Results

4. RESULTS

Reactive Oxygen Species (ROS) are universal products of aerobic metabolism. In eukaryotic cells, mitochondria are the main source of ROS. The main mitochondrial sites of ROS formation are electron carriers of respiratory chain. Reactive oxygen species can cause serious damage to many biological macromolecules such as lipids, nucleic acids and proteins, in which oxidation leads to a loss of their biological properties and eventually to cell death (Czarna and Jarmuszkiewicz, 2006).

Oxidative stress is an abnormal phenomenon occurring inside our cells and tissues, when the production of oxygen radicals exceeds their removal. Excess of free radicals damage essential macromolecules of the cell, leading to abnormal gene expression, disturbance in receptor activity, cell proliferation, immunity perturbation, mutagenesis and protein or lipofushin deposition (Favier, 2006). Reactive oxygen species play a crucial role in the physiological signal transduction and also in the pathogenesis of several human diseases such as atherosclerosis, neurodegenerative diseases, metabolic disorders, aging or cancer, amongst others (Kondoh *et al.*, 2007).

There are many pathological conditions that can be prevented or even be cured by the application of antioxidants. Food containing plenty of natural antioxidants is very important in the maintenance of health and in the prevention of many illnesses. According to nutrigenomics, the biologically active components of nutrition, including antioxidants, have an influence on the body in every single cell at all levels (Ratnam *et al.*, 2006).

Traditional medicine is an important part of human healthcare in many developing countries and also in developed countries, increasing their commercial value. Although the use of medicinal plants in therapy has been known for

centuries in all parts of the world, the demand for herbal medicine has grown dramatically in the last few decades (Kartal, 2006).

In the present study, the leaves of *Triticum aestivum* have been investigated for their antioxidant status, free radical quenching, biomolecule protective effects and their mechanism of action, using a battery of *in vitro* and *in vivo* systems. The results obtained in the four different phases of the study are presented in this chapter.

PHASE I

ANTIOXIDANT STATUS IN THE LEAVES OF *Triticum aestivum*

In the first step, the antioxidant status was assessed in the leaves of *Triticum aestivum* at three different stages of growth namely 4, 8 and 12 days after sowing (Plate 1) in order to ascertain whether any change in the antioxidant content was observed as the age of the plant increased.

ACTIVITIES OF ENZYMIC ANTIOXIDANTS IN *Triticum aestivum* LEAVES

The enzymic antioxidants analyzed in the 4th, 8th and 12th day plants of *Triticum aestivum* were superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione S-transferase (GST) and polyphenol oxidases (PPO). The activities obtained are presented in Table 1.

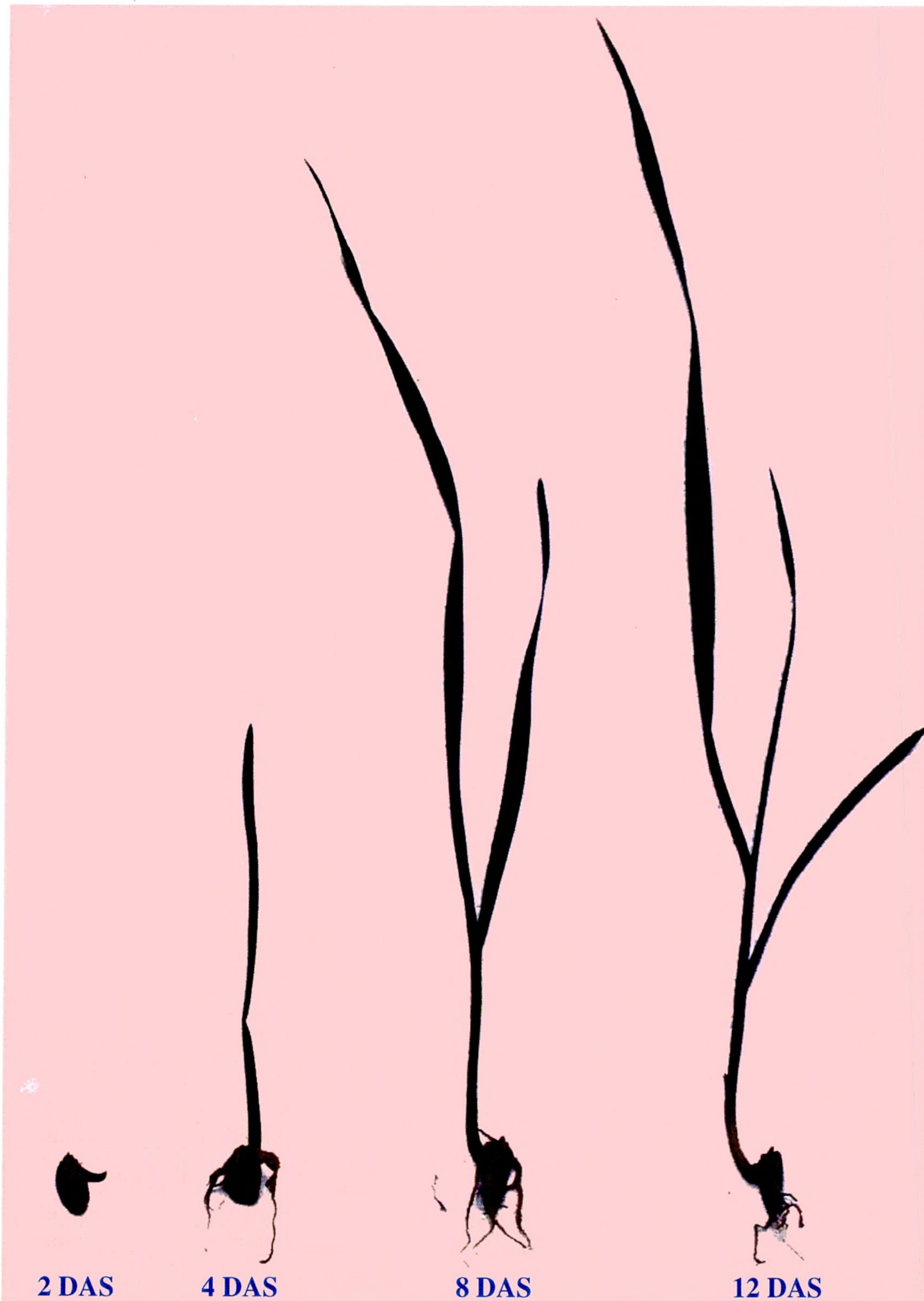


PLATE 1 : *Triticum aestivum* LEAVES AT THREE DIFFERENT STAGES OF GROWTH

TABLE 1

ACTIVITIES OF ENZYMIC ANTIOXIDANTS IN *Triticum aestivum* LEAVES

ENZYME	SAMPLE		
	4 DAS	**8 DAS**	**12 DAS**
SOD (U/g)$^{+}$	21.72 ± 1.30 ^{b}	18.32 ± 0.28 ^{a}	18.51 ± 0.53 ^{a}
CAT (U/g)*	942.50 ± 4.95 ^{b}	681.00 ± 1.41 ^{a,b}	568.00 ± 4.24 ^{a}
POD (U/g)$^{@}$	20.10 ± 1.60 ^{b}	14.80 ± 0.35 ^{a,b}	10.03 ± 0.24 ^{a}
GST (U/g)$^{\$}$	4.32 ± 0.67	4.30 ± 0.70	3.36 ± 0.62
PPO$^{\#}$ Catechol oxidase (U x 10^{-1}/g tissue)	2.80 ± 0.12 ^{b}	2.68 ± 0.04 ^{b}	2.37 ± 0.09 ^{a}
Laccase (U x 10^{-1}/g tissue)	2.55 ± 0.18	2.46 ± 0.06	2.26 ± 0.06

DAS – Days after sowing
Values are mean ± SD of triplicates

+ 1 Unit - Amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 minute
* 1 Unit - Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units
@ 1 Unit - Change in absorbance/minute at 430nm
$ 1 Unit - nmoles of CDNB conjugated/min
# 1 Unit - Amount of catechol oxidase/laccase which transforms 1 unit of dihydrophenol to quinine/minute
a - Statistically significant ($P<0.05$) compared to 4 DAS
b - Statistically significant ($P<0.05$) compared to 12 DAS

The leaves of *Triticum aestivum* possessed considerable activities of all the enzymic antioxidants studied, at all the different periods of growth selected. The leaves on the 4th day of growth was found to have maximum activity of the enzymic antioxidants studied, followed closely by the leaves on their 8th and 12th day of growth. The results showed that *Triticum aestivum* leaves, at their early stages of growth, are excellent sources of antioxidants.

LEVELS OF NON-ENZYMIC ANTIOXIDANTS IN *Triticum aestivum* LEAVES

The levels of non-enzymic antioxidants analyzed were ascorbate, tocopherol, total carotenoids, lycopene, flavonoids, reduced glutathione, total phenol and chlorophyll. The results obtained are presented in Table 2.

TABLE 2

LEVELS OF NON-ENZYMIC ANTIOXIDANTS IN *Triticum aestivum* LEAVES

PARAMETER	SAMPLE		
	4 DAS	8 DAS	12 DAS
Ascorbic acid (mg/g)	2.17 ± 0.07 [b]	1.15 ± 0.08 [a,b]	0.83 ± 0.04 [a]
Tocopherol (μg/g)	4.44 ± 0.07 [b]	3.70 ± 0.14 [a,b]	2.94 ± 0.12 [a]
Total carotenoids (mg/g)	17.92 ± 1.12 [b]	17.77 ± 0.88 [b]	12.89 ± 0.38 [a]
Lycopene (mg/g)	1.59 ± 0.72	1.01 ± 0.15	0.72 ± 0.06
Flavonoid (mg/g)	3.17 ± 0.10 [b]	2.89 ± 0.05 [a]	2.87 ± 0.10 [a]
Reduced glutathione (nmoles/g)	24.40 ± 0.98 [b]	22.97 ± 0.96	21.76 ± 0.24 [a]
Total phenol (mg/g)	0.27 ± 0.01 [b]	0.22 ± 0.02 [a,b]	0.17 ± 0.007 [a]
Total chlorophyll (mg/g)	0.70 ± 0.02 [b]	0.84 ± 0.03 [a,b]	1.07 ± 0.08 [a]

DAS – Days after sowing

Values are mean ± SD of triplicates

a - Statistically significant ($P<0.05$) compared to 4 DAS

b - Statistically significant ($P<0.05$) compared to 12 DAS

The levels of non-enzymic antioxidants such as ascorbic acid, tocopherol, reduced glutathione, total phenol, flavonoid, total carotenoid and lycopene were considerably higher in the 4th day leaf extract followed by the 8th and 12th day leaf extracts. The levels of chlorophyll were higher in the 12th day plant followed by the 8th and 4th day leaves.

PHASE II

In order to identify the nature of the active principle and the polarity into which maximum amount of antioxidants got extracted, the 4th, 8th and 12th day leaves of *Triticum aestivum* were serially extracted into solvents of increasing polarity (petroleum ether, benzene, ethyl acetate, methanol and water). The

extracts were then tested for their radical scavenging effects against a battery of oxidant moieties that included radicals like $SO^{\bullet}$, NO, DPPH and $OH^{\bullet}$, and a non-radical (H_2O_2).

RADICAL SCAVENGING EFFECTS

The different extracts of the 4th, 8th and 12th day leaves of *Triticum aestivum* were investigated for their effects on the *in vitro* generation of $SO^{\bullet}$ and NO. Figure 1 and 2 show the per cent inhibition of $SO^{\bullet}$ and NO radical generation *in vitro* by the leaves of *Triticum aestivum*.

The results of the study showed that the maximum extent of inhibition of $SO^{\bullet}$ and NO generation was mediated by the fresh juice and the methanolic extract.

The *Triticum aestivum* leaf extracts in the various solvents were tested for their radical scavenging effect against the stable free radical DPPH by a qualitative rapid dot plot assay.

The maximum DPPH quenching effect was mediated by the aqueous extract followed by methanolic and ethyl acetate extracts. The fresh aqueous extracts of 4th, 8th and 12th day leaves of *Triticum aestivum* also possessed strong DPPH scavenging ability as shown in Plate 2.

Since the results showed that the fresh juice (crude homogenate) of the leaf extracts showed the maximum inhibitory activity against various free radicals like $SO^{\bullet}$, NO and DPPH, only this extract was used in further studies. As a first step, this extract was tested against DPPH stable radicals in a quantitative photometric assay.

FIGURE 1

EFFECT OF *Triticum aestivum* LEAVES ON SO• GENERATION *in vitro*

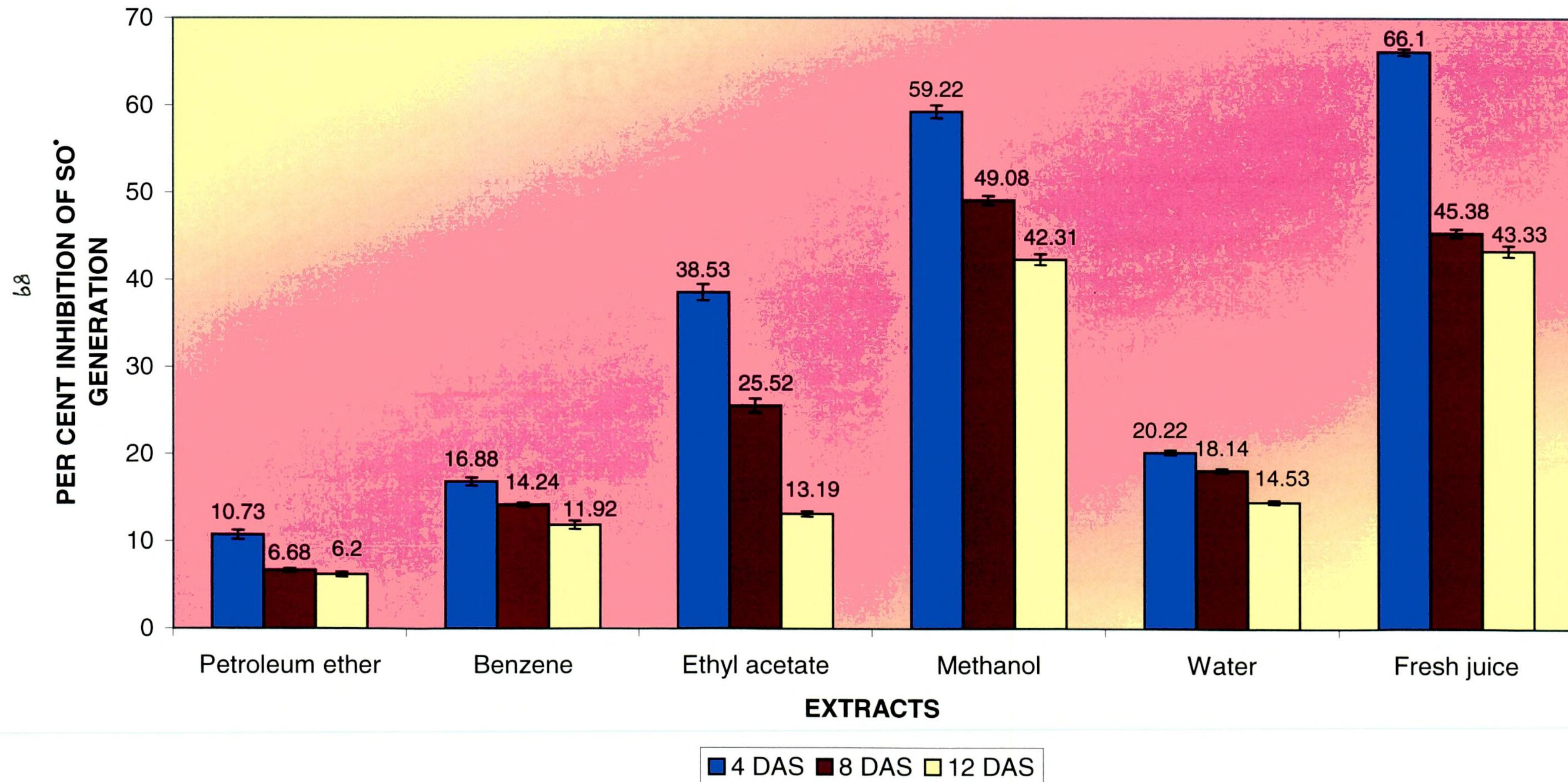
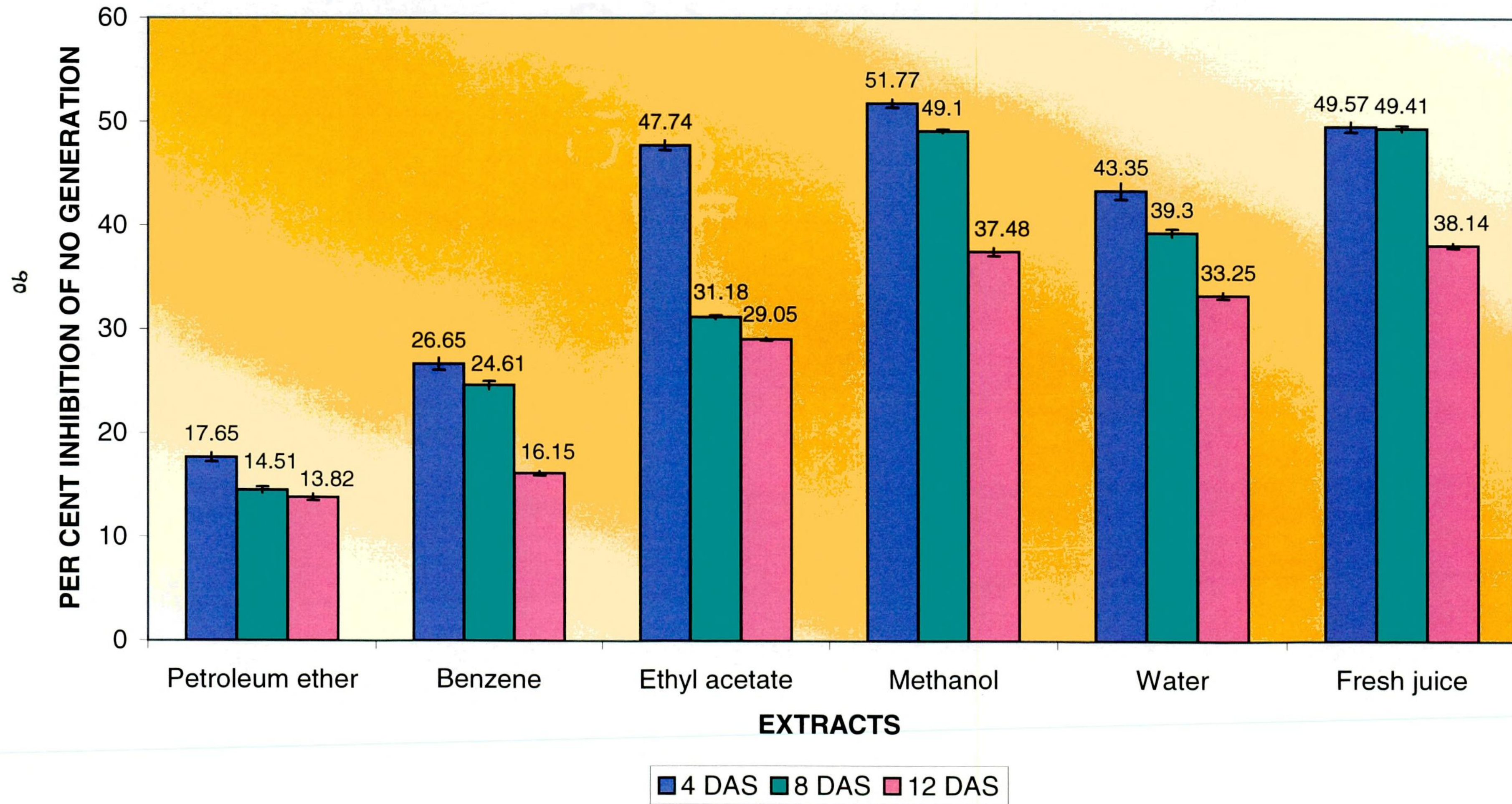
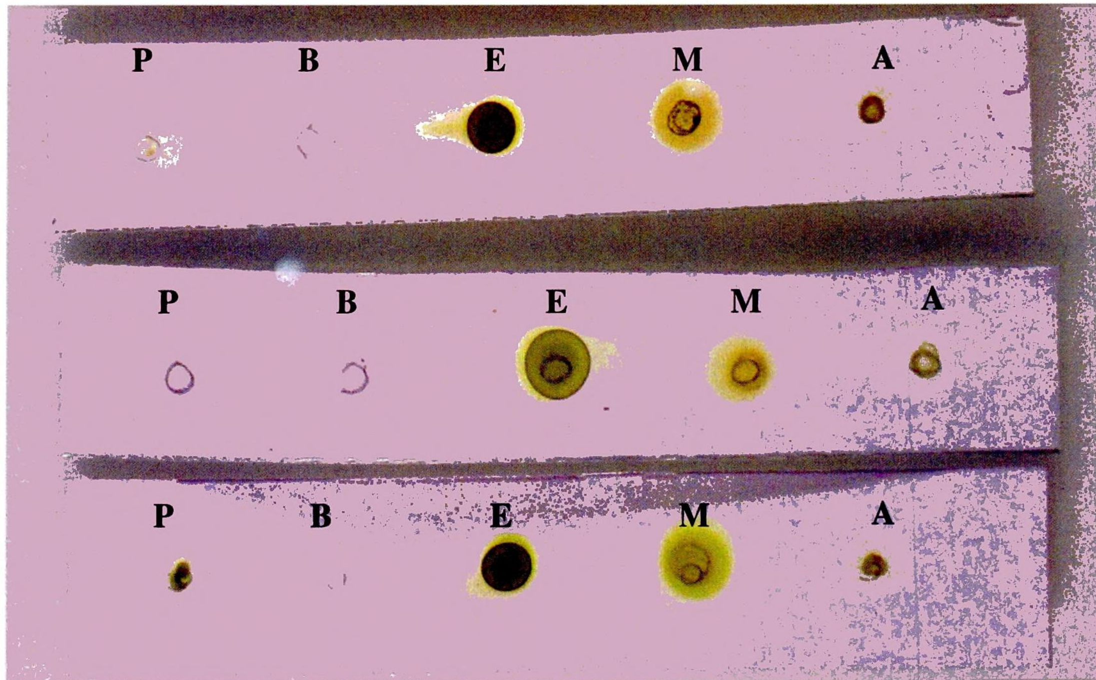


FIGURE 2
EFFECT OF *Triticum aestivum* LEAVES ON NITRIC OXIDE GENERATION *in vitro*





P – Petroleum ether
B – Benzene
E – Ethyl acetate
M – Methanol
A – Aqueous

FRESH JUICE

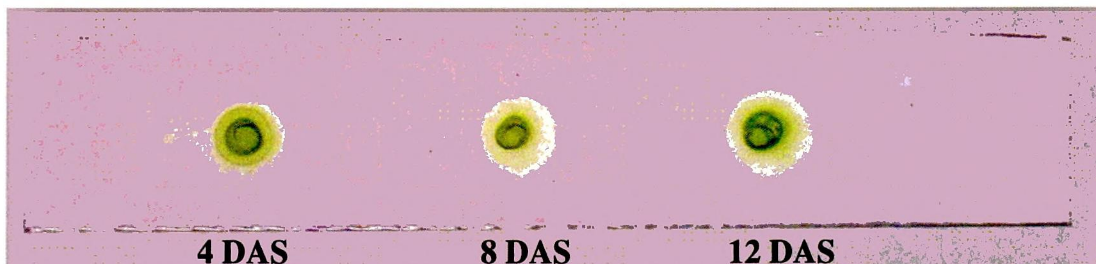


PLATE 2 : DPPH DOT PLOT ASSAY

The results obtained are presented in Figure 3.

The maximum extent of DPPH scavenging was observed by the aqueous extract of the 4th day leaves followed by the 8th and 12th day leaf extracts of *Triticum aestivum*.

The leaf extracts of the *Triticum aestivum* were also analyzed for their scavenging effects against hydroxyl radical. The amount of TBARS produced by H_2O_2 (standard oxidant) in the presence and the absence of the leaf extracts was studied. The amount of TBARS produced by H_2O_2 in the absence of the leaf extracts was fixed as 100% and the per cent extent of TBARS produced in the presence of leaf extracts were calculated. The results are presented in Figure 4.

The leaf extracts of *Triticum aestivum* were very effective in bringing down the TBARS levels, thereby decreasing the harmful effects of H_2O_2. From the values obtained, it can be deduced that the 4th day leaf extracts showed a better protection followed by the 8th and 12th day leaf extracts, against hydroxyl radical-induced damage to deoxyribose.

The aqueous extracts of the leaves of *Triticum aestivum* were then tested for their effect on the scavenging of H_2O_2. Figure 5 shows the per cent scavenging achieved with the various extracts.

The maximum scavenging of H_2O_2 was exhibited by the aqueous extract of the 4th day leaf of *Triticum aestivum*, followed closely by the 8th and 12th day leaf extracts.

FIGURE 3
EFFECT OF *Triticum aestivum* LEAVES ON DPPH RADICAL SCAVENGING ACTIVITY

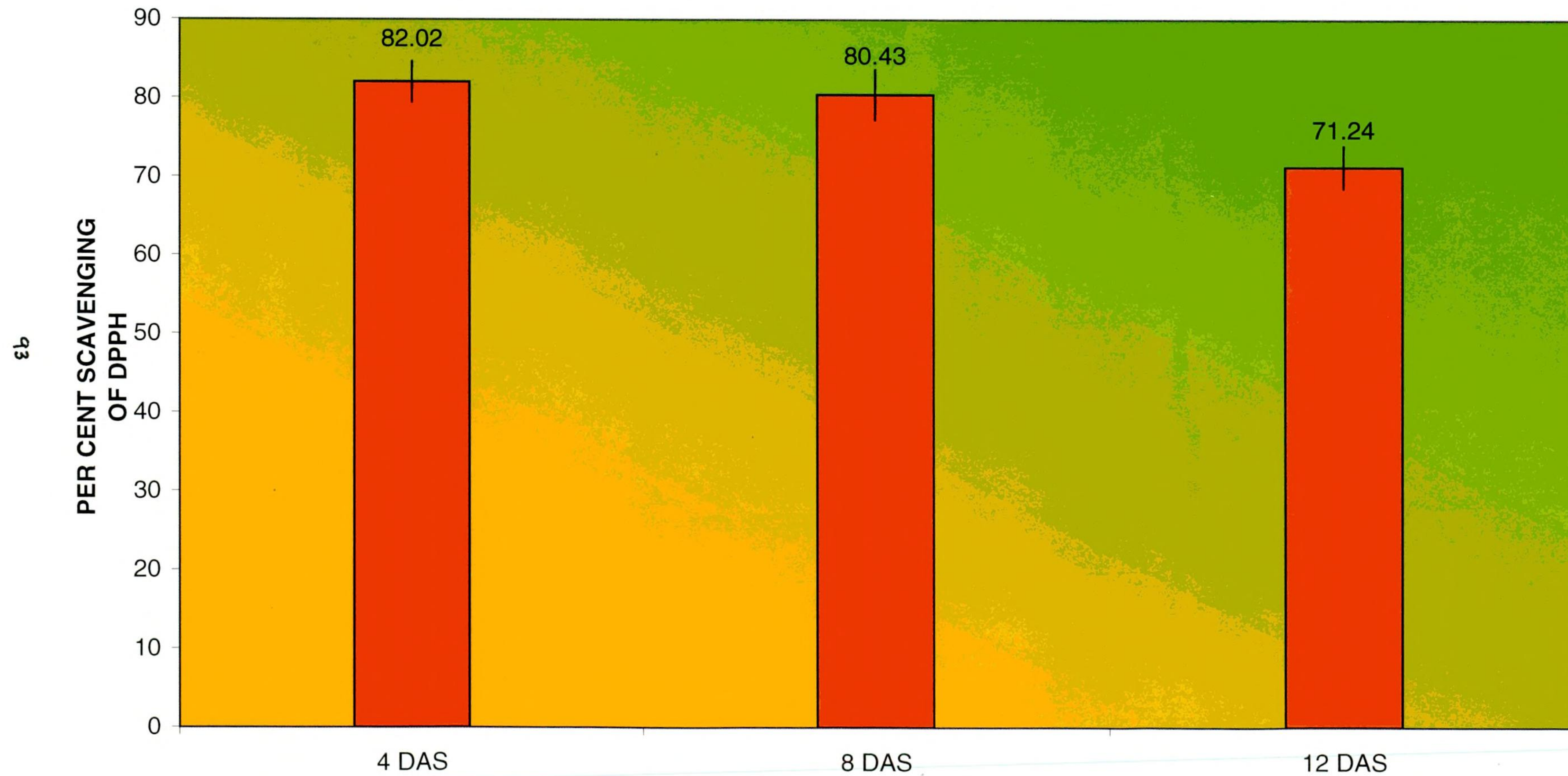


FIGURE 4
HYDROXYL RADICAL SCAVENGING ACTIVITY OF
***Triticum aestivum* LEAVES**

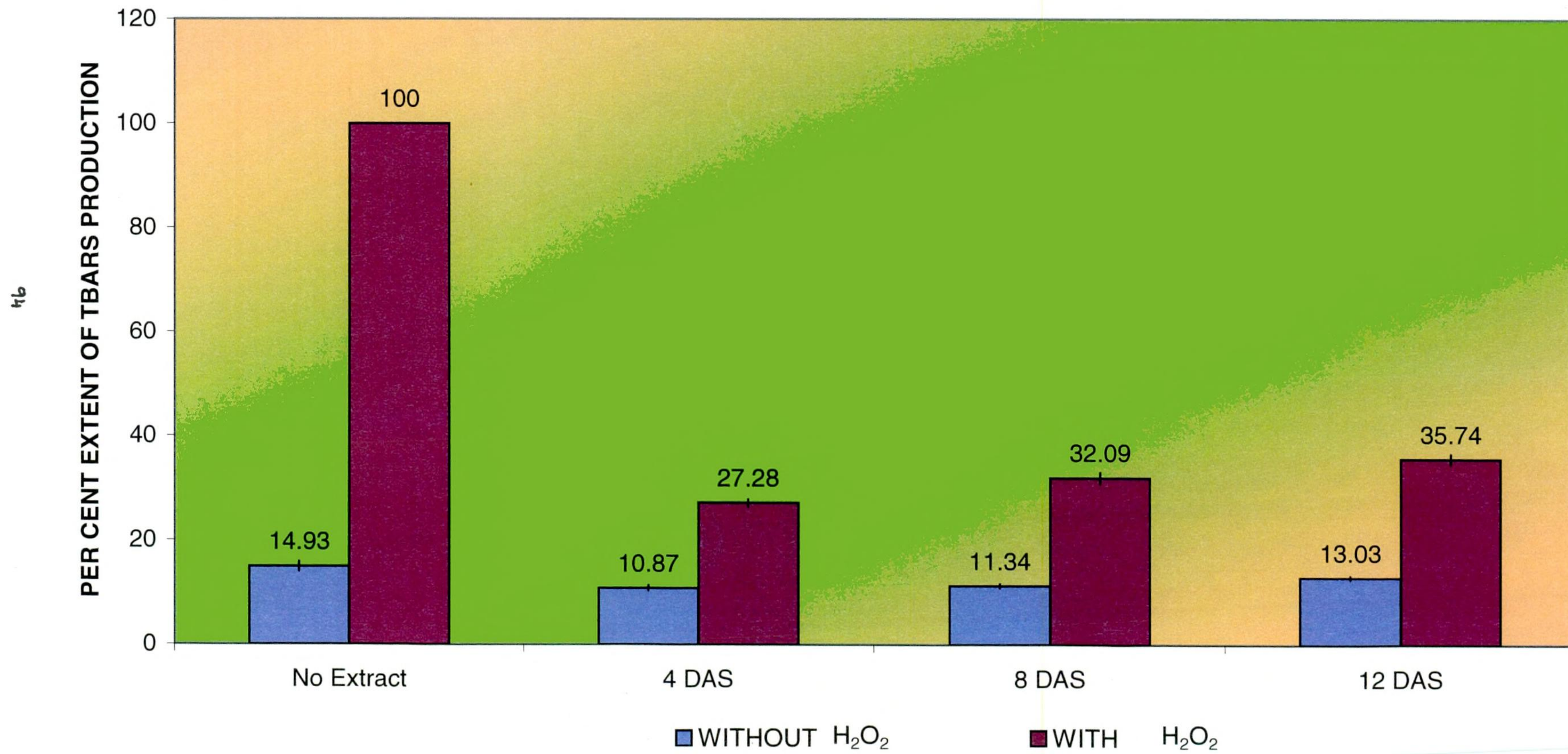
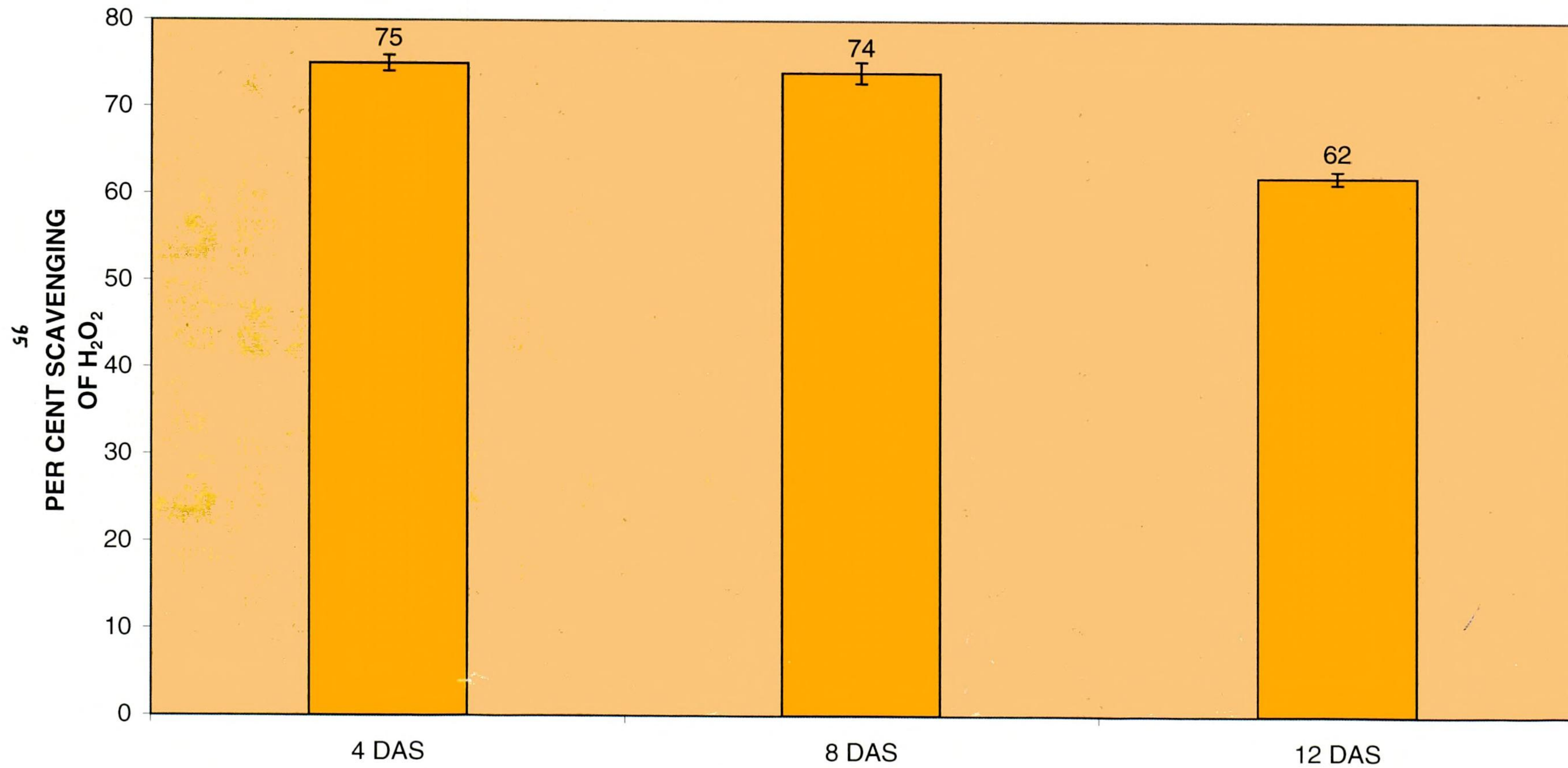


FIGURE 5

H_2O_2 SCAVENGING ACTIVITY OF *Triticum aestivum* LEAVES



EFFECT OF *Triticum aestivum* LEAVES ON OXIDATIVE DAMAGE TO BIOMOLECULES

Reactive oxygen species can mediate damage to cellular macromolecules. The primary targets of oxidative moieties are the lipid molecules, and the damage to lipids is manifested as lipid peroxidation (LPO). LPO is considered to be a major pathway by which ROS can cause tissue damage and alterations in cell membranes. Other factors affecting oxidative damage include the target molecules such as fatty acids, which are readily oxidized by ROS (Lee *et al.*, 2006b).

The ultimate targets of oxidative damage are the DNA molecules. Damage to DNA can manifest itself as mutations, leading to dire consequences. Oxidative deoxyribonucleic acid (DNA) damage from reactive oxygen species (ROS) is apparent in inflammation, malignant tumour, aging process and autoimmune diseases (Maeshima *et al.*, 2002).

Hence, in the present study, the extent of oxidative damage to lipids and DNA by standard oxidants and the effect of the *Triticum aestivum* leaves on this damage were investigated.

EFFECT OF *Triticum aestivum* LEAVES ON OXIDATIVE DAMAGE TO MEMBRANE LIPIDS

The extent of lipid peroxidation and the effects of *Triticum aestivum* leaf extracts were studied in three different membrane models. The first system comprised RBC ghosts, prepared by hypotonic lysis of RBCs which constitutes plasma membrane lipids. The second system used was goat liver homogenate which constituted a combination of lipids comprising plasma membrane and the intracellular membrane. The third system constituted goat liver slices which consisted predominantly intact cells. These different membrane preparations were

used in order to test whether the nature of the membrane lipids influenced the effects of the leaf extracts.

Lipid peroxidation (LPO) was induced in all these systems *in vitro* and the extent of inhibition of the LPO was studied in the presence of the leaf extracts. The per cent inhibition of *in vitro* LPO by leaf extracts in all the three membrane systems is presented in Figure 6.

The leaf extracts of *Triticum aestivum* inhibited LPO to considerable extent in all the three membrane preparations studied. The extent of inhibition of LPO was greater in the liver homogenate, followed by the RBC ghosts. The fourth day leaf extract of *Triticum aestivum* exhibited the maximum inhibition of LPO in all the membrane preparations.

EFFECT OF *Triticum aestivum* LEAVES ON OXIDATIVE DAMAGE TO DNA

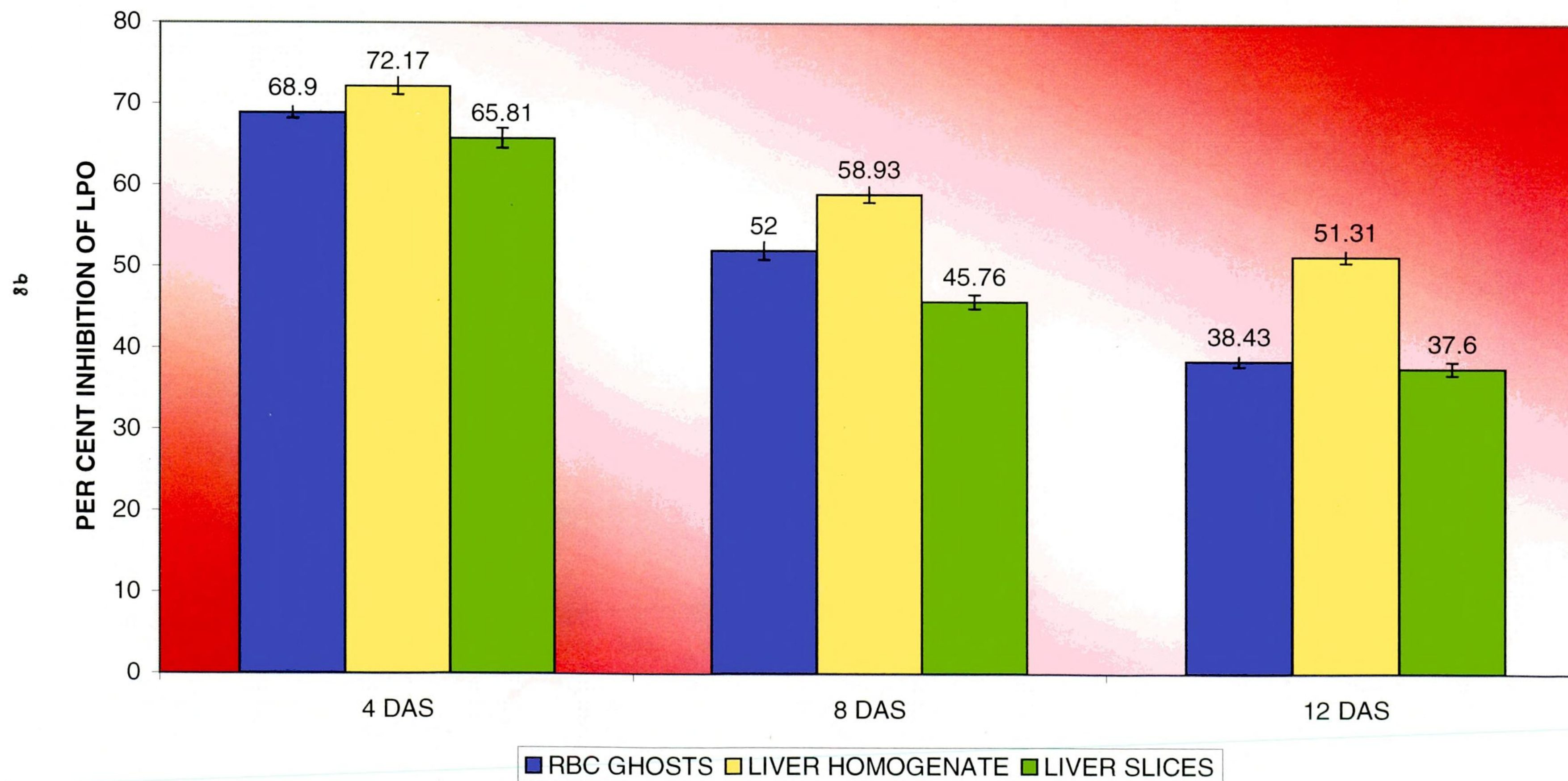
The extent of oxidative damage caused by H_2O_2 to DNA and the possible protection rendered by the leaf extracts of *Triticum aestivum* were followed in pure DNA preparations and in intact cells. Different commercially available sources of DNA falling into different hierarchies of evolution were used as pure DNA preparations. The DNA samples used were λ DNA (linear, phage), pUC18 (plasmid, circular, bacterial) and herring sperm (haploid, high molecular weight, eukaryotic) DNA.

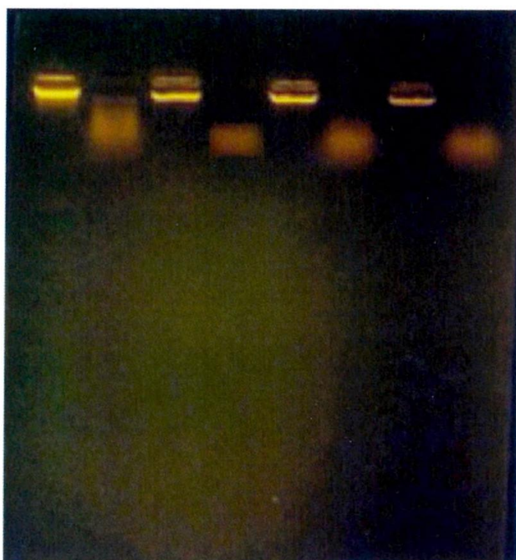
EFFECT OF *Triticum aestivum* LEAVES ON DNA DAMAGE INDUCED BY H_2O_2 TO λ DNA AND pUC18 DNA

The extent of damage to λ and pUC18 DNA by the exposure to H_2O_2 *in vitro* in the presence and the absence of leaf extracts of *Triticum aestivum* was analyzed as the differences in their migratory pattern under the influence of an electric field in a gel. The patterns of migration are presented in Plate 3.

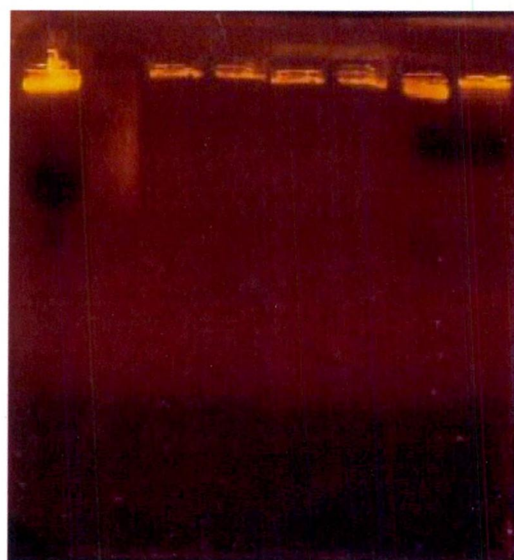
FIGURE 6

EFFECT OF *Triticum aestivum* LEAVES ON LIPID PEROXIDATION IN DIFFERENT MEMBRANE PREPARATIONS





pUC18 DNA



λ DNA

Lane 1 – Control

Lane 2 – H_2O_2

Lane 3 – 4th day leaf extract of *Triticum aestivum*

Lane 4 – H_2O_2 + 4th day leaf extract of *Triticum aestivum*

Lane 5 – 8th day leaf extract of *Triticum aestivum*

Lane 6 – H_2O_2 + 8th day leaf extract of *Triticum aestivum*

Lane 7 - 12th day leaf extract of *Triticum aestivum*

Lane 8 - H_2O_2 + 12th day leaf extract of *Triticum aestivum*

PLATE 3 : MIGRATION PATTERNS OF pUC18 AND λ DNA TREATED WITH H_2O_2 WITH AND WITHOUT THE LEAF EXTRACTS

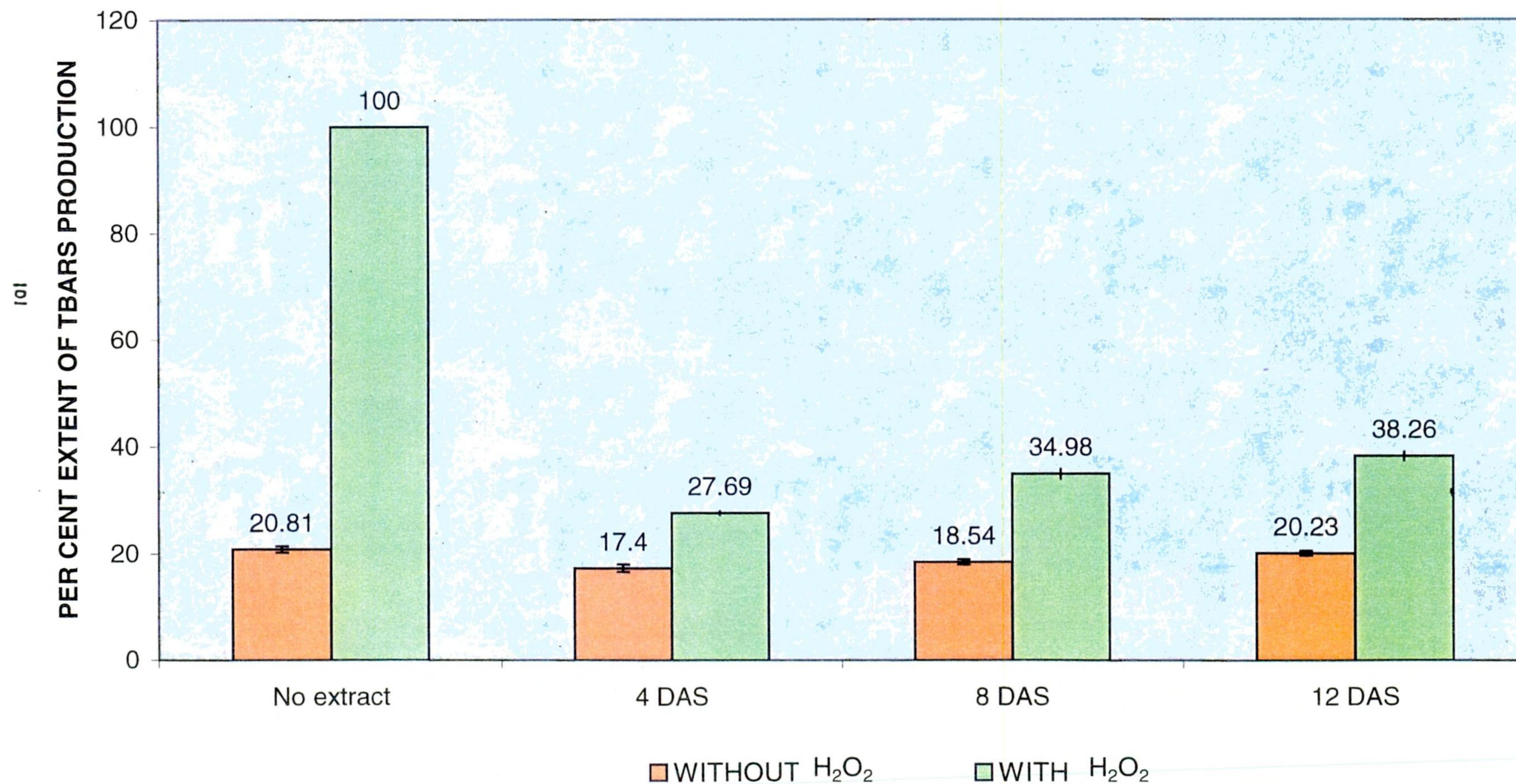
From the migration patterns obtained, it can be deduced that H_2O_2 caused damage to both λ DNA and pUC18 DNA, as reflected by the absence of a specific band in H_2O_2 treated DNA (Lane 2). The leaf extracts of *Triticum aestivum* significantly reduced the damage caused by H_2O_2 to λ DNA (Lanes 4, 6, and 8). However the reversal was not observed in the case of pUC18 DNA. *Triticum aestivum* leaves, by themselves, did not cause any DNA damage as evident from the intact DNA bands (Lanes 3, 5 and 7).

EFFECT OF *Triticum aestivum* LEAVES ON DAMAGE INDUCED IN HERRING SPERM DNA

The extent of damage to herring sperm DNA was quantified spectrophotometrically and the values are presented in Figure 7.

The extracts of *Triticum aestivum* leaves, at the 4th and 8th days of growth, decreased the extent of DNA damage even in the control group (not exposed to H_2O_2). However, the extent of DNA damage in the control group was not influenced by the 12th day leaf extract. All the three extracts, however, caused a highly significant decrease of the damage inflicted by H_2O_2 on herring sperm DNA. The effect of the 4th day extract of *Triticum aestivum* was more pronounced than the 8th and 12th day leaf extracts.

FIGURE 7
EFFECT OF *Triticum aestivum* LEAVES ON OXIDATIVE DAMAGE INDUCED IN HERRING SPERM DNA



EFFECT OF *Triticum aestivum* LEAVES ON DNA DAMAGE INDUCED BY H_2O_2 IN INTACT CELLS

The DNA damaging effect of H_2O_2 and the possible protection rendered by the extracts of *Triticum aestivum* leaves at different stages of growth were studied by following the formation of comets in intact cells. The cells employed for the study were Hep2 (laryngeal carcinoma) and KB (oral carcinoma) cells. The number of cells exhibiting DNA damage (presence of comet) per 100 cells counted in each group and is presented in Table 3.

TABLE 3

EFFECT OF *Triticum aestivum* LEAVES ON H_2O_2-INDUCED DNA DAMAGE IN Hep2 AND KB CELLS

SAMPLE	NUMBER OF COMETS PER 100 Hep2 CELLS		NUMBER OF COMETS PER 100 KB CELLS	
	WITHOUT H_2O_2	WITH H_2O_2	WITHOUT H_2O_2	WITH H_2O_2
No extract	11 ± 1	29 ± 2	12 ± 1	34 ± 2
4 DAS	6 ± 2 [a]	10 ± 1 [b,c]	8 ± 2 [a]	12 ± 1 [b,c]
8 DAS	8 ± 2	11 ± 1 [b,d]	10 ± 1	12 ± 1 [b,d]
12 DAS	9 ± 3	13 ± 2 [d]	11 ± 1 [d]	14 ± 1 [d]
	LSD (5%) = 3.314		LSD (5%) = 2.290	

DAS – Days After Sowing

Values are mean ± SD of triplicates

a - Statistically significant ($P<0.05$) compared to untreated control group

b - Statistically significant ($P<0.05$) compared to H_2O_2 treated group

c - Statistically significant ($P<0.05$) compared to respective plant group

d - Statistically significant ($P<0.05$) compared to corresponding 4th day extract treated group

H_2O_2 exposure caused a significant ($P<0.05$) increase in the number of cells with comets in both Hep2 and KB cells. The extracts of *Triticum aestivum* leaves by themselves lowered the incidence of comets with reference to untreated controls. The co-treatment of *Triticum aestivum* leaf extracts with H_2O_2 significantly decreased the number of cells expressing DNA damage. The 4th and

8th day leaf extracts of *Triticum aestivum* were very effective in completely reverting the DNA damage as evidenced by the comet cells reaching control levels in both Hep2 and KB cells.

EFFECT OF *Triticum aestivum* LEAVES ON THE VIABILITY OF Hep2 AND KB CELLS

The ultimate effect of oxidant damage to cells results in cell death. The extent of cytotoxicity of H_2O_2 in the presence and in the absence of the leaf extracts of *Triticum aestivum* was studied in Hep2 and KB cells. The MTT dye reduction assay is an accurate measure of the extent of cell death (Hazra *et al.*, 2007) which was carried out to assess cytotoxicity. The values obtained are presented in Figure 8.

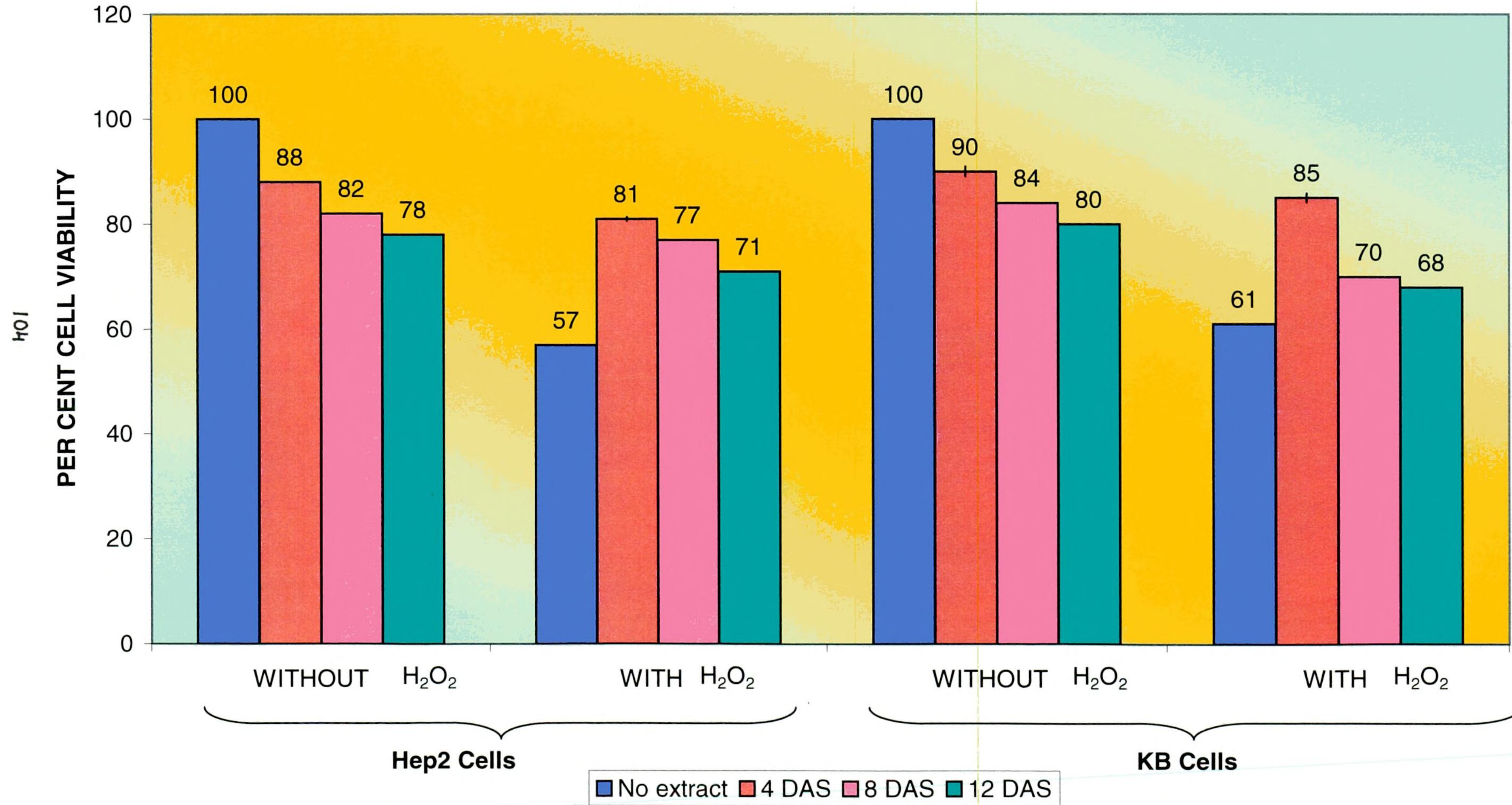
H_2O_2 exposure was highly cytotoxic to both Hep2 and KB cells. The presence of the *Triticum aestivum* leaf extracts also caused death in both cells and it is evident from the figure that they bring down the toxic effect of the oxidant effectively, as indicated by the marked increase in the viable cells. The absorbance of formazon formed in the control group was fixed as 100% viability and the per cent viability for other groups calculated relative to this.

ANTIOXIDANT POTENTIAL OF *Triticum aestivum* LEAVES IN LIVER SLICES

Having ascertained the protective effect of *Triticum aestivum* leaf extracts against oxidative damage in cell-free systems and in intact cells, the effect of the leaf extracts on the oxidative stress induced in cells in the organ was studied.

Precision-cut goat liver slices were exposed to the standard oxidant H_2O_2 in the presence and absence of *Triticum aestivum* leaf extracts. The enzymic and non-enzymic antioxidants were then analyzed in the liver slices and the results are presented below.

FIGURE 8
EFFECT OF *Triticum aestivum* LEAVES ON THE VIABILITY OF Hep2 AND KB CELLS



ENZYMIC ANTIOXIDANTS

The enzymic antioxidants analyzed were SOD, CAT, POD, GR and GST.

SOD

Table 4 depicts the SOD activity in H_2O_2 treated goat liver slices in the presence and absence of *Triticum aestivum* leaf extracts. Superoxide dismutase plays an important role in the defense against free radical damage (Hu *et al.*, 2007). H_2O_2 exposure caused a significant (P<0.05) decrease in the activity of SOD. The administration of the 4th, 8th and 12th day extracts of *Triticum aestivum* leaves caused a significant (P<0.05) increase in the SOD activity compared to untreated control. Also, the treatment of these extracts along with H_2O_2 caused a significant elevation in the SOD activity. The effect of the 4th day extract was more pronounced than the 8th and 12th day leaf extracts.

TABLE 4

EFFECT OF *Triticum aestivum* LEAVES ON SOD ACTIVITY IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	SOD ACTIVITY (UNITS/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	18.81 ± 0.09	13.18 ± 0.74 ^{a}
4 DAS	24.29 ± 0.22 ^{a}	22.96 ± 0.84 ^{a,b,c}
8 DAS	22.37 ± 0.36 ^{a,d}	22.18 ± 0.02 ^{a,b,d}
12 DAS	21.62 ± 0.65 ^{a,d}	20.44 ± 0.11 ^{a,b,c,d}

LSD (5%) - 0.566

DAS – Days After Sowing

Values are mean ± SD (n=6)

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

a - Statistically significant (P<0.05) compared to untreated control group

b - Statistically significant (P<0.05) compared to H_2O_2 treated group

c - Statistically significant (P<0.05) compared to respective plant group

d - Statistically significant (P<0.05) compared to corresponding 4th day extract treated group

CAT

Catalase catalyses the decomposition of H_2O_2 to molecular oxygen and water, thereby protecting cells from the toxic effects of H_2O_2 (Hua *et al.*, 2007). The effect of *Triticum aestivum* leaf extracts on the CAT activity in goat liver slices exposed *in vitro* to the oxidant is expressed in Table 5. The catalase activity in the H_2O_2 treated liver slices decreased significantly ($P<0.05$) compared to the untreated control. The toxic effect of H_2O_2 was negated by the concordant treatment with *Triticum aestivum* leaf extracts. *Triticum aestivum* leaves, by themselves, significantly ($P<0.05$) increased the CAT activity compared to control.

TABLE 5

EFFECT OF *Triticum aestivum* LEAVES ON CAT ACTIVITY IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	CAT ACTIVITY (UNITS/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	500.00 ± 1.50	295.43 ± 0.99 [a]
4 DAS	970.00 ± 3.13 [a]	850.00 ± 0.47 [a,b,c]
8 DAS	871.85 ±0.46 [a,d]	680.00 ± 0.78 [a,b,c,d]
12 DAS	566.60 ± 0.88 [a,d]	485.60 ± 0.86 [a,b,c,d]

LSD (5%) - 1.629

DAS – Days After Sowing

Values are mean ± SD (n=6)

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

a - Statistically significant ($P<0.05$) compared to untreated control group

b - Statistically significant ($P<0.05$) compared to H_2O_2 treated group

c - Statistically significant ($P<0.05$) compared to respective plant group

d - Statistically significant ($P<0.05$) compared to corresponding 4th day extract treated group

POD

The peroxidase activities in the H_2O_2 treated goat liver slices in the presence and absence of *Triticum aestivum* leaf extracts are presented in Table 6.

The activity of peroxidase decreased significantly (P<0.05) upon exposure to H_2O_2. Treatment with the leaf extracts of *Triticum aestivum* caused a significant increase (P<0.05) in the peroxidase activity compared to control. The extracts were very effective in reverting back the decreased peroxidase activity caused by H_2O_2 to a significant extent.

TABLE 6

EFFECT OF *Triticum aestivum* LEAVES ON POD ACTIVITY IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	POD ACTIVITY (UNITS/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	0.68 ± 0.03	0.28 ± 0.01^{a}
4 DAS	1.12 ± 0.08^{a}	$0.96 \pm 0.02^{a,b,c}$
8 DAS	$1.04 \pm 0.01^{a,d}$	$0.80 \pm 0.001^{a,b,c,d}$
12 DAS	$0.88 \pm 0.002^{a,d}$	$0.76 \pm 0.03^{a,b,c,d}$

LSD (5%) - 0.041
DAS – Days After Sowing
Values are mean ± SD (n=6)

1 Unit - Change in absorbance at 430nm/minute
a - Statistically significant (P<0.05) compared to untreated control group
b - Statistically significant (P<0.05) compared to H_2O_2 treated group
c - Statistically significant (P<0.05) compared to respective plant group
d - Statistically significant (P<0.05) compared to corresponding 4th day extract treated group

GST

The detoxification by the GST system forms one of the most important defense mechanisms. GSTs maximize the conjugation of free radicals and various lipid hydroperoxides to GSH to form water soluble products that can be easily excreted out (van Haaften *et al.*, 2003). Table 7 represents the activity of GST in goat liver slices exposed to H_2O_2 in the presence and absence of *Triticum aestivum* leaf extracts.

The activity of GST decreased significantly upon H_2O_2 assault. The leaf extracts of *Triticum aestivum* augmented the GST activities in the oxidatively stressed groups than the control values.

TABLE 7

EFFECT OF *Triticum aestivum* LEAVES ON GST ACTIVITY IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	GST ACTIVITY (UNITS/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	0.028 ± 0.003	0.012 ± 0.001 [a]
4 DAS	0.048 ± 0.003 [a]	0.038 ± 0.001 [a,b,c]
8 DAS	0.040 ± 0.002 [a,d]	0.034 ± 0.002 [a,b,c,d]
12 DAS	0.036 ± 0.001 [a,d]	0.030 ± 0.002 [b,c,d]

LSD (5%) - 0.002
DAS – Days After Sowing
Values are mean ± SD (n=6)

1 Unit - nmoles of CDNB conjugated/minute
a - Statistically significant ($P<0.05$) compared to untreated control group
b - Statistically significant ($P<0.05$) compared to H_2O_2 treated group
c - Statistically significant ($P<0.05$) compared to respective plant group
d - Statistically significant ($P<0.05$) compared to corresponding 4th day extract treated group

GR

GR is an important enzyme responsible for replenishing reduced glutathione, thus steadily providing GSH for both direct antioxidant action as well as a substrate for antioxidant (GPx) and conjugation (GST) reactions (Rogers *et al.*, 2004). The activities of GR were recorded in the liver slices exposed to H_2O_2 in the presence and the absence of *Triticum aestivum* leaf extracts and are presented in Table 8.

The co-treatment of the liver slices with *Triticum aestivum* leaf extracts and H_2O_2 caused a significant ($P<0.05$) increase in the GR activity, compared to untreated control as well as H_2O_2 treated group. This increase in GR activity was lower than the activities of GR observed in their respective leaf extracts alone

treated groups. The leaf extracts were effective in alleviating the toxicity of H_2O_2 in goat liver slices.

TABLE 8

EFFECT OF *Triticum aestivum* LEAVES ON GR ACTIVITY IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	GR ACTIVITY (UNITS/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	61.59 ± 0.89	45.63 ± 0.87 [a]
4 DAS	171.55 ± 1.39 [a]	165.05 ± 1.94 [a,b,c]
8 DAS	170.28 ± 1.76 [a]	160.80 ± 1.42 [a,b,c,d]
12 DAS	141.52 ± 1.13 [a,d]	135.63 ± 0.95 [a,b,c,d]

LSD (5%) - 1.575

DAS – Days After Sowing

Values are mean ± SD (n=6)

1 Unit - µmoles of NADPH oxidized/minute

a - Statistically significant ($P<0.05$) compared to untreated control group

b - Statistically significant ($P<0.05$) compared to H_2O_2 treated group

c - Statistically significant ($P<0.05$) compared to respective plant group

d - Statistically significant ($P<0.05$) compared to corresponding 4th day extract treated group

NON-ENZYMIC ANTIOXIDANTS

The antioxidant system of a cell is comprised of both enzymic and non-enzymic antioxidants. In the present study, the levels of vitamins C, E and A and reduced glutathione were estimated in the liver slices exposed to H_2O_2 in the presence or the absence of *Triticum aestivum* leaf extracts. The results obtained are given below.

VITAMIN C

Table 9 depicts the effect of *Triticum aestivum* leaf extracts on the levels of vitamin C in the presence of oxidant *in vitro*. The exposure of liver slices to the oxidant and the *Triticum aestivum* leaf extracts caused a very steep increase in the vitamin C levels compared to untreated control and H_2O_2 alone treated group. The

increase in the levels of vitamin C was maximally rendered by the 4th day leaf extract followed by the 8th and 12th day plants, whereas H_2O_2 treatment significantly (P<0.05) depleted the levels of vitamin C.

TABLE 9

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN C IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	VITAMIN C LEVEL (mg/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	0.313 ± 0.002	0.250 ± 0.004 [a]
4 DAS	0.409 ± 0.005 [a]	0.377 ± 0.001 [a,b,c]
8 DAS	0.389 ± 0.001 [a,d]	0.365 ± 0.021 [a,b,c,d]
12 DAS	0.353 ± 0.003 [a,d]	0.341 ± 0.006 [a,b,c,d]

LSD (5%) = 0.010
DAS – Days After Sowing
Values are mean ± SD (n=6)

a - Statistically significant (P<0.05) compared to untreated control group
b - Statistically significant (P<0.05) compared to H_2O_2 treated group
c - Statistically significant (P<0.05) compared to respective plant group
d - Statistically significant (P<0.05) compared to corresponding 4th day extract treated group

VITAMIN E

Vitamin E, a major lipid soluble antioxidant, is the most effective chain breaking antioxidant within the cell membrane, where it protects membrane functions from lipid peroxidation (Griffiths *et al.*, 2002). The levels of vitamin E observed in different treatment groups are tabulated in Table 10.

The levels of vitamin E in the liver slices after exposure to the leaf extracts showed a significant (P<0.05) increase, compared to untreated control. The levels of vitamin E were found to be higher in the 4th day extract treated group. The oxidant (H_2O_2) treatment caused a significant decrease in the levels of vitamin E

compared to untreated control. This depletion was counteracted by the leaf extracts of *Triticum aestivum*.

TABLE 10

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN E IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	VITAMIN E LEVEL (μg/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	9.28 ± 0.05	4.64 ± 0.08 [a]
4 DAS	22.42 ± 0.13 [a]	17.40 ± 1.07 [a,b,c]
8 DAS	20.49 ± 0.07 [a,d]	16.24 ± 0.89 [a,b,c,d]
12 DAS	19.72 ± 0.37 [a,d]	13.92 ± 0.47 [a,b,c,d]

LSD (5%) = 0.632

DAS – Days After Sowing

Values are mean ± SD (n=6)

a - Statistically significant ($P<0.05$) compared to untreated control group
b - Statistically significant ($P<0.05$) compared to H_2O_2 treated group
c - Statistically significant ($P<0.05$) compared to respective plant group
d - Statistically significant ($P<0.05$) compared to corresponding 4th day extract treated group

VITAMIN A

The levels of vitamin A in the liver slices exposed to H_2O_2 in the presence and absence of *Triticum aestivum* leaf extracts are represented in Table 11.

H_2O_2 treatment caused a significant depletion ($P<0.05$) in the levels of vitamin A compared to untreated control. The *Triticum aestivum* leaf extracts treatment was found to be effective in mitigating the toxicity caused by H_2O_2. The exposure of the liver slices to *Triticum aestivum* leaf extracts showed a significant ($P<0.05$) increase in the vitamin A levels.

TABLE 11

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN A IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	VITAMIN A LEVEL (µg/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	99.56 ± 1.84	62.04 ± 1.86 [a]
4 DAS	157.46 ± 3.17 [a]	133.46 ± 1.25 [a,b,c]
8 DAS	141.43 ± 2.26 [a,d]	125.86 ± 1.97 [a,b,c,d]
12 DAS	117.04 ± 1.88 [a,d]	104.40 ± 0.83 [a,b,c,d]

LSD (5%) - 2.324
DAS – Days After Sowing
Values are mean ± SD (n=6)

a - Statistically significant ($P<0.05$) compared to untreated control group
b - Statistically significant ($P<0.05$) compared to H_2O_2 treated group
c - Statistically significant ($P<0.05$) compared to respective plant group
d - Statistically significant ($P<0.05$) compared to corresponding 4th day extract treated group

GSH

The levels of reduced glutathione in the oxidant treated liver slices and the effect of the *Triticum aestivum* leaf extracts on it were analyzed and the results are tabulated in Table 12.

The oxidant (H_2O_2) treated liver slices caused a significant ($P<0.05$) decrease in the levels of reduced glutathione compared to untreated control. The depleting effect of H_2O_2 treatment was reverted back by the co-treatment with the leaf extracts of *Triticum aestivum*. The leaf extract treatments caused a significant ($P<0.05$) increase in the GSH levels, compared to untreated control, with the 4th day leaf extract showing maximum effect.

TABLE 12

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF REDUCED GLUTATHIONE IN H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	GSH LEVEL (mg/g TISSUE)	
	WITHOUT H_2O_2	WITH H_2O_2
No extract	174.40 ± 1.43	129.60 ± 0.31 [a]
4 DAS	308.00 ± 0.21 [a]	278.40 ± 0.16 [a,b,c]
8 DAS	292.80 ± 0.76 [a,d]	263.20 ± 0.05 [a,b,c,d]
12 DAS	204.00 ± 0.05 [a,d]	248.80 ± 0.55 [a,b,c,d]

LSD (5%) - 0.727

DAS – Days After Sowing

Values are mean ± SD (n=6)

a - Statistically significant ($P<0.05$) compared to untreated control group
b - Statistically significant ($P<0.05$) compared to H_2O_2 treated group
c - Statistically significant ($P<0.05$) compared to respective plant group
d - Statistically significant ($P<0.05$) compared to corresponding 4th day extract treated group

The results of the second phase of the study showed that the leaves of *Triticum aestivum* exhibited strong antioxidative effect *in vitro*. In order to confirm whether the effects observed *in vitro* did indeed occur also *in vivo* the third phase of the study was executed.

PHASE III

Phase III was the confirmatory phase, in which the antioxidant potential of the *Triticum aestivum* leaf extracts was evaluated under *in vivo* conditions. *In vivo* assessment in experimental animals provide the most physiological relevant test system and gives information that relates the effects observed under *in vitro* conditions (Tingle and Helsby, 2006).

Male Wistar rats were used as experimental models. Oxidative stress was induced in rats by a combination of ethanol and CCl_4 (as described in the previous chapter). The protective effects of the fresh extracts of *Triticum aestivum* leaves were investigated against the ethanol-CCl_4 induced oxidative stress. The effects elicited were also compared to those induced by the standard antioxidant

silymarin. Following the treatment period, the antioxidant status in the liver and kidney was evaluated. The activities of serum marker enzymes and the circulating lipid profile were also analyzed.

ASSESSMENT OF LIVER DAMAGE

Liver is an extremely important organ, which performs many complex functions within the body including carbohydrate metabolism, urea and lipid metabolism, storage of essential nutrients and biotransformation of therapeutic agents (drugs) (Brandon *et al.*, 2003).

In the liver it has been shown that the toxicity of CCl_4 is mediated by the cytochrome P450 (CYP)-dependent mixed oxidase-mediated biotransformation product, trichloromethyl free radical ($CCl_3^{\bullet}$) and subsequent derivative $Cl_3COO^{\bullet -}$) (Brent and Rumack, 1993). These free radicals combine with the cellular lipid to produce lipid peroxidation, resulting in structural changes of endoplasmic reticulum and other biomembranes and loss of metabolic activity leading to liver damage (Kadiiska *et al.*, 2000).

The extent of liver damage caused by ethanol-CCl_4 toxicity was evaluated by assessing the liver marker enzymes, bilirubin and the circulating lipid profile. The marker enzymes analyzed were transaminases (AST and ALT), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase.

The activities of AST, ALT, ALP and γ-GT and the levels of bilirubin in the serum of rats treated with CCl_4 in the presence or the absence of the 4th, 8th and 12th day leaf extracts of *Triticum aestivum* or silymarin are presented in the Tables 13, 14 and 15 respectively.

TABLE 13

EFFECT OF *Triticum aestivum* LEAVES ON SERUM TRANSAMINASE ACTIVITIES IN ETHANOL-CCl_4 TREATED RATS

TREATMENTS	SERUM AST ACTIVITY (U/L)		SERUM ALT ACTIVITY (U/L)	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	159. 10 ± 1.42 [a]	235.98 ± 2.24 [a,b]	46.57 ± 1.41 [a]	85.35 ± 0.96 [a,b]
4 DAS	110.35 ± 2.78 [a,b,c]	131.07 ± 4.78 [b,c,d,e]	31.25 ± 0.30 [a,b,c]	39.69 ± 0.76 [a,b,c,d,e]
8 DAS	111.41 ± 4.61 [a,b,c,f]	132.21 ± 2.62 [a,b,c,d,e]	31.32 ± 1.68 [a,b,c,f]	40.10 ± 0.55 [a,b,c,d,e]
12 DAS	118.58 ± 4.41 [a,b,c,f]	135.95 ± 1.47 [a,b,c,d,e]	32.21 ± 0.38 [b,c,f]	42.23 ± 0.43 [a,b,c,d,e,f]
Silymarin	106.08 ± 2.41 [a,b,c]	126.67 ± 3.13 [b,c,d]	31.23 ± 0.93 [a,b,c]	36.88 ± 1.31 [a,b,c,d]
	LSD (5%) = 3.592		LSD (5%) = 1.138	

DAS – Days after Sowing

Serum AST activity in untreated control group : 127.83 ± 1.72 U/L

Serum ALT activity in untreated control group : 32.78 ± 0.99 U/L

Values are mean ± SD (n = 6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 14

EFFECT OF *Triticum aestivum* LEAVES ON SERUM ALP AND γ-GT ACTIVITIES IN ETHANOL-CCl_4 TREATED RATS

TREATMENTS	SERUM ALP ACTIVITY (U/L)		SERUM γ-GT ACTIVITY (U/L)	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	760.17 ± 12.64 [a]	948.37 ± 14.29 [a,b]	33.43 ± 1.47 [a]	53.97 ± 5.19 [a,b]
4 DAS	477.69 ± 14.29 [a,b,c]	527.36 ± 10.96 [b,c,d]	14.68 ± 0.81 [b,c]	21.33 ± 0.56 [a,b,c,d,e]
8 DAS	486.28 ± 12.38 [a,b,c,f]	529.73 ± 8.96 [b,c,d]	14.86 ± 0.78 [b,c,f]	23.20 ± 0.71 [a,b,c,d,e]
12 DAS	505.42 ± 11.06 [b,c,f]	534.46 ± 10.00 [a,b,c,d]	15.18 ± 0.50 [b,c,f]	25.54 ± 1.23 [a,b,c,d,e,f]
Silymarin	455.23 ± 21.07 [a,b,c]	534.38 ± 10.72 [a,b,c,d]	13.37 ± 1.64 [b,c]	15.40 ± 1.15 [b,c]
	LSD (5%) = 15.001		LSD (5%) = 2.215	

DAS – Days after Sowing

Serum ALP activity in untreated control group : 519.30 ± 12.06 U/L

Serum γ-GT activity in untreated control group : 15.12 ± 1.81 U/L

Values are mean ± SD (n = 6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

Ethanol stress caused a significant ($P<0.05$) increase in the activities of serum marker enzymes and elevated the levels of bilirubin, which was further augmented by CCl_4 administration. The treatment with the 4th, 8th and 12th day fresh leaf extracts of *Triticum aestivum* were effective in restoring the activities of the oxidant stressed groups to normal levels. The 4th and 8th day extracts of *Triticum aestivum* showed a better response followed by the 12th day leaf extract. The effect exerted by them was comparable to those of the standard antioxidant silymarin.

TABLE 15

EFFECT OF *Triticum aestivum* LEAVES ON SERUM BILIRUBIN LEVELS IN ETHANOL-CCl_4 TREATED RATS

TREATMENTS	SERUM BILIRUBIN (mg/dl)	
	WITHOUT CCl_4	WITH CCl_4
Ethanol	1.46 ± 0.08^{a}	$1.93 \pm 0.08^{a,b}$
4 DAS	$0.64 \pm 0.02^{b,c}$	$0.75 \pm 0.04^{a,b,c,d,e}$
8 DAS	$0.66 \pm 0.03^{b,c,f}$	$0.79 \pm 0.06^{a,b,c,d,e}$
12 DAS	$0.72 \pm 0.02^{a,b,c}$	$0.84 \pm 0.04^{a,b,c,d,e,f}$
Silymarin	$0.51 \pm 0.01^{a,b,c,f}$	$0.65 \pm 0.02^{b,c,d}$
	LSD (5%) = 0.054	

DAS – Days After Sowing

Serum bilirubin levels in untreated control group : 0.61 ± 0.01 mg/dl

Values are mean ± SD (n = 6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

SERUM LIPID PROFILE

Serum total cholesterol, triglycerides, phospholipids and free fatty acids were analyzed and the values obtained are presented in Tables 16 and 17 respectively.

The levels of serum cholesterol, triglycerides, phospholipids and free fatty acids increased significantly after administration with ethanol. There was a profound increase in the lipid profile levels on receiving an additional single dose of CCl_4. The *Triticum aestivum* leaf extracts were effective in undoing the damage caused by CCl_4 and ethanol to the maximum extent. Better protection was rendered by the 4th day leaf extract, which was comparable to that of silymarin.

EFFECT OF *Triticum aestivum* LEAVES ON THE ANTIOXIDANT STATUS DURING OXIDATIVE STRESS

The antioxidant status was assessed in the liver (metabolic organ) and the kidney (excretory organ) of rats. Enzymic and non-enzymic antioxidants were analyzed in both these organs and the results obtained are given below.

CHANGES IN THE ACTIVITIES OF ENZYMIC ANTIOXIDANTS

The activities of the enzymic antioxidants analyzed were SOD, CAT, POD, GR, GST and G6PD during the oxidative stress induced by ethanol with CCl_4 exposure and the extent of protection rendered by *Triticum aestivum* leaf extracts were investigated in the liver and kidney of rats.

TABLE 16

EFFECT OF *Triticum aestivum* LEAVES ON SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS IN ETHANOL-CCl_4 TREATED RATS

TREATMENTS	SERUM CHOLESTEROL (mg/dl)		SERUM TRIGLYCERIDES (mg/dl)	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	107.93 ± 1.89 [a]	156.48 ± 2.70 [a,b]	174.00 ± 3.75 [a]	212.95 ± 6.77 [a,b]
4 DAS	65.50 ± 1.21 [a,b,c]	75.87 ± 2.07 [a,b,c,d]	111.91 ± 4.52 [a,b,c]	121.35 ± 3.72 [a,b,c,d]
8 DAS	67.12 ± 2.63 [b,c,f]	78.54 ± 1.30 [a,b,c,d,f]	117.93 ± 7.79 [a,b,c]	124.91 ± 2.94 [b,c,d]
12 DAS	70.36 ± 2.89 [b,c,f]	80.02 ± 1.78 [a,b,c,d,e,f]	129.77 ± 4.28 [b,c,f]	133.54 ± 5.91 [b,c,e,f]
Silymarin	62.68 ± 2.62 [a,b,c]	76.53 ± 2.29 [a,b,c,d]	111.45 ± 6.22 [a,b,c]	123.17 ± 9.48 [a,b,c,d]
	LSD (5%) = 2.593		LSD (5%) = 6.605	

DAS – Days after Sowing

Serum cholesterol level in untreated control group : 68.42 ± 2.52 mg/dl

Serum triglyceride level in untreated control group : 130.20 ± 3.62 mg/dl

Values are mean ± SD (n = 6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 17

EFFECT OF *Triticum aestivum* LEAVES ON SERUM FREE FATTY ACID AND PHOSPHOLIPID LEVELS IN ETHANOL-CCl_4 TREATED RATS

TREATMENTS	SERUM FREE FATTY ACIDS (mg/dl)		SERUM PHOSPHOLIPIDS (mg/dl)	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	203.68 ± 5.82^{a}	$249.98 \pm 9.21^{a,b}$	181.55 ± 5.00^{a}	$212.12 \pm 3.50^{a,b}$
4 DAS	$118.35 \pm 8.30^{b,c}$	$145.56 \pm 9.74^{a,b,c,d,e}$	$97.78 \pm 8.96^{a,b,c}$	$118.64 \pm 10.78^{a,b,c,d}$
8 DAS	$122.38 \pm 5.45^{b,c,f}$	$152.66 \pm 10.21^{a,b,c,d,e}$	$99.54 \pm 7.31^{b,c,f}$	$122.21 \pm 4.74^{a,b,c,d,e}$
12 DAS	$124.52 \pm 5.47^{b,c,f}$	$154.11 \pm 8.09^{a,b,c,d,e,f}$	$101.53 \pm 9.06^{b,c,f}$	$127.34 \pm 6.41^{a,b,c,d,e,f}$
Silymarin	$110.70 \pm 4.17^{a,b,c}$	$129.39 \pm 4.10^{b,c,d}$	$95.16 \pm 2.72^{a,b,c}$	$111.23 \pm 4.28^{b,c,d}$
	LSD (5%) = 8.278		LSD (5%) = 7.735	

DAS – Days after Sowing

Serum free fatty acids level in untreated control group : 124.52 ± 4.13 mg/dl

Serum phospholipids level in untreated control group : 106.56 ± 5.75 mg/dl

Values are mean ± SD (n=6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

SOD

The specific activities of SOD in the control liver and kidney were comparable to each other (Table 18). Ethanol exposure caused a depletion of SOD activity in both liver and kidney, the decrease in activities escaped statistical difference in the latter when compared to untreated control. The activities of SOD were further decreased by a single CCl_4 assault in both liver and kidney. The administration of the fresh aqueous extracts of the 4th, 8th and 12th day leaves of *Triticum aestivum* by themselves caused a significant ($P<0.05$) increase in the activity of SOD. The co-treatment of these extracts with CCl_4 boosted the SOD activity to a significant extent ($P<0.05$) when compared to the CCl_4 treated group. The response elicited by the 4th day extract was maximum and comparable to the response evoked by the standard antioxidant silymarin.

CAT

The basal specific activity of catalase was found to be higher in kidney than in liver (Table 19). From the table values, it can be deduced that ethanol stress caused a significant ($P<0.05$) decline in the activities of catalase both in liver and kidney, which was further augmented by CCl_4 exposure. The 4th, 8th and 12th day extracts of *Triticum aestivum* were very effective in reverting back the changes in the status of catalase activity caused by CCl_4 stress to a greater extent which can be evidenced by significantly higher activities of catalase found in *Triticum aestivum* leaf extract treated groups than untreated control.

TABLE 18

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF SOD IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	ACTIVITY OF SOD (U/mg protein)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	4.20 ± 0.29^{a}	$3.68 \pm 0.34^{a,b}$	4.50 ± 0.21	4.08 ± 0.25^{a}
4 DAS	$9.95 \pm 0.53^{a,b,c}$	$8.40 \pm 0.48^{a,b,c,d}$	$12.40 \pm 0.81^{a,b,c}$	$10.33 \pm 0.59^{a,b,c,d,e}$
8 DAS	$9.19 \pm 0.62^{a,b,c,f}$	$7.57 \pm 0.41^{a,b,c,d,e,f}$	$11.28 \pm 0.75^{a,b,c}$	$10.30 \pm 0.36^{a,b,c,e}$
12 DAS	$7.82 \pm 0.20^{a,b,c,f}$	$5.96 \pm 0.44^{a,b,c,d,e,f}$	$8.83 \pm 0.34^{a,b,c,f}$	$6.99 \pm 0.28^{a,b,c,d,e,f}$
Silymarin	$10.03 \pm 0.44^{a,b,c}$	$8.77 \pm 0.70^{a,b,c,d,f}$	$14.18 \pm 1.67^{a,b,c}$	$12.83 \pm 1.70^{a,b,c,d}$
	LSD (5%) = 0.532		LSD (5%) = 0.996	

DAS – Days after Sowing

Activity of SOD in untreated control liver : 5.10 ± 0.27 U/mg protein

Activity of SOD in untreated control kidney : 5.08 ± 0.37 U/mg protein

Values are mean ± SD (n = 6)

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

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 19

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF CAT IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	ACTIVITY OF CAT (U/mg protein)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	111.49 ± 1.10^{a}	$97.19 \pm 1.36^{a,b}$	186.26 ± 0.64^{a}	182.61 ± 0.85^{a}
4 DAS	$235.50 \pm 2.82^{a,b,c}$	$173.13 \pm 2.78^{a,b,c,d,e}$	$361.92 \pm 13.05^{a,b,c,f}$	$295.14 \pm 4.43^{a,b,c,d,e}$
8 DAS	$214.72 \pm 3.91^{a,b,c,f}$	$165.19 \pm 1.66^{a,b,c,d,e,f}$	$305.82 \pm 3.63^{a,b,c,f}$	$288.67 \pm 2.57^{a,b,c,d,e,f}$
12 DAS	$196.48 \pm 4.65^{a,b,c,f}$	$162.00 \pm 4.05^{a,b,c,d,e,f}$	$293.34 \pm 4.84^{a,b,c}$	$276.87 \pm 3.13^{a,b,c,d,e,f}$
Silymarin	$243.82 \pm 2.50^{a,b,c,f}$	$185.38 \pm 1.07^{a,b,c,d,e,f}$	$401.85 \pm 1.75^{a,b,c}$	$315.99 \pm 1.98^{a,b,c,d}$
	LSD (5%) = 3.226		LSD (5%) = 5.700	

DAS – Days after Sowing

Activity of CAT in untreated control liver : 153.56 ± 0.70 U/mg protein

Activity of CAT in untreated control kidney : 247.18 ± 2.42 U/mg protein

Values are mean ± SD (n=6)

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

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

POD

Higher specific activities of peroxidase were found in the kidney than the liver tissue (Table 20). Administration of ethanol decreased the activities of peroxidase to a significant extent ($P<0.05$) in both liver and kidney tissues. The activities of peroxidase were further decreased by the oxidant CCl_4 treatment. The *Triticum aestivum* leaf extracts treatment effectively counteracted the toxicity imposed by CCl_4. The 4th day extract was more potent as evidenced by the significant increase in the activities in both liver and kidney.

GST

The specific activities of GST in the control groups were comparable in both liver and kidney (Table 21). There was a significant ($P<0.05$) reduction in the GST activity upon ethanol exposure in both liver and kidney. Administration of CCl_4 further reduced the activity of GST. Treatment with *Triticum aestivum* leaf extracts at all the time points selected caused a significant elevation ($P<0.05$) in the activity of GST compared to control rats in both liver and kidney. Silymarin treated rats showed maximum activity of GST in both liver and kidney.

GR

The table values depict that the administration of ethanol caused a significant decrease ($P<0.05$) in the activities of GR in both liver and kidney tissues (Table 22). Furthermore, a single assault with CCl_4 also caused a marked decrease in GR activities. The animals fed with the *Triticum aestivum* leaf extracts caused a significant increase in the activities in both the tissues. The maximum effect was shown by the 4th day extract.

TABLE 20

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF POD IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	ACTIVITY OF POD (U/mg protein)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	7.82 ± 0.91[a]	5.58 ± 0.76 [a,b]	9.82 ± 0.82 [a,c]	5.63 ± 0.50 [a,b]
4 DAS	13.37 ± 0.51[a,b,c]	11.76 ± 0.61 [a,b,c,d,e]	15.80 ± 0.86 [a,b,c,f]	13.86 ± 0.73 [b,c,d,e]
8 DAS	12.48 ± 0.24 [a,b,c]	11.16 ± 0.37 [b,c,d,e]	14.79 ± 0.67 [b,c,f]	12.34 ± 0.49 [a,b,c,d,e,f]
12 DAS	11.31 ± 0.40 [b,c]	9.65 ± 0.23 [a,b,c,d,e,f]	12.37 ± 0.63 [a,b,c,f]	10.96 ± 0.56 [a,b,c,d,e,f]
Silymarin	15.90 ± 0.75 [a,b,c,f]	13.92 ± 0.79 [a,b,c,d]	19.57 ± 0.79 [a]	15.93 ± 0.78 [a,b,c,d]
	LSD (5%) = 0.726		LSD (5%) = 0.832	

DAS – Days after Sowing

Activity of POD in untreated control liver = 10.67 ± 0.92 U/mg protein

Activity of POD in untreated control kidney = 14.32 ± 0.94 U/mg protein

Values are mean ± SD (n=6)

1 Unit = Change in absorbance at 430nm/minute

Statistically significant ($P<0.05$) compared to

a - Untreated Control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 21

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF GST IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	ACTIVITY OF GST (U/mg protein)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	4.20 ± 0.29^{a}	$3.35 \pm 0.22^{a,b}$	4.22 ± 0.33^{a}	4.20 ± 0.19^{a}
4 DAS	$8.50 \pm 0.27^{a,b,c}$	$7.51 \pm 0.49^{a,b,c,d,e}$	$9.17 \pm 0.47^{a,b,c}$	$8.51 \pm 0.43^{a,b,c,d,e}$
8 DAS	$7.87 \pm 0.22^{a,b,c,f}$	$7.23 \pm 0.33^{a,b,c,d,e}$	$8.72 \pm 0.36^{a,b,c,f}$	$8.19 \pm 0.23^{a,b,c,d,e}$
12 DAS	$7.07 \pm 0.33^{a,b,c,f}$	$6.11 \pm 0.41^{a,b,c,d,e,f}$	$7.18 \pm 0.54^{a,b,c,f}$	$6.75 \pm 0.35^{a,b,c,d,e,f}$
Silymarin	$10.71 \pm 0.13^{a,b,c,f}$	$9.10 \pm 0.24^{a,b,c,d}$	$11.03 \pm 0.34^{a,b,c}$	$9.48 \pm 0.20^{a,b,c,d}$
	LSD (5%) = 0.346		LSD (5%) = 0.406	

DAS – Days after Sowing

Activity of GST in untreated control liver : 5.02 ± 0.14 U/mg protein

Activity of GST in untreated control kidney : 5.50 ± 0.18 U/mg protein

Values are mean ± SD (n=6)

1 Unit = nmoles of CDNB conjugated per minute

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 22

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF GR IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	ACTIVITY OF GR (U/mg PROTEIN)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	0.32 ± 0.03	0.19 ±0.02 [a]	0.28 ± 0.03 [a,c]	0.17 ± 0.02 [a]
4 DAS	0.42 ± 0.02 [a,c]	0.25 ± 0.02 [a,c,d,e]	0.53 ± 0.03 [a,b,c,f]	0.31 ± 0.03 [a,c,d,e]
8 DAS	0.40 ± 0.01 [a,c,f]	0.23 ± 0.02 [a,c,d,e]	0.50 ± 0.02 [a,b,c,f]	0.32 ± 0.02 [a,c,d,e]
12 DAS	0.35 ± 0.01 [c,f]	0.20 ± 0.03 [a,b,d,e,f]	0.44 ± 0.02 [b,c,f]	0.25 ± 0.01 [a,c,d,e,f]
Silymarin	0.57 ± 0.06 [a,b,c]	0.30 ± 0.07 [c,d]	0.67 ± 0.06 [a,b,c]	0.40 ± 0.05 [b,c,d]
	LSD (5%) = 0.040		LSD (5%) = 0.044	

DAS – Days after Sowing

Activity of GR in untreated control liver : 0.34 ± 0.02 U/mg protein

Activity of GR in untreated control kidney : 0.41 ± 0.05 U/mg protein

Values are mean ± SD (n=6)

1 Unit = µmoles of NADPH oxidized/minute

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

G6PD

Table 23 presents the activities of G6PD observed in the liver and kidney of the experimental rats.

The specific activities of G6PD were found to be high in the liver than in the kidney. The activities of GR depicted in the table portray that the ethanol administration led to a significant ($P<0.05$) decrease in the activities of G6PD. CCl_4 treatment further decreased the G6PD activity. The co-administration of the leaf extracts of *Triticum aestivum* effectively mitigated the toxic effect of CCl_4 and significantly increased the G6PD activities, compared to control in both the organs studied. The maximum activity of G6PD was observed in silymarin treated group.

NON-ENZYMIC ANTIOXIDANTS

The non-enzymic antioxidants analyzed were vitamin C, vitamin E, vitamin A, GSH and protein thiols. The results obtained are presented below.

VITAMIN C

The levels of ascorbic acid in the liver and kidney of rats exposed to CCl_4 in the presence and the absence of *Triticum aestivum* leaf extracts or silymarin are presented in Table 24.

The basal levels of vitamin C were higher in kidney than in the liver. From the values, it can be clearly observed that ethanol treatment by itself caused a significant ($P<0.05$) depletion of vitamin C in both liver and kidney. Treatment with CCl_4 further decreased the levels of vitamin C in both the tissues studied. The rats fed with the *Triticum aestivum* leaf extracts were found to possess higher content of ascorbic acid in both the tissues, and the leaf extract treatments effectively nullified the damage caused by CCl_4.

TABLE 23

EFFECT OF *Triticum aestivum* LEAVES ON THE ACTIVITY OF G6PD IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	ACTIVITY OF G6PD (U/mg protein)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	8.42 ± 0.14 [a]	5.80 ± 0.35 [a,b]	5.22 ± 0.27 [a]	3.55 ± 0.34 [a,b]
4 DAS	15.49 ± 0.64 [a,b,c,f]	13.95 ± 0.28 [a,b,c,d]	9.53 ± 0.46 [a,b,c,f]	8.95 ± 0.46 [b,c,d,e]
8 DAS	15.12 ± 0.67 [a,b,c,f]	13.70 ± 0.99 [a,b,c,d,e]	9.27 ± 0.34 [a,b,c]	8.73 ± 0.35 [b,c,d,e]
12 DAS	14.16 ± 0.47 [a,b,c]	13.57 ± 0.56 [a,b,c,e]	8.93 ± 0.52 [b,c]	8.63 ± 0.37 [b,c,e]
Silymarin	15.64 ± 0.69 [a,b,c]	14.55 ± 0.53 [a,b,c,d]	11.76 ± 0.48 [a,b,c]	10.13 ± 0.34 [a,b,c,d]
	LSD (5%) = 0.693		LSD (5%) = 0.454	

DAS – Days after Sowing

Activity of G6PD in untreated control liver : 12.59 ± 0.76 U/mg protein

Activity of G6PD in untreated control kidney : 8.56 ± 0.30 U/mg protein

Values are mean ± SD (n=6)

1 Unit = The amount of enzyme which causes an initial change of 0.01 OD/minute

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 24

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN C IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	LEVELS OF VITAMIN C (mg/g tissue)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	97.46 ± 5.95 [a]	87.28 ± 2.46 [a,b]	107.27 ± 3.93 [a]	91.52 ± 4.32 [a,b]
4 DAS	294.91 ± 1.03 [a,b,c]	264.75 ± 1.45 [a,b,c,d,e]	323.26 ± 1.34 [a,b,c,f]	294.58 ± 3.01 [a,b,c,d,e]
8 DAS	278.84 ± 1.44 [a,b,c,f]	258.68 ± 1.72 [a,b,c,d,e]	305.56 ± 1.61 [a,b,c,f]	278.19 ± 1.84 [a,b,c,d,e,f]
12 DAS	257.21 ± 1.45 [a,b,c,f]	234.92 ± 2.13 [a,b,c,d,e,f]	296.88 ± 1.84 [a,b,c]	249.68 ± 2.21 [a,b,c,d,e,f]
Silymarin	328.08 ± 5.33 [a,b,c,f]	279.63 ± 7.32 [a,b,c,d]	363.65 ± 6.88 [a,b,c]	303.33 ± 4.32 [a,b,c,d]
	LSD (5%) = 7.037		LSD (5%) = 5.719	

DAS – Days after Sowing

Levels of vitamin C in untreated control liver : 146.78 ± 16.35 mg/g tissue

Levels of vitamin C in untreated control kidney : 150.62 ± 11.81 mg/g tissue

Values are mean ± SD (n=6)

Statistically significant ($P<0.05$) compared to

- a - Untreated control
- b - Ethanol alone treated group
- c - Ethanol and CCl_4 treated group
- d - Respective plant / silymarin control
- e - Ethanol + CCl_4 + silymarin treated group
- f - Ethanol + CCl_4 + 4th day extract treated group

VITAMIN E

Tocopherol levels were estimated in the liver and kidney of the rats treated with ethanol and CCl_4 in the presence and absence of *Triticum aestivum* leaf extracts or silymarin. The values obtained are listed in Table 25. The basal levels of vitamin E were higher in kidney than in the liver. Ethanol supplement caused a considerable increase in the vitamin E levels in both liver and kidney when compared to untreated control. On the other hand, CCl_4 exposure caused a significant ($P<0.05$) decrease in the levels of vitamin E in both the liver and the kidney on comparison with untreated control. Treatment with the leaf extracts of *Triticum aestivum* by themselves, significantly ($P<0.05$) increased the vitamin E levels in both the tissues compared to control and ethanol-CCl_4 treated groups. It is evident from the table that the plant extracts help in suppressing the damage caused by CCl_4 in both liver and kidney.

VITAMIN A

Much higher levels of vitamin A were observed in the liver of the untreated control animals than in the kidney (Table 26). The vitamin A levels were found to decrease drastically in ethanol treated groups. The levels were profoundly decreased further in both the liver and kidney tissues on receiving a single dose of CCl_4. Treatment with the leaf extracts of *Triticum aestivum* caused a significant ($P<0.05$) elevation of vitamin A levels in both the liver and kidney tissues.

GSH

Reduced glutathione is a major representative of non-protein thiols. The levels of GSH in the liver and kidney of rats exposed to ethanol and CCl_4 in the presence and absence of *Triticum aestivum* leaf extracts or silymarin are given in Table 27.

TABLE 25

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN E IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	LEVELS OF VITAMIN E (µg/g tissue)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	374.68 ± 5.84 [a]	334.08 ± 6.88 [a,b]	306.24 ± 3.28 [c]	193.70 ± 5.99 [a,b]
4 DAS	405.55 ± 6.52 [a,b,c]	364.96 ± 13.14 [a,c,b]	423.54 ± 7.48 [a,b,c]	385.85 ± 14.53 [a,b,c,d]
8 DAS	402.33 ± 7.81 [a,b,c,f]	364.58 ± 14.78 [a,c,d]	422.17 ± 5.85 [a,b,c,f]	360.64 ± 12.03 [a,b,c,d,e]
12 DAS	393.20 ± 9.12 [a,b,c,f]	336.75 ± 8.23 [a,b,d,e,f]	397.84 ± 11.06 [a,b,c,f]	356.79 ± 13.40 [a,b,c,d,e,f]
Silymarin	417.54 ± 5.12 [a,b,c,f]	361.92 ± 9.84 [a,c,d]	431.91 ± 4.74 [a,b,c]	404.86 ± 5.42 [a,b,c]
	LSD (5%) = 13.827		LSD (5%) = 28.206	

DAS – Days after Sowing

Levels of vitamin E in untreated control liver : 263.27 ± 25.97 µg/g tissue

Levels of vitamin E in untreated control kidney : 281.88 ± 30.80 µg/g tissue

Values are mean ± SD (n=6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 26

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF VITAMIN A IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	LEVELS OF VITAMIN A (µg/g tissue)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	686.17 ± 36.32 [a]	261.59 ± 82.93 [a,b]	152.86 ± 34.68 [a]	112.19 ± 46.97 [a]
4 DAS	1069.72 ± 18.78 [b,c,f]	802.46 ± 8.23 [a,b,c,d]	280.85 ± 6.87 [a,b,c,f]	202.91 ± 5.73 [a,b,c,d]
8 DAS	1051.52 ± 6.08 [b,c,f]	763.70 ± 6.09 [a, c,d,e]	249.63 ± 5.35 [a,b,c,f]	194.07 ± 3.63 [a,c,d]
12 DAS	980.35 ± 7.12 [a,b,c,f]	721.22 ± 6.17 [a,c,d,e]	227.07 ± 7.15 [a,b,c]	191.81 ± 6.69 [a, c]
Silymarin	1077.88 ± 221.73 [b,c,f]	869.57 ± 71.13 [a,b,c,d]	307.86 ± 40.91 [a,b,c]	229.97 ± 42.75 [a,b,c,d]
	LSD (5%) = 101.092		LSD (5%) = 40.058	

DAS – Days after Sowing

Levels of vitamin A in untreated control liver : 1149.20 ± 135.29 µg/g tissue

Levels of vitamin A in untreated control kidney : 369.74 ± 77.70 µg/g tissue

Values are mean ± SD (n=6)

Statistically significant ($P<0.05$) compared to

- a - Untreated control
- b - Ethanol alone treated group
- c - Ethanol and CCl_4 treated group
- d - Respective plant / silymarin control
- e - Ethanol + CCl_4 + silymarin treated group
- f - Ethanol + CCl_4 + 4th day extract treated group

TABLE 27

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF GSH IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	LEVELS OF GSH (mg/g tissue)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	22.61 ± 3.11^{a}	$12.25 \pm 1.64^{a,b}$	19.87 ± 0.96^{c}	$8.32 \pm 1.20^{a,b}$
4 DAS	$41.89 \pm 3.69^{a,b,c}$	$33.60 \pm 1.19^{b,c,d}$	$25.80 \pm 1.91^{a,b,c,f}$	$16.73 \pm 0.18^{a,b,c,d}$
8 DAS	$39.59 \pm 1.46^{a,b,c,f}$	$32.42 \pm 1.60^{b,c,d}$	$25.58 \pm 1.43^{a,b,c,f}$	$16.34 \pm 0.84^{a,b,c,d}$
12 DAS	$36.31 \pm 1.42^{a,b,c}$	$29.26 \pm 1.35^{a,b,c,d,e,f}$	$23.72 \pm 1.63^{b,c,f}$	$14.88 \pm 0.58^{a,b,c,d,e}$
Silymarin	$46.88 \pm 2.88^{a,b,c,f}$	$35.75 \pm 5.02^{b,c,d}$	$29.36 \pm 3.93^{a,b,c}$	$18.26 \pm 3.21^{a,b,c,d}$
	LSD (5%) = 3.456		LSD (5%) = 2.308	

DAS – Days after Sowing

Levels of GSH in untreated control liver : 32.75 ± 5.36 mg/g tissue

Levels of GSH in untreated control kidney : 21.85 ± 2.79 mg/g tissue

Values are mean ± SD (n=6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

The levels of GSH were significantly ($P<0.05$) decreased upon ethanol stress, which was further significantly reduced on CCl_4 assault in both the liver and kidney of rats. Treatment with the leaf extracts of *Triticum aestivum* increased the GSH content in both the tissues studied. The effects evoked by the *Triticum aestivum* leaf extracts were comparable to those induced by silymarin.

PROTEIN THIOLS

Protein thiols were estimated in the liver and kidney, as the reactive thiol groups are players in the antioxidant defense. Table 28 presents the values obtained.

The protein thiol levels were higher in the kidney than in the liver. The levels were depleted significantly ($P<0.05$) from untreated control levels by both ethanol and CCl_4 treatments. The co-exposure of the leaf extracts of *Triticum aestivum* significantly increased the protein thiol levels in both the liver and kidney. Silymarin evoked a still higher response in improving the protein thiol levels in both organs.

EXTENT ON LIPID PEROXIDATION

The ultimate targets of oxidative moieties in the cell are the macromolecules, especially, the lipids, proteins and DNA. Lipids are the easy targets, oxygen free radicals can interact with lipid molecules in cell membrane in a process called lipid peroxidation, which in turn results in the generation of additional reactive molecules especially malondialdehyde. Therefore, the extent of lipid peroxidation is considered as a direct measure of the extent of oxidative stress suffered by the system (Huang *et al.*, 2005). In tune with this, lipid peroxidation was followed as the amount of thiobarbituric acid reactive substances (TBARS) formed, in the liver of the treated animals. The estimation could not be carried out in the kidney due to the exhaustion of the tissue for the estimation of the other parameters. The results are presented in Table 29.

TABLE 28

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF PROTEIN THIOLS IN THE LIVER AND KIDNEY OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	LEVELS OF PROTEIN THIOLS (mg/g tissue)			
	LIVER		KIDNEY	
	WITHOUT CCl_4	WITH CCl_4	WITHOUT CCl_4	WITH CCl_4
Ethanol	7.31 ± 0.74	6.13 ± 0.83 [a]	8.80 ± 0.99 [a,c]	7.73 ± 0.97 [a,b]
4 DAS	12.76 ± 1.08 [a,b,c]	10.14 ± 0.59 [a,b,c,d,e]	12.65 ± 0.59 [a,b,c,f]	11.33 ± 0.49 [b,c,d,e]
8 DAS	11.33 ± 0.61 [a,b,c,f]	9.80 ± 0.72 [a,b,c,d,e]	12.09 ± 0.68 [b,c]	10.63 ± 0.61 [a,b,c,e,f]
12 DAS	9.52 ± 0.78 [a,b,c]	8.47 ± 0.59 [a,b,e,f]	10.98 ± 0.59 [a,b,c]	10.14 ± 0.59 [b,c,d,e]
Silymarin	14.44 ± 2.13 [a,b,c,f]	13.23 ± 0.57 [a,b,c,d]	15.78 ± 0.78 [a,b,c]	14.33 ± 0.50 [a,b,c,d]
	LSD (5%) = 1.119		LSD (5%) = 0.886	

DAS – Days after Sowing

Levels of protein thiols in untreated control liver : 8.34 ± 0.79 mg/g tissue

Levels of protein thiols in untreated control kidney : 11.08 ± 1.29 mg/g tissue

Values are mean ± SD (n=6)

Statistically significant ($P<0.05$) compared to

a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
d - Respective plant / silymarin control
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

The oxidative stress imposed by both ethanol and CCl_4 administration was evident by the increased TBARS level in the liver, compared to the untreated control. The administration of the leaf extracts of *Triticum aestivum* improved the antioxidant status by causing a decrease in the TBARS levels to a significant extent.

TABLE 29

EFFECT OF *Triticum aestivum* LEAVES ON THE LEVELS OF TBARS IN THE LIVER OF RATS EXPOSED *in vivo* TO ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS

TREATMENTS	EXTENT OF TBARS FORMATION (μM EQUIVALENT OF MDA)	
	LIVER	
	WITHOUT CCl_4	WITH CCl_4
Ethanol	27.50 ± 1.15	30.51 ± 0.85 ^{b}
4 DAS	19.27 ± 0.90 ^{a,b,c}	20.29 ± 1.20 ^{a,b,c,e}
8 DAS	21.98 ± 1.48 ^{a,b,c}	22.71 ± 0.69 ^{a,b,c,f}
12 DAS	24.72 ± 1.03 ^{a,b,c,f}	25.21 ± 1.01 ^{a,c,f}
Silymarin	23.05 ± 0.65 ^{a,b,c}	24.10 ± 0.82 ^{a,b,c}
	LSD (5%) = 2.072	

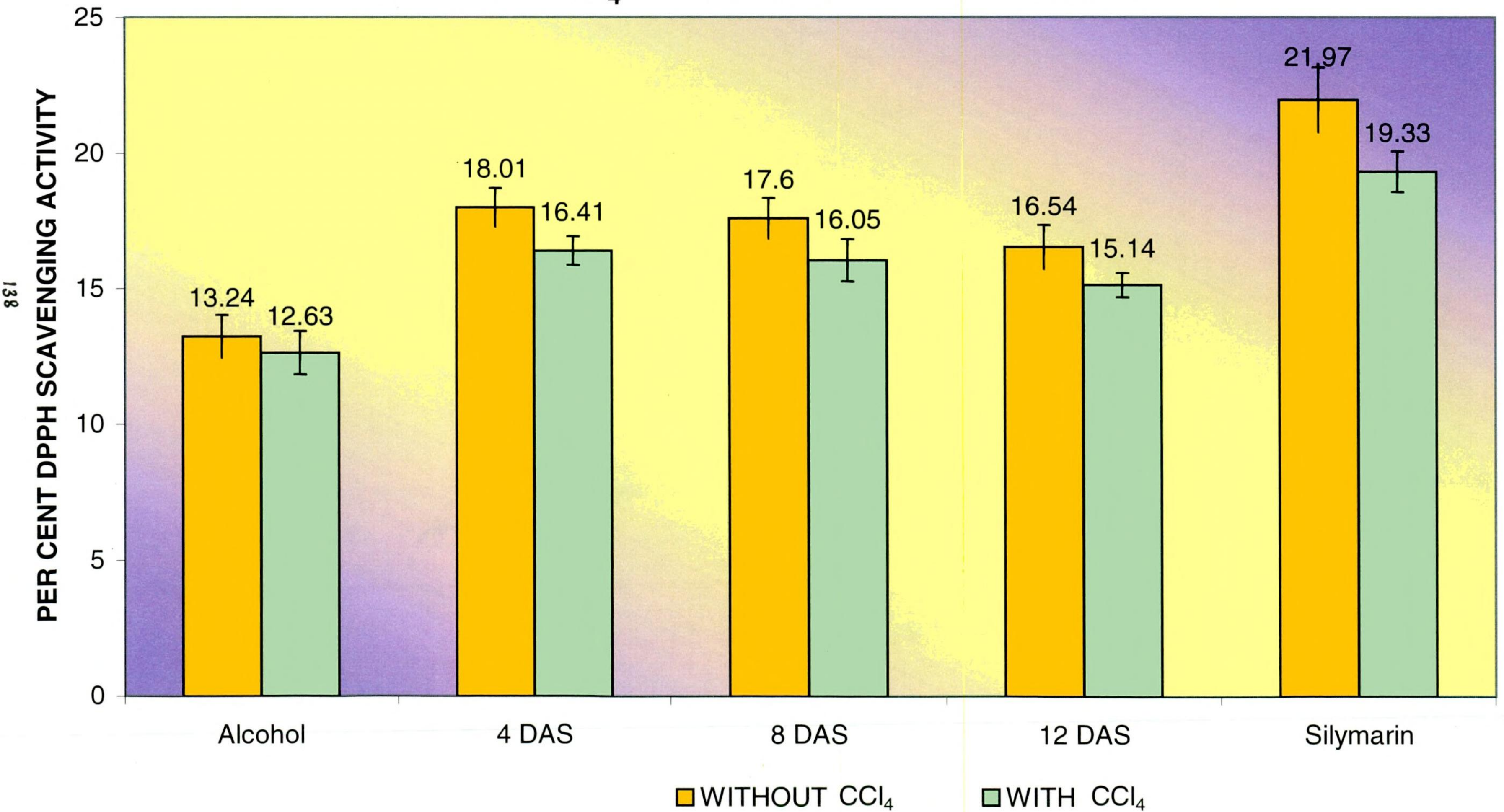
DAS – Days After Sowing
Levels of TBARS in untreated control : 29.33 ± 4.92
Values are mean ± SD (n=6)
Statistically significant ($P<0.05$) compared to
a - Untreated control
b - Ethanol alone treated group
c - Ethanol and CCl_4 treated group
e - Ethanol + CCl_4 + silymarin treated group
f - Ethanol + CCl_4 + 4th day extract treated group

RADICAL SCAVENGING ACTIVITY

The total antioxidant status of the tissues treated with ethanol and CCl_4 in the presence and absence of *Triticum aestivum* leaf extracts was assessed by the ability of the tissue homogenate to scavenge the stable free radical DPPH. The assay was carried out only in the liver and the per cent scavenging activity is presented in Figure 9.

FIGURE 9

EFFECT OF *Triticum aestivum* LEAVES ON THE DPPH RADICAL SCAVENGING ACTIVITY IN THE LIVER OF RATS EXPOSED TO ALCOHOL-CCl_4 INDUCED OXIDATIVE STRESS



The DPPH scavenging activity was not significantly affected in ethanol and CCl_4 treated groups. However, the treatment with *Triticum aestivum* leaf extracts caused a significant increase in DPPH scavenging activity over the controls. The effect elicited by the 4th day extract of *Triticum aestivum* was comparable with silymarin.

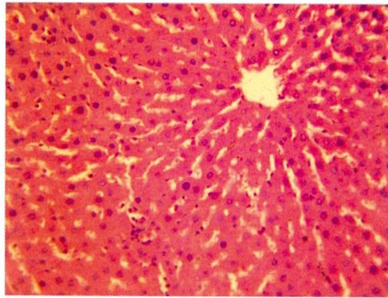
EFFECT OF *Triticum aestivum* LEAF EXTRACTS ON THE HEPATIC AND RENAL HISTOLOGY

CHANGES IN THE LIVER

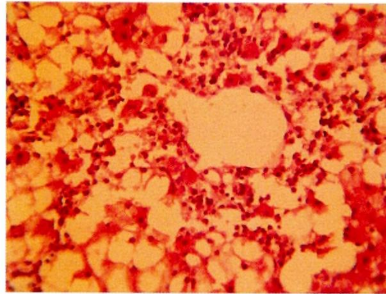
The untreated control rats showed normal hepatic architecture with hepatocytes showing normal cord pattern with central vein and portal tracts (Plate 4).

In the ethanol alone treated rats, the liver showed centrilobular infiltration with focal necrosis of hepatocytes. The liver of rats treated with ethanol and CCl_4 showed fatty changes and focal necrosis of hepatocytes away from the central vein. The necrosis was confluent in some areas. Preserved hepatocytes were seen around the central vein.

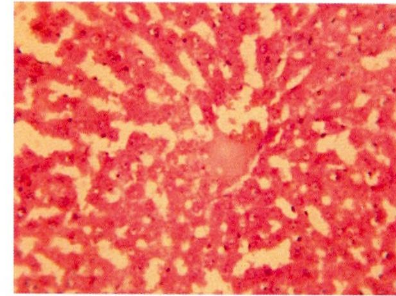
When the rats were administered with the 4th, 8th and 12th day extracts of *Triticum aestivum,* a significant reversal in the histological architecture was observed. The liver sections of rats treated with the 4th day extract showed hepatocytes with normal cord pattern with central vein and portal tracts, which was similar to the picture observed in the untreated control liver. When the animals were administered with the 8th day extract of *Triticum aestivum*, the liver showed perivenular single cell necrosis around central veins, whereas in the case of 12th day extract treated groups, the liver showed periportal hepatocytes with normal features. These changes were comparable to silymarin treated liver sections. The silymarin treated controls and the leaf extract treated controls exhibited normal pattern.



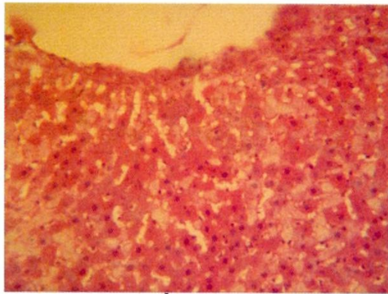
Control



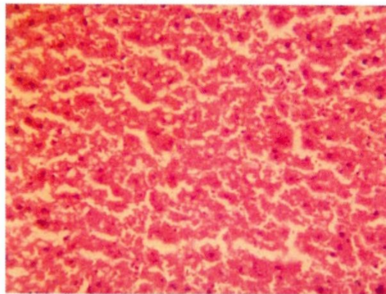
Ethanol



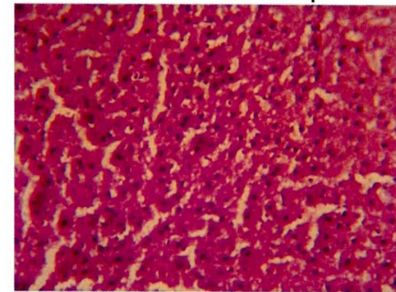
Ethanol + CCl_4



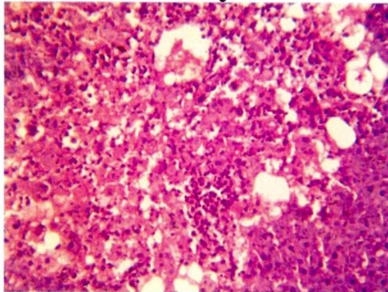
Ethanol + 4th day leaf extract



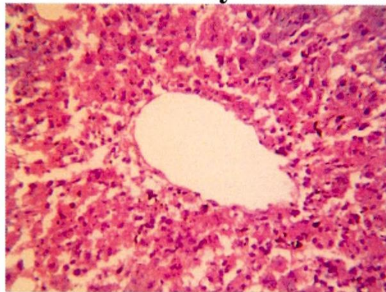
Ethanol + 8th day leaf extract



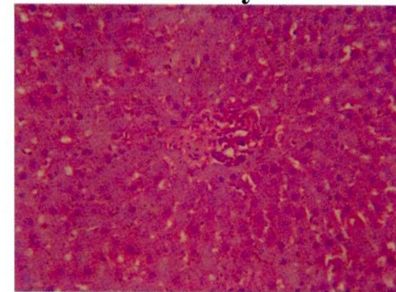
Ethanol + 12th day leaf extract



Ethanol + CCl_4 + 4th day leaf extract

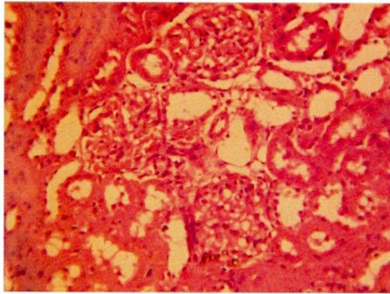


Ethanol + CCl_4 + 8th day leaf extract

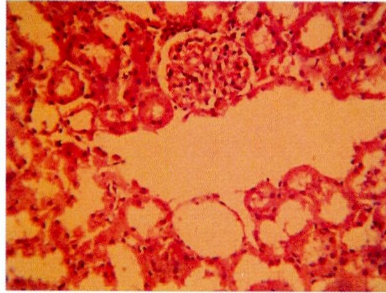


Ethanol + CCl_4 + 12th day leaf extract

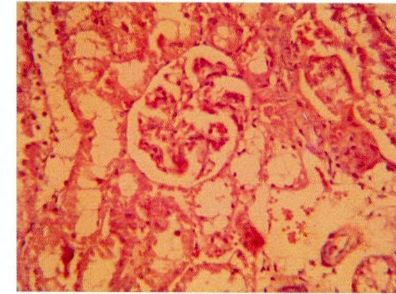
PLATE 4 : HISTOPATHOLOGICAL ARCHITECTURE IN THE LIVER OF CONTROL AND EXPERIMENTAL ANIMALS



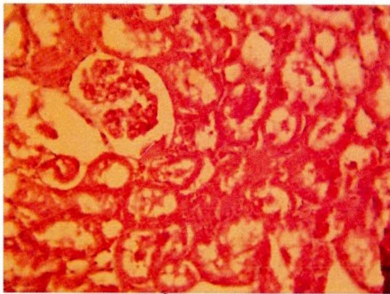
Control



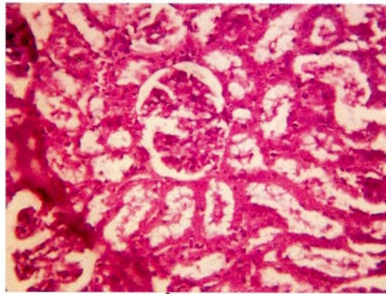
Ethanol



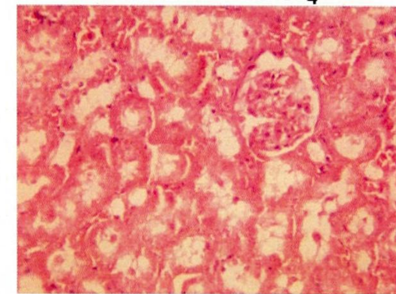
Ethanol + CCl_4



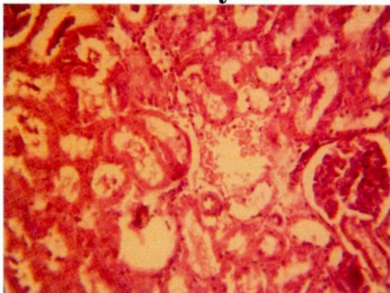
Ethanol + 4th day leaf extract



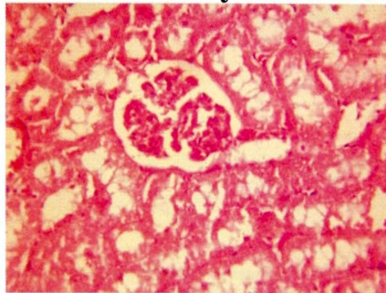
Ethanol + 8th day leaf extract



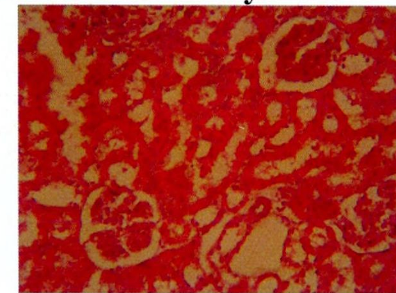
Ethanol + 12th day leaf extract



Ethanol + CCl_4 + 4th day leaf extract



Ethanol + CCl_4 + 8th day leaf extract



Ethanol + CCl_4 + 12th day leaf extract

PLATE 5 : HISTOPATHOLOGICAL ARCHITECTURE IN THE KIDNEY OF CONTROL AND EXPERIMENTAL ANIMALS

CHANGES IN THE KIDNEY

The untreated control rats showed normal glomeruli, proximal convoluted tubules and vessels (Plate 5). However, suffused glomeruli tuffs were seen in the ethanol and CCl_4 treated groups. When the 4th, 8th and 12th day extracts of *Triticum aestivum* were administered to oxidant stressed groups, the kidney showed normal features with no significant pathologies. Plant extract treated controls and silymarin treated controls exhibited normal kidney architecture. The silymarin treated groups also showed normal histological features.

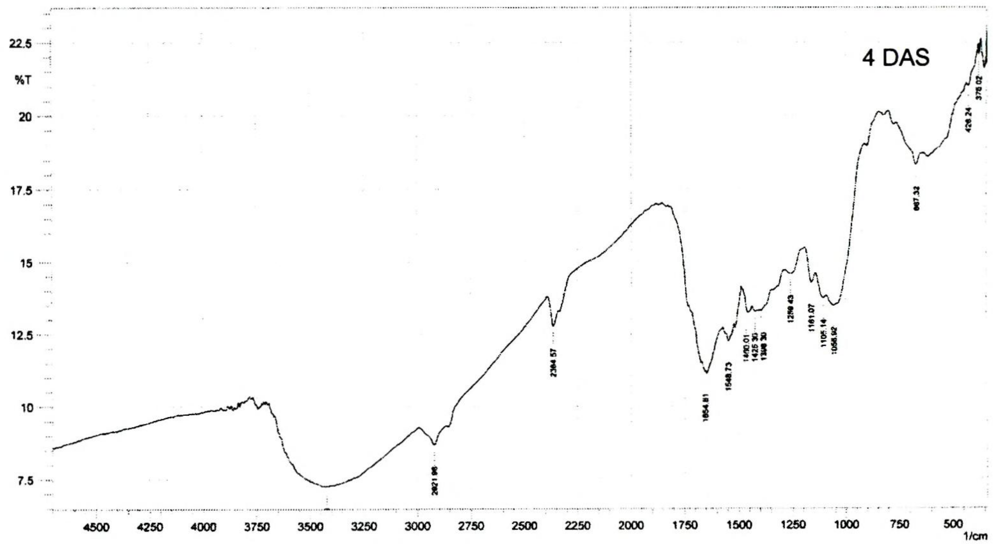
PHASE IV

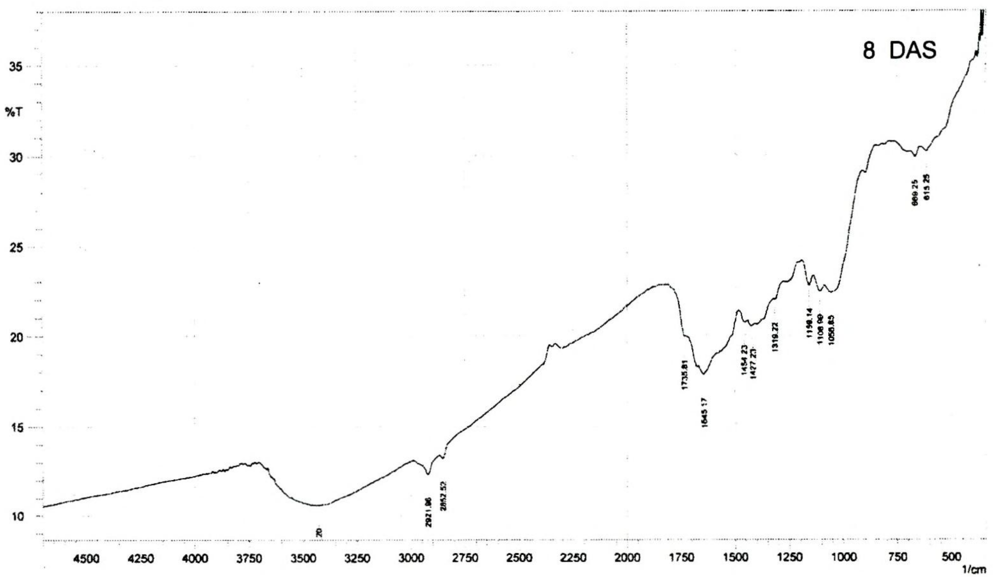
Having ascertained the antioxidant potential of the *Triticum aestivum* leaf extracts on the time periods selected both *in vitro* and *in vivo*, a preliminary phytochemical screening was done in order to identify the chemical nature of the active component present that is responsible for rendering the leaves its antioxidant property. Qualitative analysis of the extracts revealed the presence of alkaloids, phenolics and flavonoids in the 4th, 8th and 12th day leaf extracts of *Triticum aestivum.*

In order to confirm the nature of the active component observed, spectral analysis (FT-IR, ^{1}H NMR and GC-MS) were carried out.

FT-IR ANALYSIS

The infrared spectrum (Figure 10) exhibited absorptions at 3200-3500 (br), 2921, 2360, 1540, 1398 which showed the presence of hydroxyl, alkyl and isopropyl groups.





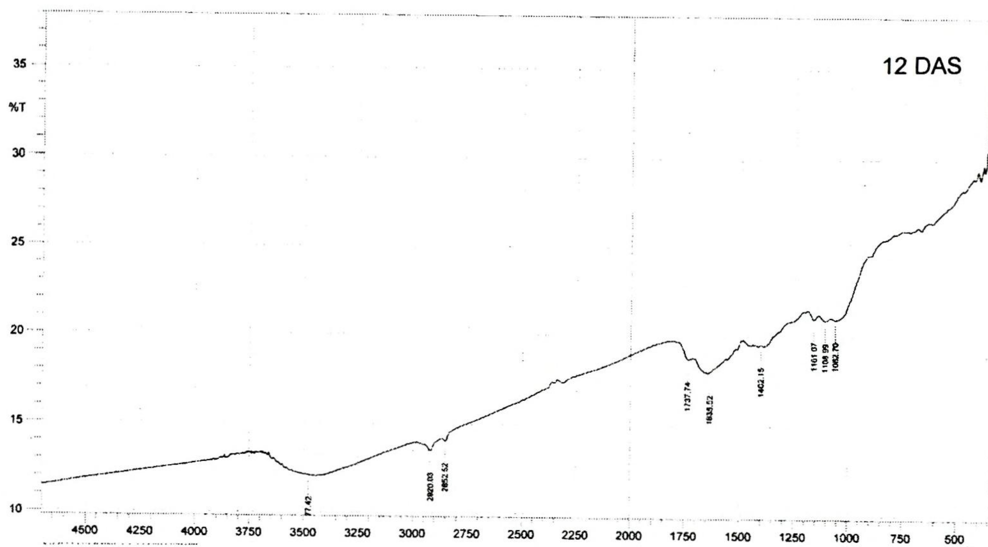


Figure - 10: FT-IR Analysis of *Triticum aestivum* leaves

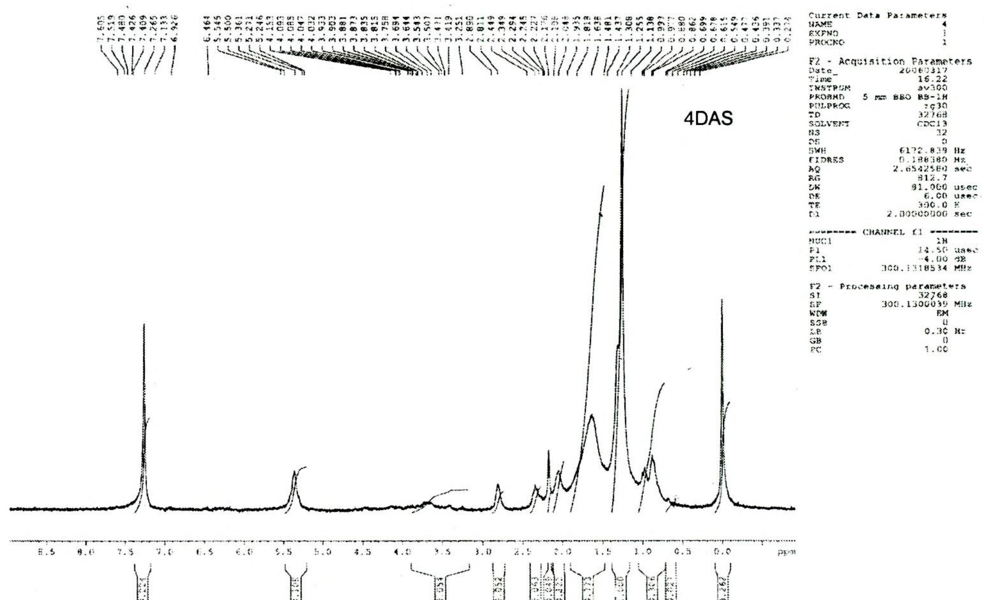
^{1}H-NMR ANALYSIS

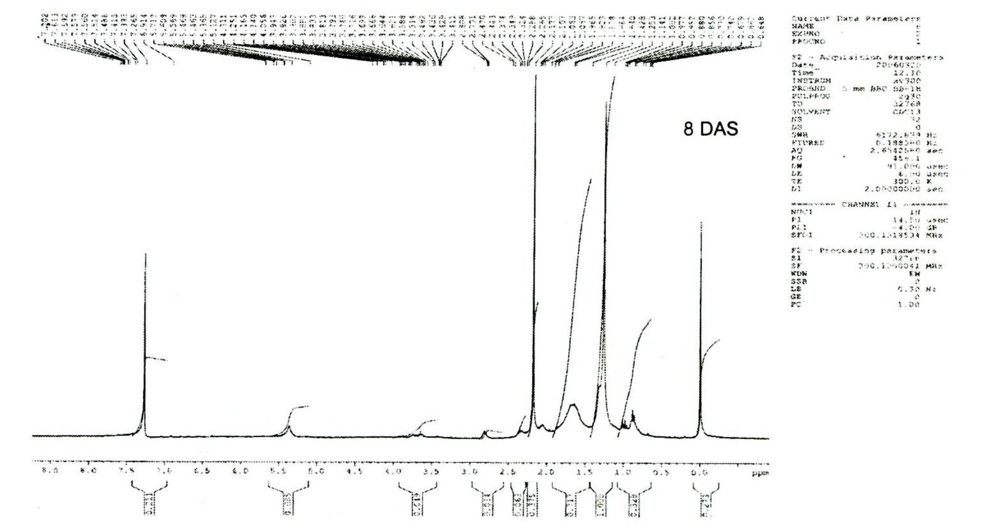
The ^{1}H spectrum of the crude extracts of *Triticum aestivum* leaves is shown in Fig. 11. The ^{1}H-NMR spectrum showed a doublet at δ5.36 and multiplet at δ3.54, which are the characteristic patterns of the Δ^5-3β-hydroxy steroids. The signals in the region δ0.67 - δ2.49 suggested the presence of $-CH_2$ and $-CH_3$ entity of the steroid nucleus. The peaks at δ0.69 and at δ1.02 were accountable for 18 $-CH_3$ and 19 $-CH_3$ groups respectively. From these data, it was inferred that the compound in all probability might be a steroid. The peaks in the aliphatic regions also showed the probability of the existence of alkaloids. GC-MS was conducted to confirm this inference.

GC-MS ANALYSIS

The GC-MS analysis of the crude extract was carried out to identify the nature of the components present. The presence of steroidal alkaloids were evident from the chromatogram of the crude extract (Fig. 12).

The mass spectrum showed peaks at *m/z* 414, 400, 269, 253, 251, 221, 215, 147, 131, 99, 77, 69 and 55 which demonstrated the presence of Δ^5-3β-hydroxy steroid nucleus with saturated side chains. The peaks suggested the presence of steroidal alkaloids and phytosterols. The suspected phytosterols were β-sitosterol and campesterol. Thus, the spectral studies identified the presence of steroidal alkaloids.





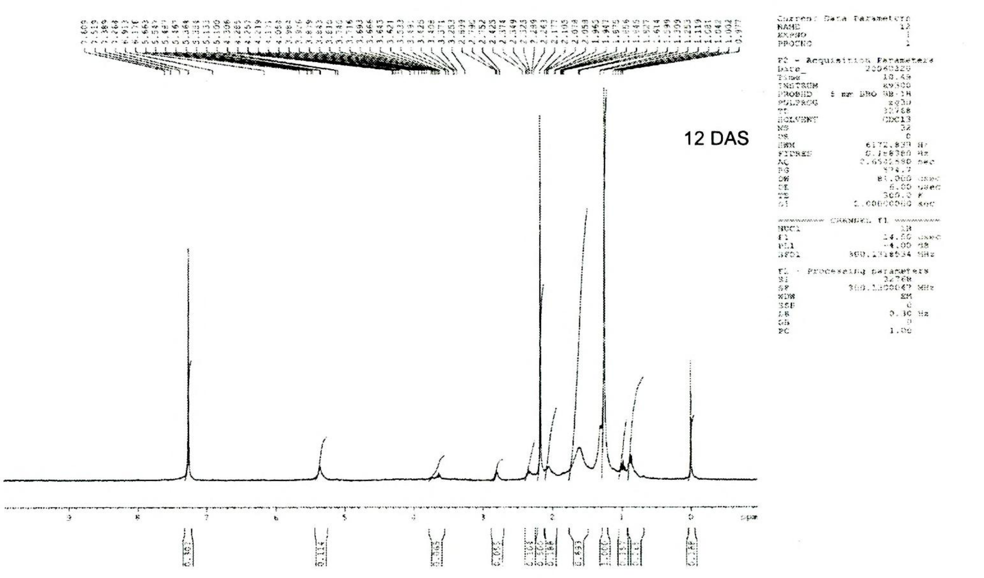
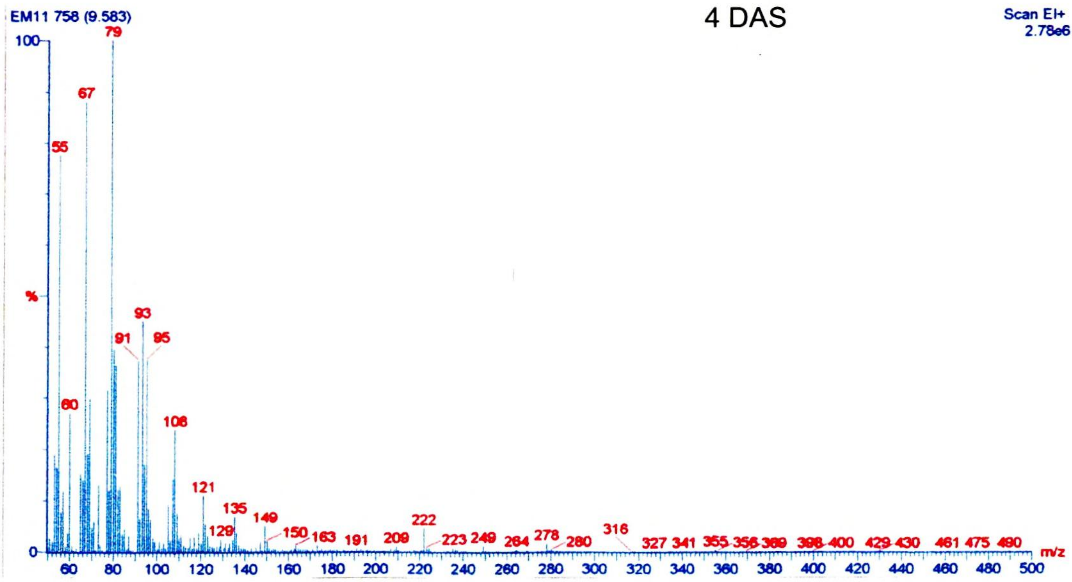
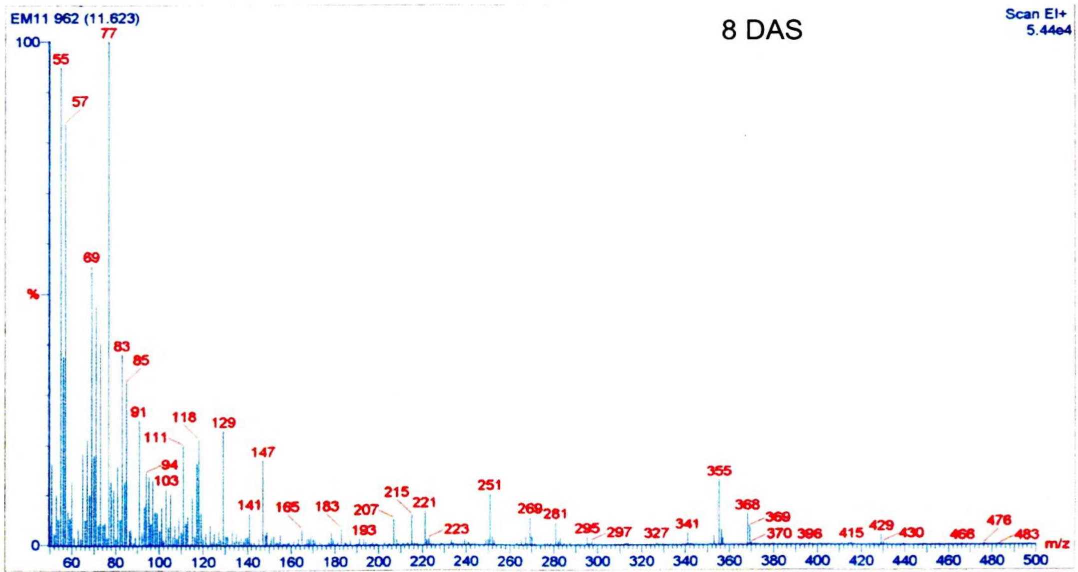


Figure - 11: ^{1}H-NMR Spectrum of *Triticum aestivum* leaves





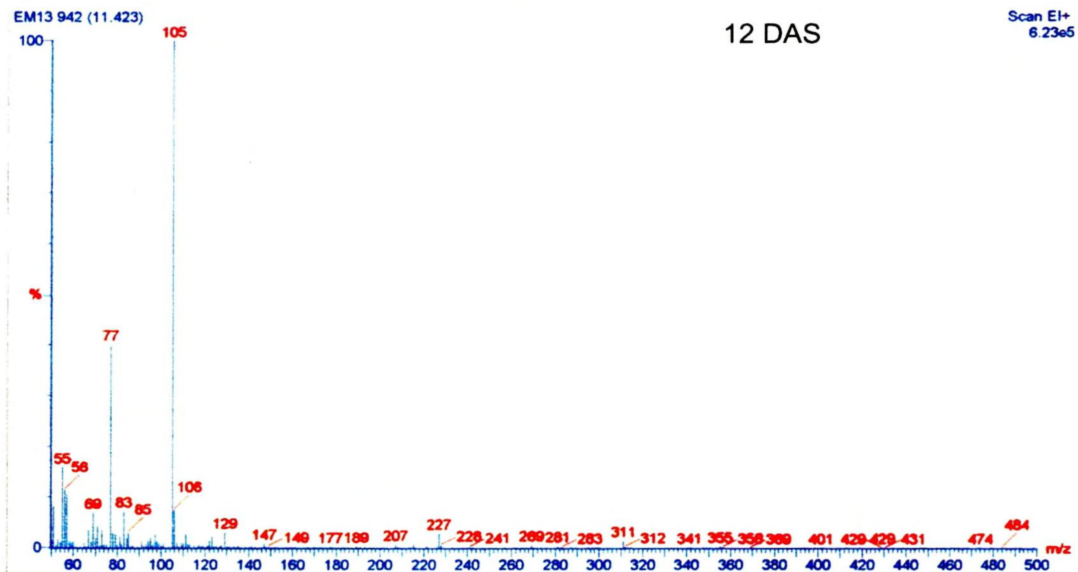


Figure-12: GC-MS Analysis of *Triticum aestivum* leaves

Thus, in the present study, the results of Phase I showed that the *Triticum aestivum* leaves as a rich source of antioxidants. The second phase proved the free radical scavenging properties and the antioxidant potential of the *Triticum aestivum* leaves under *in vitro* conditions This antioxidant effect was further confirmed under *in vivo* conditions in phase III. In this phase, the hepatoprotective and nephroprotective effects of the leaf extracts were also proved. The active principle rendering the antioxidant properties were probed in phase IV, which showed the presence of steroidal alkaloids as the major constituents. Further analyses are required to identify all the active principles involved.

The salient observations made in the study and the results presented in this chapter, are discussed in the light of the relevant available literature in the next chapter.