		WITHOUT OXIDANT	WITH H_2O_2		WITH CCl_4	
			LOW DOSE	HIGH DOSE	LOW DOSE	HIGH DOSE
Male	No extract	1.07 ± 0.04	0.75 ± 0.05	0.61 ± 0.07	0.84 ± 0.06	0.72 ± 0.10
	Triticum aestivum	1.02 ± 0.10	0.83 ± 0.04	0.63 ± 0.04	0.93 ± 0.01	0.81 ± 0.04
Female	No extract	0.84 ± 0.06	0.56 ± 0.05	0.32 ± 0.04	0.62 ± 0.03	0.52 ± 0.00
	Triticum aestivum	0.75 ± 0.05	0.64 ± 0.06	0.42 ± 0.06	0.72 ± 0.00	0.54 ± 0.06

The values are means ± SD of triplicates
1 Unit = Change in absorbance at 430 nm per minute

Co-administration of ursolic acid increased the activity of SOD, CAT and GPx and enhanced the antioxidant capacity in the heart of rats chronically administered with ethanol (Saravanan and Pugalendi, 2003). Liu *et al.* (2006) reported that the methanolic extracts of *Ginkgo biloba* significantly increased the GPx activity upon CCl_4 intoxication in the liver and plasma of rats.

A study conducted by Berenguer *et al.* (2006) indicated the gastroprotective effects of *Rhizophora mangle* in diclofenac-induced ulcers in rats, which was suggested to be related to its antioxidant properties, which increased the activity of GSH-Px and SOD.

The present study shows that the extract of *Triticum aestivum* was very effective in reverting back the changes in the status of peroxidase activity caused by oxidative stress induced by H_2O_2 and CCl_4.

VITAMIN C

Vitamin C provides antioxidant protection by being an electron donor. Vitamin E can use this electron to recycle itself when oxidized. Vitamin C is regarded as the first line natural antioxidant defense in plasma. It is a water soluble potent chain breaking antioxidant (Iqbal *et al.*, 2004).

The results of the vitamin C level in Drosophila exposed to oxidative stress in the presence and absence of *Triticum aestivum* leaf extract is shown in Table 4. There was significant decrease in the level of vitamin C in the H_2O_2 and CCl_4 treated groups. The lowest level of vitamin C was observed in H_2O_2 treated groups, specifically in the females.

TABLE IV

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN C IN THE CONTROL AND TREATED *Drosophila melanogaster*

SEX	GROUP	VITAMIN C (µg / 30mg of flies)				
		WITHOUT OXIDANT	WITH H_2O_2		WITH CCl_4	
			LOW DOSE	HIGH DOSE	LOW DOSE	HIGH DOSE
Male	No extract	4.13 ± 0.37	3.06 ± 0.14	2.36 ± 0.23	3.43 ± 0.50	3.16 ± 0.18
	Triticum aestivum	3.89 ± 0.24	3.55 ± 0.14	2.83 ± 0.60	3.67 ± 0.18	3.25 ± 0.10
Female	No extract	3.56 ± 0.18	2.23 ± 0.22	2.20 ± 0.20	3.06 ± 0.14	2.90 ± 0.00
	Triticum aestivum	3.32 ± 0.01	3.06 ± 0.15	2.97 ± 0.40	3.16 ± 0.05	3.07 ± 0.15

The values are means ± SD of triplicates

Both the doses of oxidants caused a similar reduction in the level of vitamin C in females. However, a slight dose-response was observed in the males, especially with CCl_4. Untreated control and plant extract treated groups showed nearly the same level of vitamin C.

Treatment with the aqueous extract of *Triticum aestivum* increased the level of vitamin C in both H_2O_2 and CCl_4 treated groups. Normal level was recovered in the group where the plant extract was co-treated with the lower dose of CCl_4. The level of vitamin C was reversed to near normal level in the female flies treated with the lower dose of CCl_4 and plant extract.

The levels of vitamin C were found to be slightly lower in the females than in the males. Reversal to normal level is more marked in males than in the females.

Among the non-enzymic antioxidants, vitamins C and E are the most widely studied. There are several studies reporting the changes in the levels of vitamin C in oxidative stress conditions and their modulation by herbal components.

The study by Dhanalakshmi *et al.* (2006) showed that the levels of vitamin C were significantly decreased in cold stress induced rats. This cold stress induced decline in the vitamin C was significantly prevented by pretreatment of Triphala. This indicated that vitamin C effectively intercepts oxidants in the aqueous phase before they attack and cause detectable oxidative damage.

The oral administration of rutin improved the antioxidant status of streptozotocin-induced diabetic rats by increasing enzymic (SOD and CAT)

and non-enzymic (GSH, vitamins C and E) antioxidants (Kamalakannan and Prince, 2006). A reduction in tissue ascorbic acid level was observed in streptozotocin induced diabetic rats, which was significantly reverted by the administration of *Casearia esculenta* root extract (Prakasam *et al.*, 2005).

The results of the present study are in agreement with these reports, and show that vitamin C is sensitive to both the doses of the oxidants under study (H_2O_2 and CCl_4). The aqueous extract of *Triticum aestivum* counteracted the oxidative stress condition induced by both H_2O_2 and CCl_4.

VITAMIN E

Vitamin E is a fat soluble vitamin that mainly prevents the attack of reactive oxygen species of the membrane PUFA. Vitamin E (tocopherol) is probably the most important lipid soluble antioxidant protecting membranes, lipids and lipoproteins (Vanbakel *et al.*, 2000). Some clinical trials have also reported the beneficial effects of vitamin E supplementation in the secondary prevention of cardiovascular events (Meydani, 2000). Driver and Georgeou (2003) proved that antioxidant compounds such as vitamin E, which scavenge oxygen radicals are associated with increased lifespan in fruit flies.

According to the results obtained in the present investigation (Table 5), both the oxidants (H_2O_2 and CCl_4) caused a decrease in the levels of vitamin E compared to the untreated control. In the flies exposed to H_2O_2, the level of vitamin E was found to be lower than the ones exposed to CCl_4. The decrease in the level of vitamin E was similar in the males and females exposed to the higher dose of H_2O_2.

TABLE V

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN E IN THE CONTROL AND TREATED *Drosophila melanogaster*

SEX	GROUP	VITAMIN E (µg / 30mg of flies)				
		WITHOUT OXIDANT	WITH H_2O_2		WITH CCl_4	
			LOW DOSE	HIGH DOSE	LOW DOSE	HIGH DOSE
Male	No extract	6.50 ± 0.12	3.30 ± 0.10	1.30 ± 0.01	3.41 ± 0.10	2.90 ± 0.30
	Triticum aestivum	4.76 ± 0.13	4.20 ± 0.00	2.69 ± 0.04	4.30 ± 0.11	3.40 ± 0.02
Female	No extract	3.46 ± 0.18	2.40 ± 0.29	1.20 ± 0.09	2.62 ± 0.05	2.50 ± 0.00
	Triticum aestivum	3.30 ± 0.06	2.91 ± 0.08	1.69 ± 0.07	3.11 ± 0.11	2.91 ± 0.08

The values are means ± SD of triplicates

The untreated control group showed a slightly elevated level of vitamin E than the group treated with the plant extract alone. The depletion of vitamin E was reverted to near normal levels by the co-treatment with the aqueous extract of *Triticum aestivum* leaves in the male flies exposed to the lower dose of H_2O_2 and CCl_4. There was a marked increase in the level of vitamin E in the CCl_4 treated group (at higher dose) but the levels did not reach the control values. The level of vitamin E was increased to some extent in the females exposed to the higher dose of H_2O_2, but were comparatively lower than the controls in the lower dose treated group. The level of vitamin E was in general higher in males than in the females.

Lower concentrations of vitamins E, C and GSH have been reported in the 7,12-dimethylbenz(a)anthracene (DMBA) induced hamster buccal pouch carcinoma. This was suggested to be due to their utilisation by the tumor tissues for their growth or to combat the deleterious effect of excessively generated lipid peroxides. Oral administration of *Clerodendron inerme* enhanced the antioxidant capacity by increasing the levels of GSH, which keeps the cellular level of vitamins C and E in their active forms (Manoharan *et al.*, 2006).

Sivalokanathan *et al.* (2006) reported decreased levels of vitamins C and E in N-nitrosodiethylamine induced hepatocellular carcinoma in rats. This altered level was recovered by the administration of an ethanolic extract of *Terminalia arjuna* bark.

Piperine, the major alkaloid present in *Piper nigrum* and *Piper longum*, significantly elevated the vitamin E level in the lung and liver of cancer bearing mice (Selvendiran *et al.*, 2003). Administration of grape

(*Vitis vinifera* L.) leaf extract significantly increased the non-enzymic antioxidant levels in the liver and kidney of alcohol administered rats (Pari and Suresh, 2008).

Oral administration of cyanobacterial phycoerythrin in CCl_4 intoxicated rats increased the level of vitamin E and vitamin C, and decreased the malondialdehydes, lipid hydroperoxides and conjugated dienes, indicating their protective effects against oxidative stress induced by CCl_4 (Soni *et al.*, 2008).

In agreement with these studies, our results also showed that the administration of *Triticum aestivum* leaf extract can restore the levels of vitamin E, which were depleted by the oxidant (H_2O_2 and CCl_4) treatment.

REDUCED GLUTATHIONE

Reduced glutathione (GSH) is an important antioxidant that limits oxidative damage caused by reactive oxygen species (ROS). The reduced glutathione level of the untreated control and the treated groups were analysed and the results are given in Table 6.

The level of reduced glutathione decreased in the flies exposed to H_2O_2 and CCl_4, compared to the control group. There was only a slight decrease in the GSH level in the male group treated with the lower dose of CCl_4. There was a marked decrease in the level of reduced glutathione in the females exposed to both the doses of H_2O_2. The lowest level of GSH was observed in the female flies exposed to the higher dose of CCl_4.

TABLE VI

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF REDUCED GLUTATHIONE IN THE CONTROL AND TREATED *Drosophila melanogaster*

SEX	GROUP	REDUCED GLUTATHIONE (nmoles / 30mg of flies)				
		WITHOUT OXIDANT	WITH H_2O_2		WITH CCl_4	
			LOW DOSE	HIGH DOSE	LOW DOSE	HIGH DOSE
Male	No extract	3.47 ± 0.33	2.91 ± 0.14	2.64 ± 0.10	3.26 ± 0.24	3.06 ± 0.08
	Triticum aestivum	3.37 ± 0.21	3.18 ± 0.00	2.93 ± 0.00	3.35 ± 0.00	3.19 ± 0.00
Female	No extract	2.90 ± 0.20	2.19 ± 0.63	1.80 ± 0.00	2.41 ± 0.49	2.17 ± 0.06
	Triticum aestivum	2.63 ± 0.32	2.40 ± 0.00	1.90 ± 0.00	2.58 ± 0.00	2.39 ± 0.00

The values are means ± SD of triplicates

There was no significant difference in the levels of GSH in the plant extract treated group. The levels were found to be increased on co-administration of plant extract along with the oxidants H_2O_2 and CCl_4. Reversal to near normal level was obtained in the group exposed to the lower dose of CCl_4 in the presence of the plant extract. There was a marked increase in GSH level in the males exposed to H_2O_2 upon co-administration of the plant extract. Females showed decreased levels of reduced glutathione when compared to males.

The biological antioxidant defense system involving superoxide dismutase, glutathione and catalase, showed a significant increase, with their levels close to the control with a decrease in the lipid peroxide content upon administration of *Desmodium gangeticum* extract and its phenolics in arthritic rats, indicating the antioxidant property of the extracts under arthritic condition (Govindarajan *et al.*, 2006).

Treatment of *Annona squamosa* extract significantly increased the level of GSH in diabetic rats (Kaleem *et al.*, 2006). The GPx activity and GSH content increased significantly upon supplementation with a combination of *Withania somnifera*, *Convolvulus pleuricauas* and *Aloe vera*, alleviating the neuronal injury induced by kainic acid in female Swiss albino mice (Parihar and Hemnani, 2003).

The decreased GSH content in the alloxan induced diabetic rats were increased by the aqueous extract of *Cassia fistula* Linn. flowers (Manonmani *et al.*, 2005). Pretreatment of rats with the ethanolic extract of *Zizipus*

mauritiana leaf protected against CCl_4-induced hepatic injury by maintaining the levels of glutathione and vitamin E (Dahiru *et al.*, 2005).

The levels of reduced glutathione were significantly decreased in Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice when compared to the control group. These levels were found to be increased on administration of a methanolic extract of *Mucuna puriens*. This study demonstrated that the extract increased the lifespan of EAC bearing mice and decreased lipid peroxidation and thereby augmented the endogenous antioxidants (Rajeshwar *et al.*, 2005).

Adult *Drosophila melanogaster* treated with different concentrations of $MgCl_2$ showed significant increase in the activities of SOD and CAT, as well as in the concentration of GSH (Matkovics *et al.*, 1997).

In light of these studies, the results of the present study show that the aqueous extract of *Triticum aestivum* helps in increasing the GSH levels in the male and female Drosophila exposed to H_2O_2 and CCl_4, indicating that GSH protects the system against oxidative stress.

Thus, the study clearly demonstrates that the exposure to oxidants (H_2O_2 and CCl_4) causes a significant depletion of the major antioxidant components in *Drosophila melanogaster*. Adminsitration of *Triticum aestivum* leaf extract restores the depleted levels of both enzymic and non-enzymic antioxidants. These results show that the leaves of *Triticum aestivum* exert a significant antioxidant action against oxidative stress induced by H_2O_2 and CCl_4 *in vivo*.

It can be concluded from the present study that *Drosophila melanogaster* can be effectively used as an *in vivo* model system to assess the effects of oxidative stress and their modulation by herbal extracts and their components. This study, thus, validates the use of the fruit fly in studies assessing the oxidant-antioxidant status. The study gains significance in that there are no studies reported earlier, validating *Drosophila melanogaster* as an *in vivo* model for studying antioxidant modulation.

The results obtained in this study are summarized and the conclusions drawn therein are presented in the next chapter.