RESULTS

4.0 RESULTS

The plant *Solanum nigrum* which is used as a leafy vegetable was analyzed for its antioxidant and anticancer effects using various *in vitro* and *in vivo* models. This investigation was performed in four phases and the results obtained are presented in this chapter.

This research work was started with analyzing the antioxidant content (Phase I) of the leaves of two varieties of *Solanum nigrum* which are designated as BBL and RBL (for plants bearing black berries and red berries respectively).

PHASE I

EVALUATION OF ANTIOXIDANT CONTENT OF *Solanum nigrum* LEAVES

ENZYMIC ANTIOXIDANTS

The activities of enzymic antioxidants, SOD, CAT, Px, GPx, GR, G6PD, AAO and PPO were analysed in the leaves of the two varieties of *Solanum nigrum*. The results obtained are presented in Table 1.

TABLE 1

ACTIVITIES OF ENZYMIC ANIOXIDANTS IN *Solanum nigrum* LEAVES

PARAMETER		BBL	RBL
SOD (U^a/g)		8.90 ± 0.14*	6.13 ± 0.22
CAT (U^b/g)		74.98 ± 8.05*	54.05 ± 7.14
Px (U^c/g) x 10^2		3.05 ± 0.29	6.13 ± 0.71*
GPx (U^d/g) x 10^{-2}		2.10 ± 0.12	2.80 ± 0.15*
GR (U^e/g)		1.37 ± 0.05	3.33 ± 0.47*
G6PD (U^f/g)		3.79 ± 0.33	4.98 ± 0.46*
AAO (U^g/g)		50.78 ± 4.54	54.53 ± 3.51
PPO (U^h/g	Catechol oxidase	56.40 ± 1.27*	29.20 ± 1.26
	Laccase	50.40 ± 1.30*	26.20 ± 1.29

Values are mean ± SD n=6 *Statistically significant ($p<0.05$) compared to the other variety

[a]1unit - Activity of enzyme that exhibits 50% inhibition of NBT reduction/minute

[b]1unit - µmoles of H_2O_2 utilized/minute

[c]1unit - µmoles of pyrogallol oxidized/minute

[d]1unit - µmoles of GSH utilized/minute

[e]1unit - µmoles of NADPH oxidized/minute

[f]1unit - Activity of enzyme which causes a change in OD of 0.01/minute at 340nm

[g]1unit - Change in OD/minute at 620 nm

[h]1unit - Activity of catechol oxidase / laccase that transforms 1 unit of dihydrophenol to quinone/minute

Both the varieties of *Solanum nigrum* leaves exhibited considerable level of activities of the enzymic antioxidants tested. The SOD, CAT and PPO activities were significantly greater in BBL than in RBL. The activities of non-specific peroxidase (Px) and the glutathione dependent enzymes (GPx, GR, and G6PD) were significantly elevated in RBL when compared to BBL. There was no significant difference in AAO activity between the two varieties.

DETERMINATION OF NON-ENZYMIC ANTIOXIDANTS

The level of non-enzymic antioxidants such as ascorbic acid, tocopherol, total carotenoids, reduced glutathione, total phenols and chlorophyll were estimated and the values obtained are represented in Table 2.

TABLE 2

LEVELS OF NON-ENZYMIC ANTIOXIDANTS IN *Solanum nigrum* LEAVES

PARAMETER	BBL	RBL
Ascorbic acid (mg/g tissue)	6.67 ± 0.61	7.03 ± 0.47
Tocopherol (µg/g tissue)	0.34 ± 0.04*	0.22 ± 0.03
Total carotenoids (µg /g tissue)	50.09 ± 1.17*	26.47 ± 2.01
Glutathione (reduced) (µmoles/g tissue)x 10^{-2}	3.60 ± 0.21	5.70 ± 0.25*
Total phenols (mg/g tissue)	12.79 ± 0.41*	10.87 ± 0.65
Chlorophyll (mg/ g tissue)	1.85 ± 0.07*	1.60 ± 0.06

Values are mean ± SD n=6 * Statistically significant ($p<0.05$) compared to the other variety.

From the results it was observed that a significant difference was not observed in the ascorbate content between BBL and RBL. Tocopherol, total carotenoids, total phenols and chlorophyll recorded a significantly higher level in BBL than RBL. Glutathione content was significantly greater in RBL when compared to BBL.

QUANTIFICATION OF ANTIOXIDANT MINERALS

Selected antioxidant minerals, namely, Cu, Zn, Mn and Se were quantified and the results obtained are showed in Table 3.

TABLE 3

QUANTITY OF ANTIOXIDANT MINERALS IN THE *Solanum nigrum* LEAVES

PARAMETER	BBL	RBL
Copper (μg/g)	0.083 ± 0.053*	0.043 ± 0.015
Manganese (μg/g)	0.066 ± 0.011	0.081 ± 0.012
Zinc (μg/g)	0.960 ± 0.214	1.127 ± 0.315
Selenium (μg/g)	BDL	BDL

Values mean ± SD n=3 * Statistically significant ($p<0.05$) compared to the other variety

Copper was significantly higher in BBL than RBL. There was no significant difference in the levels of zinc and manganese between these two varieties. Selenium was below detectable level (BDL) in both the varieties.

The results obtained from the first phase showed that the leaves of the two varieties of *Solanum nigrum* were found to be good sources of antioxidants. In order to evaluate the antioxidant potential exhibited by the two varieties of *Solanum nigrum* leaves, they were analyzed for the antioxidant response evoked in various *in vitro* models. The results obtained are presented in the following phase.

PHASE II

In this phase, the following analyses were done.

- Scavenging effect of the leaves of *Solanum nigrum* on various sources of free radicals and non-radical oxidant molecules
- Protective effect on oxidative lipid and DNA damage
- Antioxidant response evoked in *in vivo* simulated *in vitro* model (liver slices)
- *In vitro* cytotoxic effect on selected cancer cell lines

SCAVENGING EFFECT OF *Solanum nigrum* LEAVES ON VARIOUS SOURCES OF FREE RADICALS AND OXIDANT MOLECULES

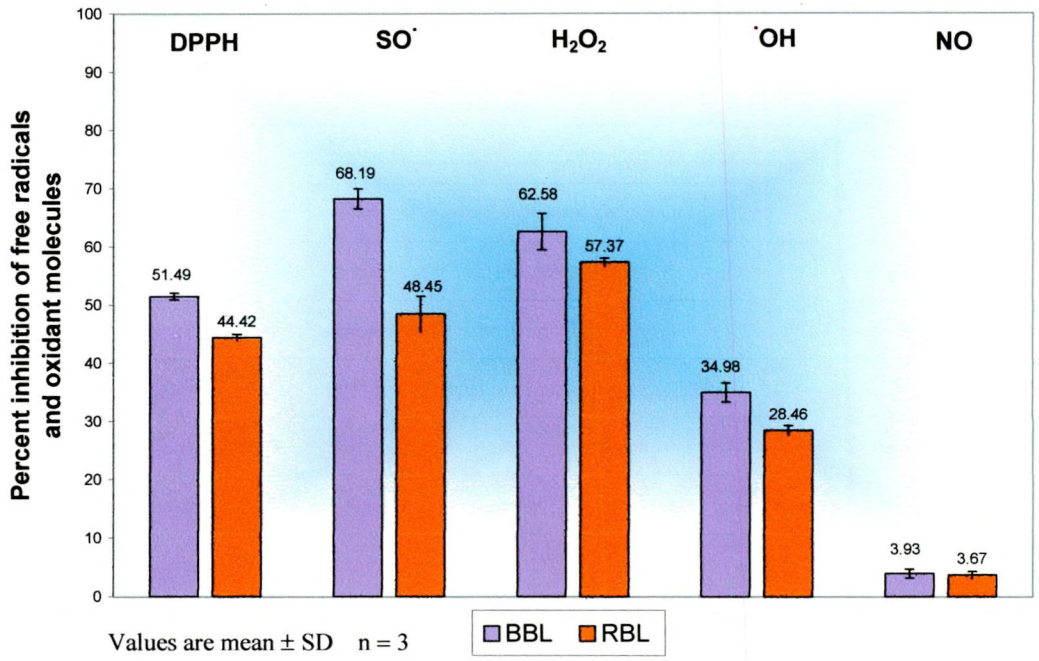
One of the major mechanisms by which antioxidants act is by scavenging the oxidants, thereby protecting the cellular environment from their harmful effects. Therefore, in the present study, the effect of the leaf extracts on a spectrum of oxidants namely, DPPH, $SO^{\bullet}$, H_2O_2, $^{\bullet}OH$ and NO were studied. The extent of inhibition of lipid peroxidative products (TBARS) formed were also determined in an *in vitro* system.

SCAVENGING OF FREE RADICALS

Quantification of DPPH radical scavenging activity of the crude aqueous extracts of BBL and RBL were analysed and these extracts were further subjected to various other experimental systems to analyse their effect on the generation of superoxide radical ($SO^{\bullet}$), scavenging of hydrogen peroxide (H_2O_2), quenching of hydroxyl radical ($^{\bullet}OH$) and generation of nitric oxide (NO). The results obtained are depicted in Figure 10.

FIGURE -10

EFFECT OF *Solanum nigrum* LEAVES ON FREE RADICALS AND OXIDANTS



The outcome of the experiments revealed that the crude aqueous extracts of BBL and RBL exhibited effective scavenging activity on DPPH, $SO^{\bullet}$ and $^{\bullet}OH$

radicals, and the non-radical H_2O_2 molecule. However, both the extracts did not show effective scavenging activity on NO generation.

EFFECT OF *Solanum nigrum* LEAVES ON OXIDATIVE DAMAGE TO BIOMOLECULES

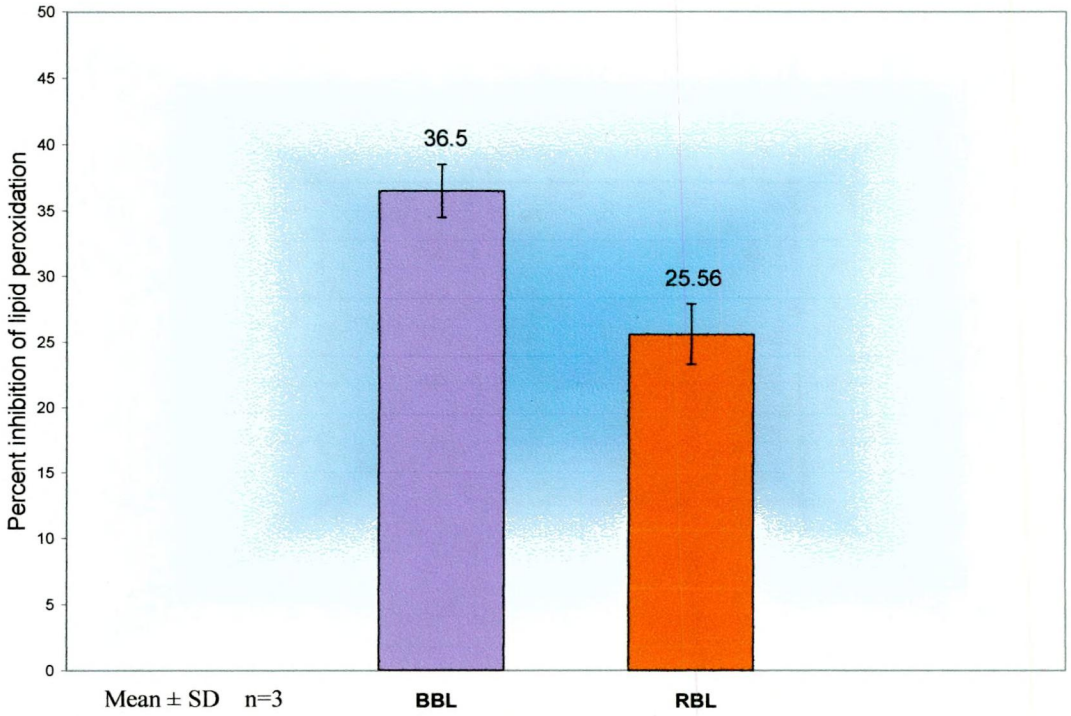
The primary targets of oxidants are the membrane lipids, as membrane is the macromolecular assembly that immediately comes in contact with the generated oxidants. The ultimate targets, however, are the DNA molecules, the damage of which result in mutations, which can manifest themselves into various diseases, including cancer.

EFFECT OF *Solanum nigrum* LEAVES ON LIPID PEROXIDATION

The extent of inhibition of lipid peroxidation by BBL and RBL was analysed using the goat liver homogenate and the results obtained are presented in Figure 11.

FIGURE -11

EFFECT OF *Solanum nigrum* LEAVES ON *in vitro* LIPID PEROXIDATION



Both the leaf extracts caused an effective inhibition on the extent of LPO and the BBL was found to exert a better protective effect than RBL.

PREVENTIVE EFFECT OF *Solanum nigrum* LEAVES ON OXIDATIVE DNA DAMAGE

A number of intrinsic and extrinsic mutagens induce structural damage in cellular DNA. These DNA damages are cytotoxic, miscoding or both and are believed to be the origin of cell lethality, tissue degeneration, ageing and cancer (Gros *et al.*, 2002). In the present study, the extent of DNA damage by the standard oxidant (H_2O_2) and the preventive action of the leaf extracts were evaluated using various sources of DNA, falling into different evolutionary hierarchies. The DNA sources used were λ DNA (linear, phage DNA), pUC 18 plasmid DNA (circular, bacterial DNA) and herring sperm DNA (haploid, high molecular weight genomic DNA). In addition, DNA of live cells was also studied.

EFFECT OF *Solanum nigrum* LEAVES ON DNA DAMAGE INDUCED BY H_2O_2 IN λ PHAGE DNA AND pUC 18 PLASMID DNA

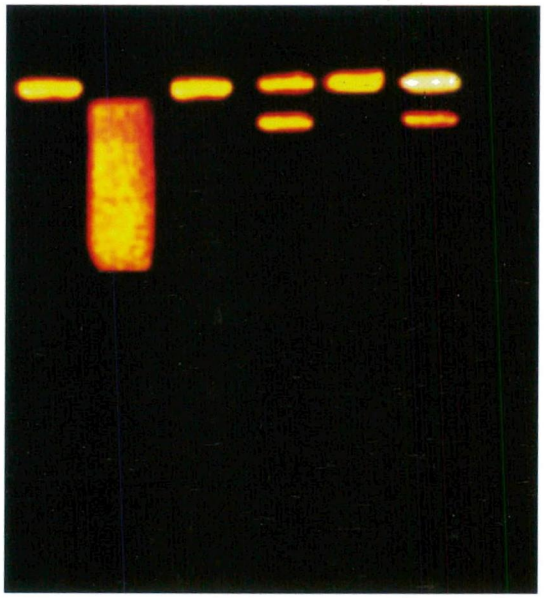
The migration pattern indicated that the λ phage DNA and pUC 18 plasmid DNA were severely damaged in the presence of H_2O_2, resulting in very small fragments that cannot be visualized on the gel (Plate 2). The aqueous extracts of BBL and RBL significantly reduced the DNA damage induced by H_2O_2 to λ phage DNA and pUC 18 plasmid DNA. The leaf extracts themselves did not cause any damage to λ phage DNA and pUC 18 plasmid DNA molecules.

EFFECT OF *Solanum nigrum* LEAVES ON THE DAMAGE INDUCED BY H_2O_2 TO HERRING SPERM DNA

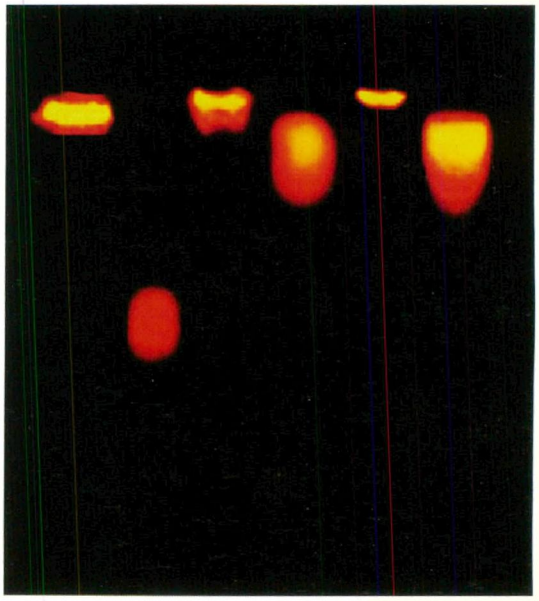
The preventive effect of *Solanum nigrum* leaves on DNA damage induced by H_2O_2 to herring sperm DNA was quantified spectrophotometrically and the results obtained are shown in Figure 12.

PLATE 2

MIGRATION PATTERN OF λ DNA AND pUC18 DNA TREATED WITH BBL AND RBL EXTRACTS IN THE PRESENCE AND ABSENCE OF H_2O_2



λDNA

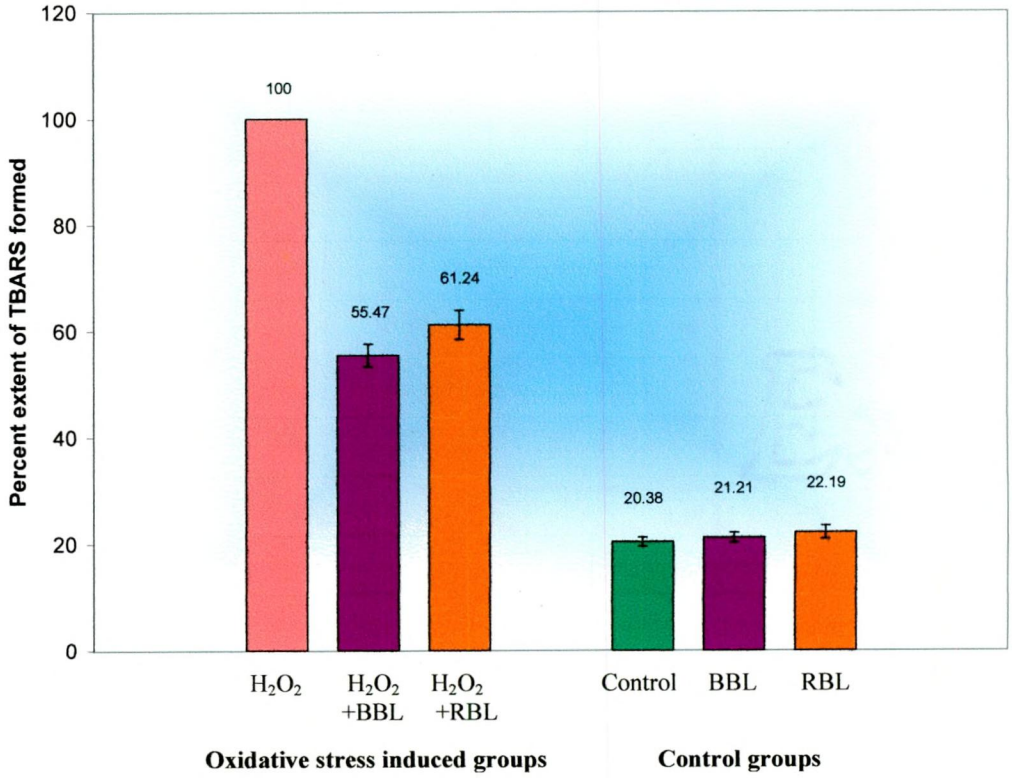


pUC18 DNA

Lane 1 - Control
Lane 2 - H_2O_2
Lane 3 - BBL alone
Lane 4 - BBL + H_2O_2
Lane 5 - RBL alone
Lane 6 - RBL + H_2O_2

Figure 12

EFFECT OF *Solanum nigrum* LEAVES ON H_2O_2 INDUCED DAMAGE TO HERRING SPERM DNA



Exposure of H_2O_2 to herring sperm DNA caused a significant DNA damage over control. Aqueous extracts of BBL and RBL counteracted the effect induced by H_2O_2. A marked protective effect was exhibited by BBL when compared to RBL.

EFFECT OF *Solanum nigrum* LEAVES ON DNA DAMAGE INDUCED BY H_2O_2 IN LIVE CELLS

The effect of *Solanum nigrum* leaf extracts on DNA damage induced by H_2O_2 in intact KB oral carcinoma cells was studied using the comet assay and the results are recorded in Table 4.

TABLE 4

EFFECT OF *Solanum nigrum* LEAVES ON H_2O_2-INDUCED DNA DAMAGE IN KB ORAL CARCINOMA CELLS

SAMPLE	NUMBER OF COMETS PER 100 CELLS	
	CONTROL	H_2O_2 – TREATED
No extract	12 ± 2	25 ± 2^{a}
BBL extract	10 ± 1	13 ± 1^{b}
RBL extract	11 ± 2	$16 \pm 2^{a,b}$

Values are mean ± SD n = 3 LSD (5%) = 3.081
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to H_2O_2 treated group

H_2O_2 caused a significant ($p<0.05$) increase in the number of cells bearing comets, demonstrating significant DNA damage. BBL and RBL extracts caused a marked decrease in the number of comet bearing cells. Additionally, and these extracts did not cause excess damage over the control, indicating that they are safe for use.

The results obtained in the above studies showed that the crude aqueous extracts of BBL and RBL were found to be effective in counteracting the oxidative damage to biological molecules (lipids and DNA) in cell free system, liver homogenate and intact cells. As a next step, the influence of the leaf extracts on antioxidant status of tissue slices was studied.

ANTIOXIDANT POTENTIAL OF *Solanum nigrum* LEAVES IN GOAT LIVER SLICES - AN *in vivo* SIMULATED *in vitro* MODEL

In order to reduce the number of animals in research, the scientists are encouraged to follow three Rs which include reduction, refinement and replacement (frame-uk.demon.co.uk).

Goat liver slices are used in this experiment because it is one of the alternative methods to replace the animal. In the present dissertation, the liver slices were carefully maintained in an *in vivo* simulated *in vitro* environment.

The antioxidant status of the crude aqueous extracts of *Solanum nigrum* leaves was analayzed by assaying enzymic antioxidants, determining the non-enzymic antioxidants and estimating the extent of lipid peroxidation in goat liver slices exposed to the standard oxidant CCl_4 in the presence and the absence of the leaf extracts.

ACTIVITY OF SUPEROXIDE DISMUTASE (SOD)

SOD, an important primary defense enzyme, catalyses the dismutation of superoxide radicals to oxygen and hydrogen peroxide. The change in the activity of SOD under the influence of the leaf extracts in the presense and absence of CCl_4 is represented in Table 5.

TABLE 5

EFFECT OF *Solanum nigrum* LEAVES ON SOD ACTIVITY IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	SOD ACTIVITY (Units*/ g tissue)	
	CONTROL	CCl_4 – TREATED
No extract	30.12 ± 1.68	21.73 ± 2.56^{a}
BBL extract	$47.90 \pm 2.24^{a,c}$	$46.71 \pm 4.32^{a,b,c}$
RBL extract	39.29 ± 3.02^{a}	$29.80 \pm 3.16^{b,d}$

Values are mean ± SD n=6 LSD (5%) = 3.848
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group
c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group
d – Statistically significant ($p<0.05$) compared to respective plant control

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

The liver slices assaulted with CCl_4 showed a significant ($p<0.05$) decrease in SOD activity. The treatment with BBL and RBL significantly elevated the levels but did not reverse them to the control value. BBL was found to exhibit a marked increase in the SOD activity than RBL when compared to untreated control and the CCl_4 treated groups. Both the extracts exhibited a significant increase in SOD activity over the untreated control.

ACTIVITY OF CATALASE (CAT)

Catalase, a heme protein, is considered biologically essential in the reduction of hydrogen peroxide. The influence of the crude aqueous extracts of *Solanum nigrum* leaves on the activity of CAT was studied in liver slices in the presence and absence of CCl_4. The results obtained are presented in Table 6.

TABLE 6

EFFECT OF *Solanum nigrum* LEAVES ON CAT ACTIVITY IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	CAT ACTIVITY (UNITS / g tissue)	
	CONTROL	CCl_4 – TREATED
No extract	358.95 ± 5.23	233.73 ± 25.11[a]
BBL extract	381.32 ± 12.01[a,c]	314.85 ± 6.46[a,b,c,d]
RBL extract	365.32 ± 7.94	280.29 ± 11.82[a,b,d]

Values are mean ± SD n=6 LSD (5%) = 15.579

a – Statistically significant ($p<0.05$) compared to untreated control group

b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group

c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group

d – Statistically significant ($p<0.05$) compared to respective plant control

1 Unit = μmoles of H_2O_2 utilized / minute

CCl_4 exposure significantly ($p<0.05$) reduced the activity of catalase. Treatment with BBL and RBL extracts significantly elevated these levels when compared to CCl_4 treatment group. BBL was found to possess better activity than RBL, in the presence and the absence of oxidative stress. BBL and RBL extracts significantly elevated the activity of catalase by themselves when compared to the untreated control.

ACTIVITY OF GLUTATHIONE PEROXIDASE (GPx)

Glutathione peroxidase is an another major antioxidant enzyme counteracting the action of oxidative molecules. Table 7 shows the changes in the activity of GPx in liver slices treated with leaf extracts and/or CCl_4.

TABLE 7

EFFECT OF *Solanum nigrum* LEAVES ON GPx ACTIVITY IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	GPx ACTIVITY (UNITS / g tissue)	
	CONTROL	CCl_4 - TREATED
No extract	0.088 ± 0.006	0.069 ± 0.004^{a}
BBL extract	0.087 ± 0.003^{c}	$0.084 \pm 0.046^{b,c}$
RBL extract	0.108 ± 0.002^{a}	0.092 ± 0.010

Values are mean ± SD n=6 LSD (5%) = 0.007

a – Statistically significant ($p<0.05$) compared to untreated control group

b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group

c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group

1 Unit = μ moles of GSH H_2O_2 utilized / minute

Exposure to CCl_4 showed a significant ($p<0.05$) depletion of the GPx activity, which was counteracted by the treatment with the extracts of BBL and RBL. RBL exhibited a significantly ($p<0.05$) greater activity than BBL in the presence and the absence of CCl_4. BBL and RBL treatments significantly elevated the GPx activity over untreated control groups.

ACTIVITY OF GLUTATHIONE S-TRANSFERASE (GST)

The GST family of enzymes catalyses the conjugation of a variety of structurally diverse compounds with reduced glutathione. The GST activity was analyzed and the results are reported in Table 8

GST activity was depleted upon exposure to CCl_4 ($p<0.05$). Extracts of BBL and RBL significantly improved the GST activity when compared to the CCl_4 treated group. RBL extract showed a significantly elevated activity of GST when compared to BBL treated group both in the presence and the absence of the CCl_4.

TABLE 8

EFFECT OF *Solanum nigrum* LEAVES ON GST ACTIVITY IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	GST ACTIVITY (UNITS / g tissue)	
	CONTROL	CCl_4 - TREATED
No extract	51.89 ± 0.97	30.92 ± 3.36[a]
BBL extract	58.51 ± 4.59[a,c]	35.49 ± 3.49[a,b,c,d]
RBL extract	65.84 ± 1.28[a]	49.47 ± 5.44[b,d]

Values are mean ± SD n=6 LSD (5%) = 4.211
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group
c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group
d – respective plant control

1 Unit = µmoles of CDNB conjugated / minute

ACTIVITY OF GLUTATHIONE REDUCTASE (GR)

GR is an important enzyme in maintaining the levels of thiol groups in the cells. Therefore, the activity of GR was assessed and the results obtained are shown in Table 9.

TABLE 9

EFFECT OF *Solanum nigrum* LEAVES ON GR ACTIVITY IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	GR ACTIVITY (UNITS / g tissue)	
	CONTROL	CCl_4 - TREATED
No extract	6.37 ± 1.36	4.65 ± 0.45[a]
BBL extract	7.04 ± 0.55	5.66 ± 0.55[b,c,d]
RBL extract	7.27 ± 0.55[a]	6.62 ± 0.17[b]

Values are mean ± SD n=6 LSD (5%) = 0.832
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group
c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group
d – respective plant control

1 Unit = µmoles of NADPH oxidized / minute

GR activity was significantly ($p<0.05$) reduced upon exposure to CCl_4. Treatment with the extracts of BBL and RBL exerted a significant increase in GR activity when compared to CCl_4 intoxicated group. Treatment with RBL extract exhibited significantly higher activity when compared with BBL extract treated group, both in the presence and absence of CCl_4. BBL and RBL extracts improved the GR activity when compared to untreated control.

ACTIVITY OF GLUCOSE 6-PHOSPHATE DEHYDROFERASE (G6PD)

G6PD is an important enzyme for the generation of NADPH, which is utilized for the regeneration of various antioxidant molecules. Table 10 the results obtained for the activity of G6PD in the various treatment groups.

TABLE 10

EFFECT OF *Solanum nigrum* LEAVES ON G6PD ACTIVITY IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	G6PD ACTIVITY (UNITS / g tissue)	
	CONTROL	CCl_4 – TREATED
No extract	2.19 ± 0.12	1.70 ± 0.71^{a}
BBL extract	$2.50 \pm 0.06^{a,c}$	$1.90 \pm 0.06^{a,d}$
RBL extract	2.74 ± 0.36^{a}	$1.99 \pm 0.05^{b,d}$

Values are mean ± SD n=6 LSD (5%) = 0.239
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group
d – Statistically significant ($p<0.05$) compared to respective plant control

1 Unit = Change in OD of 0.01 / minute

Exposure to CCl_4 showed a significant ($p<0.05$) depletion of G6PD activity. This effect was counteracted by the extracts of BBL and RBL. Significantly elevated levels of G6PD was observed in RBL and BBL extract treated groups than the untreated control.

The results indicated that the enzyme activities were modulated significantly by the crude aqueous extracts of BBL and RBL. This experiment was followed by the determination of non-enzymic antioxidants.

DETERMINATION OF NON-ENZYMIC ANTIOXIDANTS

In the present work, the effect of crude aqueous extracts of BBL and RBL on various non-enzymic antioxidants namely vitamins C, E and A, and reduced glutathione were estimated in the liver slices exposed to CCl_4.

VITAMIN C (ASCORBIC ACID) LEVEL

The results of the effect of the extracts of *Solanum nigrum* leaves on vitamin C levels exposed to CCl_4 are presented in Table 11.

TABLE 11

EFFECT OF *Solanum nigrum* LEAVES ON VITAMIN C LEVEL IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	VITAMIN C (mg / g tissue)	
	CONTROL	CCl_4 - TREATED
No extract	1.90 ± 0.05	1.09 ± 0.08^{a}
BBL extract	$2.65 \pm 0.04^{a,c}$	$1.56 \pm 0.04^{a,b,d}$
RBL extract	2.35 ± 0.44^{a}	$1.72 \pm 0.04^{b,d}$

Values are mean ± SD n=6 LSD (5%) = 0.218
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group
c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group
d – Statistically significant ($p<0.05$) compared to respective plant control

When the liver slices were stressed with CCl_4, the vitamin C level was reduced significantly ($p<0.05$). Extracts of BBL and RBL caused a significant elevation in vitamin C level when compared to untreated control and oxidative stress induced (CCl_4) groups. The vitamin C level was more pronounced in the RBL treated groups than in the BBL treated ones under conditions of oxidative stress.

VITAMIN E (TOCOPHEROL) LEVEL

The level of vitamin E obtained are presented in Table 12.

TABLE 12

EFFECT OF *Solanum nigrum* LEAVES ON VITAMIN E LEVEL IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	VITAMIN E (µg / g tissue)	
	CONTROL	CCl_4 - TREATED
No extract	114.58 ± 1.80	93.79 ± 2.39[a]
BBL extract	137.90 ± 8.55[a,c]	106.92 ± 1.54[a,b,c,d]
RBL extract	121.58 ± 4.48[a]	99.24 ± 0.353[a,b,d]

Values are mean ± SD n=6 LSD (5%) = 4.922
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group
c – Statistically significant ($p<0.05$) compared tocorresponding RBL extract treated group
d – Statistically significant ($p<0.05$) compared to respective plant control

The vitamin E content was significantly ($p< 0.05$) depleted in the liver slices assaulted with CCl_4. The levels were significantly higher in BBL and RBL extract treated groups than the CCl_4 intoxicated and untreated control groups. BBL extract treated group showed a more significant rise in the level of vitamin E than the RBL extract treated group.

VITAMIN A LEVEL

Vitamin A, a fat soluble vitamin, plays a role in trapping the peroxyl free radicals. The results observed are tabulated below (Table 13).

Significantly ($p<0.05$) lower levels of vitamin A were observed in CCl_4 assaulted lives slices. This level was improved significantly when the liver slices were treated with leaf extracts. BBL extracts exhibited significantly higher levels when compared to untreated control. Vitamin A content was more pronounced in BBL extract treated groups than the RBL extract treated ones.

TABLE 13

EFFECT OF *Solanum nigrum* LEAVES ON VITAMIN A LEVEL IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	VITAMIN A (mg/g tissue)	
	CONTROL	CCl_4 - TREATED
No extract	2.44 ± 0.11	0.72 ± 0.43^{a}
BBL extract	$2.95 \pm 0.09^{a,c}$	$1.73 \pm 0.06^{a,b,c,d}$
RBL extract	2.51 ± 0.10	$1.43 \pm 0.10^{a,b,d}$

Values are mean ± SD n=6 LSD (5%) = 0.101

a – Statistically significant ($p<0.05$) compared to untreated control group

b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group

c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group

d – Statistically significant ($p<0.05$) compared to respective plant control

REDUCED GLUTATHIONE (GSH) LEVEL

The level of reduced glutathione in the oxidant induced liver slices and the effect of the leaf extract on them are depicted in Table 14.

TABLE 14

EFFECT OF *Solanum nigrum* LEAVES ON GLUTATHIONE LEVEL IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

SAMPLE	GSH (μmoles / g tissue)	
	CONTROL	CCl_4 – TREATED
No extract	0.123 ± 0.0083	0.081 ± 0.0094^{a}
BBL extract	0.150 ± 0.0088^{a}	$0.116 \pm 0.0109^{a,b,d}$
RBL extract	0.160 ± 0.0112^{a}	$0.123 \pm 0.0064^{b,d}$

Values are mean ± SD n=6 LSD (5%) = 0.011

a – Statistically significant ($p<0.05$) compared to untreated control group

b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group

c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group

d – Statistically significant ($p<0.05$) compared to respective plant control

The level of reduced glutathione reflected the same trend as vitamin C. Reduced glutathione level dropped significantly ($p<0.05$) in the liver slices exposed to CCl_4.

A significant increase was observed in the liver slices treated with BBL and RBL extracts when compared with CCl_4 treated and untreated control groups. RBL extract treatment exhibited significantly higher levels of GSH than BBL treatment both, in the presence and absence of CCl_4.

LIPID PEROXIDATION (LPO)

Lipid peroxidation is the oxidative deterioration of lipids. A large number of toxic byproducts are formed in this process, which can be quantified in terms of thiobarbituric acid reactive substances (TBARS). The extent of LPO in the various treatment groups are presented in Table 15.

TABLE 15

EFFECT OF *Solanum nigrum* LEAVES ON THE LPO LEVEL IN CCl_4 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

TREATMENTS	LEVELS OF TBARS FORMED (nmoles of MDA formed /g tissue)	
	CONTROL	CCl_4 - TREATED
No extract	2.89 ± 0.23	3.45 ± 0.14^{a}
BBL extract	2.36 ± 0.03^{a}	$2.63 \pm 0.21^{a,b,c,d}$
RBL extract	2.54 ± 0.07^{a}	$2.90 \pm 0.38^{b,c}$

Values are mean ± SD n=6 LSD (5%) = 0.250

a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to CCl_4 alone treated group
c – Statistically significant ($p<0.05$) compared to corresponding RBL extract treated group
d – Statistically significant ($p<0.05$) compared to respective plant control

The extent of lipid peroxidation was found to be increased significantly ($p<0.05$) upon exposure to CCl_4. There was a significant drop in the extent of LPO, when the extracts of the leaves were administered along with CCl_4. An interesting observation made was that BBL and RBL extracts decreased even the basal levels of TBARS (compared to untreated control group).

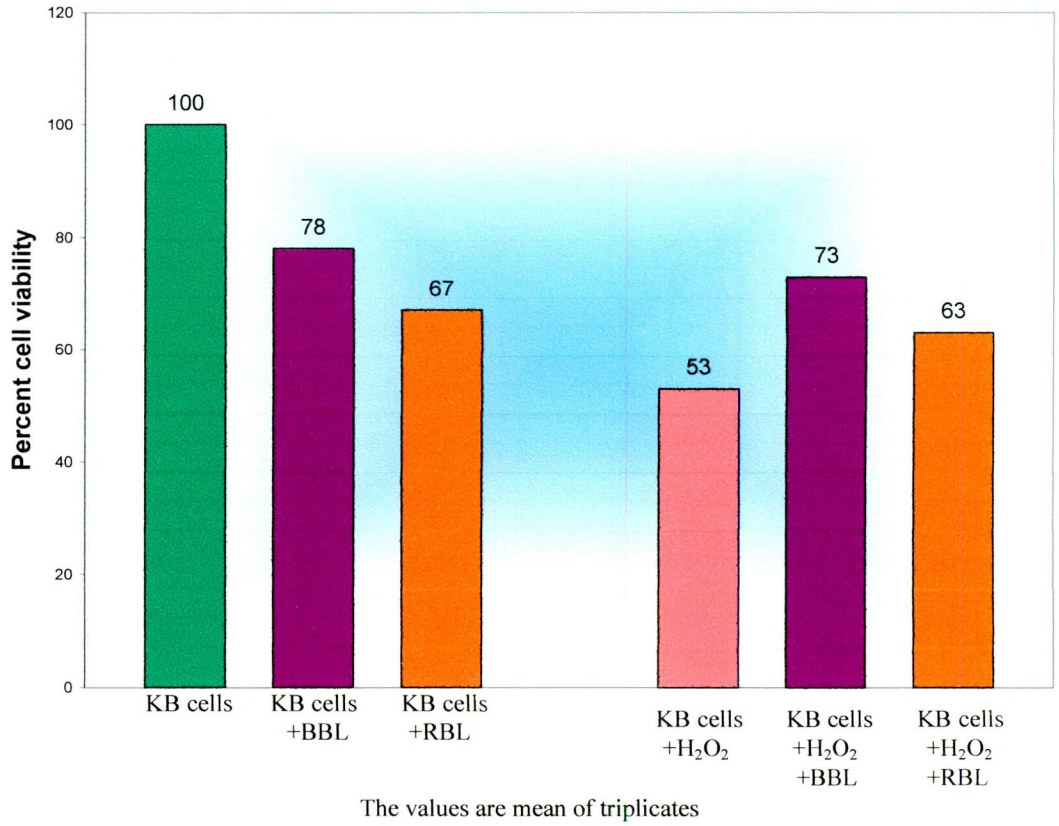
The outcome of the second phase revealed the marked efficiency of BBL and RBL extracts in scavenging free radicals and protecting the cellular biomolecules. The leaves improved the antioxidant (both enzymic and non-enzymic) status of the liver slices exposed to oxidative stress. Thus, the antioxidant protection offered by the leaves became evident in the *in vitro* systems.

In vitro CYTOTOXIC EFFECT OF *Solanum nigrum* LEAVES ON KB CELLS (Oral carcinoma cells)

The cytotoxic effect of the crude aqueous extracts of BBL and RBL using KB cells in the presence and absence of the oxidant, H_2O_2 was found. The results of the experiments are represented in Figure 13.

FIGURE 13

***In vitro* CYTOTOXIC EFFECT OF *Solanum nigrum* LEAVES ON KB CELLS**



The values are mean of triplicates

The absorbance of formazon in the control group was fixed as 100% viability and percent viability for other groups were calculated relative to this value.

BBL and RBL extracts were found to exhibit a considerable level of cytotoxic activity on KB (oral carcinoma cells) cell line, indicating their anticancer effect. Among the two, the RBL extract was found to possess a better cytotoxic effect than the BBL extract.

PHASE III

Since several factors like hormonal control, xenobiotic metabolism and interorgan interactions can interfere under *in vivo* conditions, the results obtained in the second phase needed to be confirmed using the *in vivo* system. Therefore, a study was conducted to confirm the antioxidant potential of the crude leaf extracts in experimental rats and anticancer effect in mice, which constituted the third phase of the study.

Female Wistar albino rats were used in this phase. Oxidative stress was induced by ethanol-CCl_4 system. Ethanol was administered for 20 days to activate the cytochrome P450 2E1 (CYP2E1) followed by a single subcutaneous administration of CCl_4 on the 21st day. This regime was planned in order to maximize the oxidative stress induced by CCl_4, at the same time avoiding the acute toxicity, by using a sub-acute dose. Since CCl_4 is converted to the oxidative moiety ($CCl_3^{\bullet}$) by CYP2E1, its induction with alcohol maximized the release of oxidative species from the subacute dose of CCl_4. The effect of leaf extracts were studied under unstressed and oxidant-stressed conditions and compared with a standard antioxidant, silymarin.

To assess the hepatoprotective activity, the liver function maker enzymes and circulating lipid profile were analysed in serum. Antioxidant status was determined by analyzing the enzymic and non-enzymic antioxidants, and lipid peroxidation products in the liver.

EFFECT OF *Solanum nigrum* LEAVES ON LIVER FUNCTION MARKER ENZMES AND LIPID PROFILE IN SERUM

The liver function marker enzymes analysed were transaminases (AST and ALT) and alkaline phosphatase (ALP). The results obtained are recorded in Tables 16, 17 and 18 respectively.

TABLE 16

EFFECT OF *Solanum nigrum* LEAVES ON THE SERUM AST ACTIVITIY IN ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	AST ACTIVITY (U/L)	
	CONTROL	ETHANOL + CCl_4
No extract	39.61 ± 1.37	128.19 ± 0.62[a]
BBL extract	41.76 ± 1.53	81.42 ± 1.97[a,b,c,d,e,f]
RBL extract	44.76 ± 1.24[a]	102.94 ± 1.80[a,b,d,e,f]
Silymarin	42.83 ± 1.51[a]	73.90 ± 3.99[a,b,e,f]

AST activity in ethanol alone treated group= 43.60 ± 0.39

Values are mean ± SD n=6 LSD (5%) = 2.182
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

TABLE 17

EFFECT OF *Solanum nigrum* LEAVES ON THE SERUM ALT ACTIVITIY IN ETHANOL- CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	ACTIVITY ALT (U/L)	
	CONTROL	ETHANOL + CCl_4
No extract	26.03 ± 0.52	65.97 ± 4.22[a,b]
BBL extract	27.78 ± 3.08[c]	37.33 ± 1.20 [a,b,c,d,e,f]
RBL extract	28.14 ± 2.52	45.26 ± 3.41[a,b,c,e,f]
Silymarin	26.82 ± 1.39	30.87 ± 1.89[a,b,c,f]

Ethanol alone treated group 31.21 ± 1.05

Values are mean± SD n = 6 LSD (5%) = 2.885
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

TABLE 18

EFFECT OF *Solanum nigrum* LEAVES ON THE ACTIVITY OF SERUM ALP IN ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	ALP ACTIVITY (U/L)	
	CONTROL	ETHANOL + CCl_4
No extract	75.53 ± 3.69	126.17 ± 2.61[a]
BBL extract	77.52 ± 3.78[a]	95.59 ± 1.36[a,b,c,d,e,f]
RBL extract	75.15 ± 3.86	102.95 ± 5.6[a,b,c,e,f]
Silymarin	75.98 ± 2.17[a]	85.62 ± 2.94[a,b,c]

Ethanol alone treated group 83.16±2.69

Values are mean± SD n = 6 LSD (5%) = 3.871
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

Ethanol administration significantly ($p<0.05$) elevated the activities and these levels were further augmented by CCl_4 treatment. Treatment with extracts of BBL and RBL brought down the activities significantly compared to oxidant stress induced groups. The best response was exhibited by silymarin. BBL extract was found to exert a better performance than the RBL extract.

The circulating levels of the lipids were also estimated and the values obtained are presented in Table 19 and 20.

The levels of triglycerides and cholesterol were significantly ($p<0.05$) elevated in ethanol alone and ethanol-CCl_4 treated groups. Treatment with BBL and RBL extracts brought down the levels significantly. Silymarin evoked the best response. Among the two extracts, BBL was found to have a better effect than RBL on cholesterol levels, while RBL evoked a better response on TG levels.

TABLE 19

EFFECT OF *Solanum nigrum* LEAVES ON THE LEVEL OF SERUM CHOLESTEROL IN ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	CHOLESTEROL (mg/dl)	
	CONTROL	ETHANOL+CCl_4
No extract	58.50 ± 2.07	92.17 ± 4.26[a]
BBL extract	61.17 ± 2.14	69.67 ± 3.78 [a,b,c,d,e,f]
RBL extract	60.17 ± 6.34	72.50 ± 3.62[a,b,c,e,f]
Silymarin	56.50 ± 4.09	65.67 ± 5.13[a,b,c,d,f]

Ethanol alone treated group = 79.00±3.23

Values are mean± SD n = 6 LSD (5%) = 4.714
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

TABLE 20

EFFECT OF *Solanum nigrum* LEAVES ON THE LEVEL OF SERUM TRIGLYCERIDES IN ETHANOL-CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	TRIGLYCERIDES (mg/dl)	
	CONTROL	ETHANOL+CCl_4
No extract	60.00 ± 5.97	109.17 ± 5.67[a]
BBL extract	60.33 ± 6.50	85.83 ± 4.75[a,c,d,e,f]
RBL extract	62.33 ± 4.08	74.17 ± 4.71[a,b,c,e,f]
Silymarin	59.33 ± 7.87	67.00 ± 7.77[a,b,c,f]

Ethanol alone treated group = 91.50±4.89

Values are mean ± SD n=6 LSD (5%) = 6.908
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

EFFECT OF *Solanum nigrum* LEAVES ON HEPATC ANTIOXIDANT STATUS IN OXIDATIVE STRESS INDUCED RATS

The antioxidant potential was assessed by analyzing the activities of oxidizing enzymes, enzymic and non-enzymic antioxidants, and the lipid peroxidative products in the liver.

ACTIVITIES OF CYTOCHROMES b_5 AND P450

The enzymes cytochrome b_5 and cytochrome P450 are found to play a major role in metabolizing the ethanol and CCl_4, and the levels were assessed in liver tissues. The results are depicted in Tables 21 and 22 respectively.

The levels of cytochrome b5 and cytochrome P450 were significantly ($p<0.05$) higher in ethanol alone and ethanol-CCl_4 treatments. Administration of the extracts of BBL and RBL, and silymarin significantly lowered the levels compared to oxidative stress induced animals.

TABLE 21

EFFECT OF *Solanum nigrum* LEAVES ON THE ACTIVITIES OF HEPATIC CYTOCHROME b_5 IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	Cytochrome b_5 (µmoles/mg protein)	
	CONTROL	ETHANOL + CCl_4
No extract	43.69 ± 2.92	119.12 ± 7.34[a]
BBL extract	43.79 ± 2.80	65.25 ± 3.79[a,b,c,d,e,f]
RBL extract	51.70 ± 3.85	79.58 ± 3.34[a,b,c,e,f]
Silymarin	47.54 ± 3.89	56.36 ± 3.59[a,b,c,d,f]

Ethanol alone treated group = 89.02 ± 3.23

Values are mean ± SD n=6 LSD (5%) = 4.733
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

TABLE 22

EFFECT OF *Solanum nigrum* LEAVES ON THE ACTIVITIES OF CYTOCHOROME P450 IN ETHANOL – CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	Cytochrome P450 (μmoles/mgprotein)	
	CONTROL	ETHANOL + CCl_4
No extract	75.98 ± 3.17	187.67 ± 9.05[a]
BBL extract	86.69 ± 4.89	106.13 ± 5.20[a,b,c,d,e,f]
RBL extract	94.64 ± 4.90	149.03 ± 5.32[a,c,e,f]
Silymarin	77.13 ± 5.11	95.44 ± 6.65[a,b,c,d,f]

Ethanol alone treated group = 146.31±5.36

Values are mean ± SD n=6 LSD (5%) = 6.651
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

ANTIOXIDANT ENZYMES

The antioxidant enzymes SOD, CAT, GPx and GST were assayed in the liver and the results obtained are presented below.

SOD

The results obtained for the activity of SOD in the various treatment groups are summarized in Table 23.

The activity of SOD was significantly ($p<0.05$) decreased in ethanol alone and ethanol CCl_4 treated groups. The administration of extracts of BBL, RBL or silymarin caused a significant increase when compared to oxidative stress induced groups. BBL extract treatment was significantly greater than RBL extract treatment. However, both the leaf extracts were not as effective as silymarin in inducing the activity of SOD.

TABLE 23

EFFECT OF *Solanum nigrum* LEAVES ON THE ACTIVITY OF HEPATIC SOD IN ETHANOL – CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	SOD (Units / mg protein)	
	CONTROL	ETHANOL +CCl_4
No extracts	2.87 ± 0.43	$1.69 \pm 0.10^{a,b}$
BBL extracts	3.26 ± 0.13^{a}	$2.63 \pm 0.17^{c,d,f}$
RBL extract	3.08 ± 0.17^{a}	$2.11 \pm 0.19^{a,b,c,e,f}$
Silymarin	4.90 ± 0.45^{a}	$2.88 \pm 0.18^{b,c,f}$

Ethanol alone treated group = 2.51 ± 0.03

Values are mean± SD n = 6 LSD (5%) = 0.308
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

1 unit = Activity of enzyme that causes 50% reduction in NBT oxidation

CAT

The activity of catalase of different treatment groups are depicted in Table 24.

Catalase activity reflected the same trend observed for the activity of SOD. The activities of catalase were significantly ($p<0.05$) decreased upon exposure to oxidative stress. This level was significantly raised in animals treated with the extracts of BBL and RBL, and silymarin. Treatment with BBL extract exerted significantly greater activities in the presence and absence of oxidative stress. Silymarin was found to have pronounced effect on catalase activity.

TABLE 24

EFFECT OF *Solanum nigrum* LEAVES ON THE ACTIVITY OF HEPATIC CAT IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	CAT ACTIVITY (Units/ mg protein)	
	CONTROL	ETHANOL +CCl_4
No extract	56.92 ± 3.17	38.79 ± 2.29[a]
BBL extract	62.78 ± 3.99[a]	51.61 ± 3.35[a,b,c,e,f]
RBL extract	57.44 ± 5.64	43.71 ± 2.98[a,b,d,e,f]
Silymarin	69.58 ± 2.90[a]	54.26 ± 0.18[b,c,f]

Ethanol alone treated group = 32.99±2.49

Values are mean± SD n = 6 LSD (5%) = 3.848
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

I Unit = µmoles of H_2O_2 utilized/minute

GPx

The activity of glutathione peroxidase in the liver of rats exposed to oxidative stress are summarized in Table 25.

From the results it was observed that ethanol treatment caused a significant decrease in the activity of GPx and a further significant depletion was obtained when assaulted with CCl_4. Treatment with the extracts of BBL and RBL, and silymarin caused a significant increase in the activity of GPx. Among the two leaf extracts, RBL was found to cause a slight increase in the activity compared to BBL

TABLE 25

EFFECT OF *Solanum nigrum* LEAVES ON THE ACTIVITY OF HEPATIC GPx IN ETHANOL –CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	GPx ACTIVITY (Units / mg protein)	
	CONTROL	ETHANOL +CCl_4
No extract	21.96 ± 0.93	15.83 ± 0.30[a]
BBL extract	20.30 ± 1.09	18.03 ± 0.41[a,c,e,f]
RBL extract	21.41 ± 1.77	19.15 ± 0.85[a,b,c,f]
Silymarin	24.74 ± 1.03	20.08 ± 0.17[a,b,c,f]

Ethanol alone treated group = 17.94± 0.12

Values are mean± SD n = 6 LSD (5%) = 1.140
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

I Unit = µmoles of GSH utilized/minute

GST

GST, a xenobiotic conjugating enzyme, was assayed in the liver of rats subjected to the different treatments. The results obtained are presented in Table 26.

The results obtained for the activity of GST followed the same trend as GPx. Significant ($p<0.05$) decrease in the activity of GST was observed in ethanol-CCl_4 treated groups. These effects were effectively counteracted by the administration of leaf extracts and silymarin. Treatment with RBL extract showed a significantly greater induction in GST activity compared to the treatment with BBL extract.

TABLE 26

EFFECT OF *Solanum nigrum* LEAVES ON THE ACTIVITY OF HEPATIC GST IN ETHANOL – CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	GST ACTIVITY (Units / mg protein)	
	CONTROL	ETHANOL + CCl_4
No extract	2.97 ± 0.14	$0.78 \pm 0.06^{a,b}$
BBL extract	2.64 ± 0.39	$1.15 \pm 0.78^{a,b,c,d,f}$
RBL extract	3.31 ± 0.34	$1.64 \pm 0.81^{a,b,d,e,f}$
Silymarin	4.41 ± 0.31^{a}	$2.49 \pm 0.17^{a,b,e,f}$

Ethanol alone treated group = 1.12 ± 0.15

Values are mean± SD n = 6 LSD (5%) = 0.182
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

I Unit = µmoles of CDNB conjugated/minute

NON-ENZYMIC ANTIOXIDANTS

The non-enzymic antioxidants analyzed were vitamin C, vitamin E, vitamin A and GSH.

VITAMIN C

The levels of vitamin C in the various treatment groups are presented in Table 27.

Ethanol, by itself, caused a significant decrease in the levels of vitamin C. A further significant ($p<0.05$) decrease was observed with CCl_4 treatment. Co-administration of the leaf extracts and silymarin caused a significant elevation in the levels of vitamin C. Significant difference was not observed between the BBL and RBL extracts treatment.

TABLE 27

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC VITAMIN C LEVEL IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	VITAMIN C (mg/g tissue)	
	CONTROL	ETHANOL+CCl_4
No extract	9.89 ± 0.42	$7.22 \pm 0.14^{a,b}$
BBL extract	9.97 ± 0.32	$8.22 \pm 0.12^{a,b,c,d,e,f}$
RBL extract	9.88 ± 0.16	$8.65 \pm 0.12^{a,b,c,e}$
Silymarin	10.15 ± 0.18	$8.89 \pm 0.11^{a,c,f}$

Ethanol alone treated group = 9.02 ± 0.21

Values are mean± SD n = 6 LSD (5%) = 0.259
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

VITAMIN E

The levels of vitamin E obtained in the different treatment groups are given in Table 28.

TABLE 28

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC VITAMIN E LEVEL IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	VITAMIN E (µg / g tissue)	
	CONTROL	ETHANOL +CCl_4
No extract	112.48 ± 2.83	$77.69 \pm 2.61^{a,b}$
BBL extract	$116.34 \pm 2.56^{a,c}$	$93.02 \pm 1.56^{a,b,c,d,e,f}$
RBL extract	112.81 ± 3.34	$87.93 \pm 0.71^{a,b,c,e,f}$
Silymarin	123.26 ± 2.86^{a}	$95.88 \pm 2.27^{a,b,c,f}$

Ethanol alone treated group = 99.43 ± 2.95

Values are mean± SD n = 6 LSD (5%) = 2.940
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

The values portray that the administration of ethanol-CCl_4 caused a significant ($p<0.05$) decrease in vitamin E levels. Simultaneous treatment with BBL and RBL extracts, and silymarin significantly elevated the level when compared to the oxidative stress induced groups with the plant or antioxidant supplement. BBL extract treatment showed significantly higher levels than RBL extract treatment.

VITAMIN A

The levels of vitamin A analyzed are depicted in Table 29.

TABLE 29

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC VITAMIN A LEVEL IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	VITAMIN A (mg / g tissue)	
	CONTROL	ETHANOL +CCl_4
No extract	1.21 ± 0.71	$0.51 \pm 0.05^{a,b}$
BBL extract	1.22 ± 0.57	$0.81 \pm 0.05^{a,c,d,e,f}$
RBL extract	1.10 ± 0.09	$0.63 \pm 0.03^{a,b,c,e,f}$
Silymarin	1.65 ± 0.13^{a}	$1.00 \pm 0.12^{a,b,c,f}$

Ethanol alone treated group = 0.81 ± 0.052

Values are mean± SD n = 6 LSD (5%) = 0.091
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

Results obtained for vitamin A reflected the same trend as that of vitamin E. The levels decreased significantly ($p<0.05$) upon exposure to ethanol-CCl_4. These levels were found to be improved significantly in the presence of leaf extracts and silymarin. Among the leaf extracts, BBL extract treatment was found to result in a higher quantity of vitamin A.

GSH

The levels of reduced glutathione in the different treatment groups are shown in Table 30.

TABLE 30

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC REDUCED GLUTATHIONE (REDUCED) LEVEL IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	GSH (nmoles / g tissue)	
	CONTROL	ETHANOL + CCl_4
No extract	3.24 ± 0.14	$1.80 \pm 0.39^{a,b}$
BBL extract	3.38 ± 0.18	$2.63 \pm 0.92^{a,c,e,f}$
RBL extract	3.45 ± 0.16^{a}	$2.83 \pm 0.15^{a,b,c,e,f}$
Silymarin	3.89 ± 0.07^{a}	$3.16 \pm 0.09^{b,c,f}$

Ethanol alone treated group = 2.57 ± 0.09

Values are mean± SD n = 6 LSD (5%) = 0.206
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

From the tabulated values, it was found that oxidative stress caused a significant ($p<0.05$) reduction in the level of glutathione. These levels were significantly elevated by the co-administration of the leaf extracts as well as silymarin. However, only silymarin was effective in bringing the values on par with the untreated controls.

LIPID PEROXIDATION

Lipid peroxidation was measured in terms of their products formed, namely conjugated dienes, hydroperoxides and malondialdehyde. The results obtained are presented in Tables 31, 32 and 33 respectively.

TABLE 31

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC CONJUGATED DIENES IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	LEVELS OF CONJUGATED DIENES (nmoles of CD formed / mg protein)	
	CONTROL	ETHANOL + CCl_4
No extract	0.262 ± 0.010	0.647 ± 0.021[a,b]
BBL extract	0.248 ± 0.049	0.430 ± 0.037[a,b,c,d,e,f]
RBL extract	0.280 ± 0.014	0.468 ± 0.041[a,b,c,e,f]
Silymarin	0.262 ± 0.008	0.324 ± 0.009[a,c,f]

Ethanol alone treated group = 0.316 ± 0.008

Values are mean± SD n = 6 LSD (5%) = 0.027
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

TABLE 32

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC HYDROPEROXIDES IN ETHANOL – CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	LEVELS OF HYDROPEROXIDES (nmoles of HP formed / mg protein)	
	CONTROL	ETHANOL + CCl_4
No extract	0.206 ± 0.005	0.600 ± 0.044[a,b]
BBL extract	0.220 ± 0.018	0.317 ± 0.022[a,c,d,e,f]
RBL extract	0.230 ± 0.031	0.423 ± 0.031[a,b,c,e.f]
Silymarin	0.203 ± 0.005	0.244 ± 0.007[a,b,c.f]

Ethanol alone treated group = 0.307±0.0163

Values are mean± SD n = 6 LSD (5%) = 0.0273
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

TABLE 33

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC LIPID PEROXIDATION IN ETHANOL–CCl_4 INDUCED OXIDATIVE STRESS IN RATS

TREATMENTS	LEVELS OF TBARS FORMED (nmoles MDA / mg protein)	
	CONTROL	ETHANOL + CCl_4
No extract	0.52 ± 0.009	$0.96 \pm 0.026^{a,b}$
BBL extract	0.52 ± 0.014	$0.69 \pm 0.010^{a,b,c,d,e,f}$
RBL extract	0.55 ± 0.020^{a}	$0.78 \pm 0.011^{a,b,c,e,f}$
Silymarin	0.39 ± 0.013^{a}	$0.49 \pm 0.015^{a,b,c,f}$

Ethanol alone treated group = 0.623±0.008

Values are mean± SD n = 6 LSD (5%) = 0.017
a – Statistically significant ($p<0.05$) compared to untreated control group
b – Statistically significant ($p<0.05$) compared to ethanol alone treated group
c – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 treated group
d – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + RBL extract treated group
e – Statistically significant ($p<0.05$) compared to ethanol + CCl_4 + silymarin treated group
f – Statistically significant ($p<0.05$) compared to respective plant control / silymarin

The levels of lipid peroxidative products, namely CD, HP and TBARS, were found to be significantly ($p<0.05$) increased in ethanol treated group and further augmentation was observed in the ethanol-CCl_4 treated group. Co-administration of leaf extracts and silymarin caused a significant reduction. Silymarin was found to be the most potent antioxidant among the three treatments. Among the two extracts, BBL treatment was found to have significantly better antiperoxidative activity.

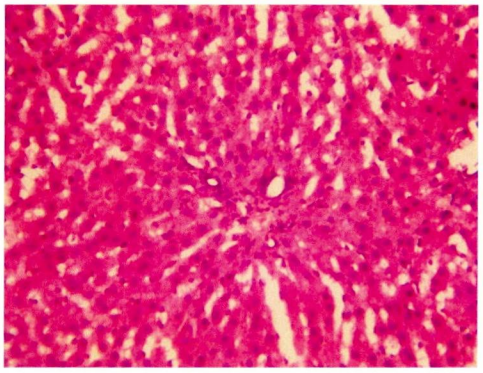
EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC HISTOLOGY

Histopathological pictures of the liver of various treatment groups are presented in plate 3.

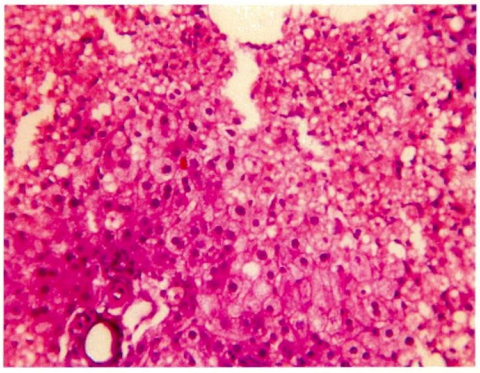
Control rat liver showed normal hepatocytes, normal portal tracts and normal central vein. The liver of the rats treated with ethanol-CCl_4 (oxidative stress) showed hepatocellular necrosis, fatty changes in hapatocytes and there is sparing of hepatocytes around the portal tract. When the oxidative stress was induced in rats treated with BBL and RBL, the abnormally modified histological architecture was found to be lesser when compared to ethanol-CCl_4 treated group.

PLATE 3

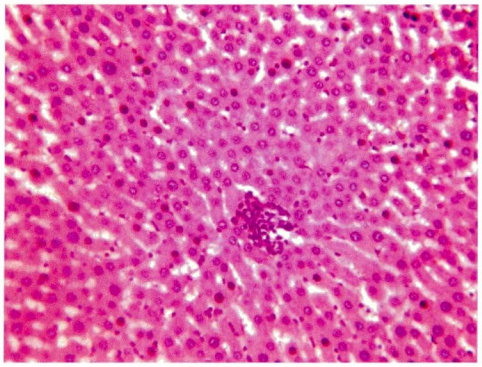
HISTOPATHOLOGICAL ARCHITECTURE IN THE LIVER OF CONTROL AND EXPERIMENTAL RATS (H & E 100x)



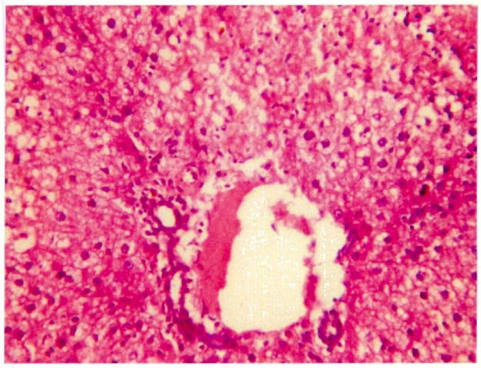
CONTROL



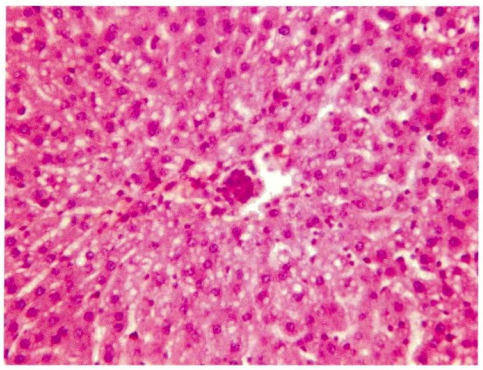
ETHANOL + CCl_4



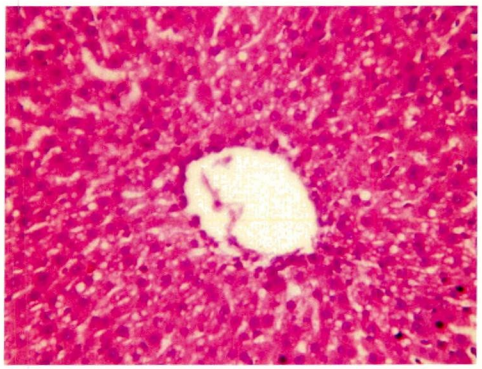
BBL ALONE



ETHANOL + CCl_4 + BBL



RBL ALONE



ETHANOL + CCl_4 + RBL

They exhibited considerable reduction in necrosis and fatty changes with sparse periportal lymphocytic infiltration. The silymarin-treated group showed almost normalisation of fatty accumulation and necrosis. The liver sections of rats treated with BBL and RBL alone showed the presence of normal hepatocytes, portal tract and central vein.

The results of the phase III obtained revealed the leaves of *Solanum nigrum* to exhibit a strong antioxidant activity against the oxidative stress induced by ethanol-CCl_4. Results from the earlier phase also showed that the leaves could protect lipids and DNA against oxidative damage. It is known that diseases like cancer result from repeated assault to DNA, oxidative damage being predominant among them. Thus, in the next step of the study, the possible antioxidant effect of the leaf extracts was analyzed in experimental animals against Dalton's Lymphoma Ascites (DLA) tumour.

EFFECT OF *Solanum nigrum* LEAVES ON DLA TUMOUR INDUCED IN SWISS ALBINO MICE

DLA cells were administered to Swiss albino mice in a single injection. Plant extracts were administered as explained in the experimental procedure section. The animals were monitered for excessive weight loss, weakness and peritoneal cavity swelling (Plate 4), which indicated tumour development.

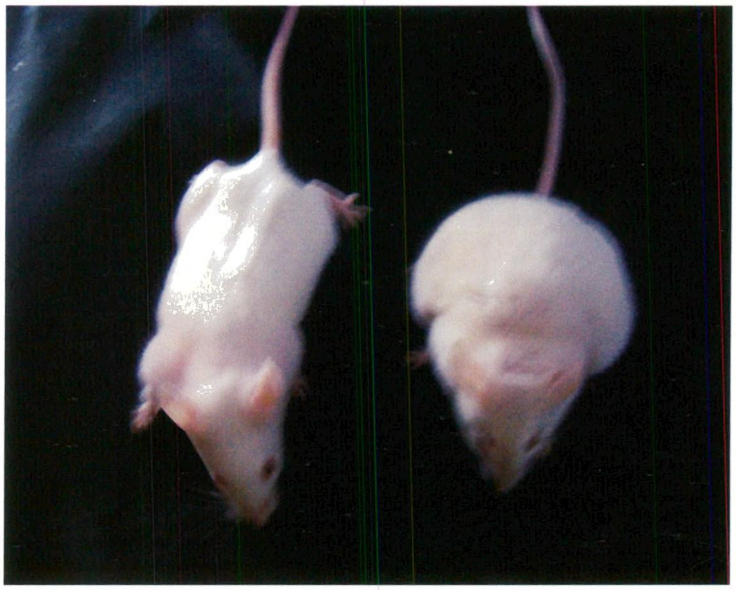
MEAN SURVIVAL PERIOD

The mean survival time was recorded in the experimental set up containing four animals in each group (Table 34).

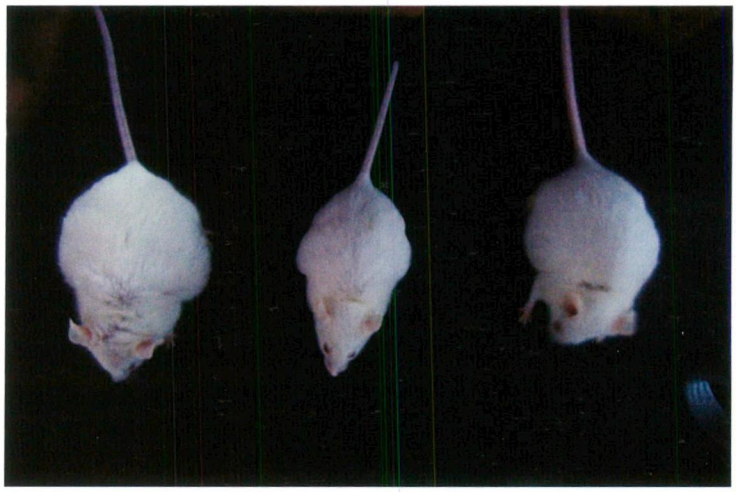
The DLA tumour induced animals showed a high mortality rate compared to the other groups. The tumour-transplantated mice survived for 24 days. The survival time significantly increased to 45 days and 39 days when treated with BBL and RBL respectively.

PLATE 4

DLA TUMOUR BEARING MICE



NORMAL DLA



DLA DLA + BBL DLA + RBL

TABLE 34

EFFECT OF *Solanum nigrum* LEAVES ON THE LIFE SPAN OF DLA TUMOUR INDUCED MICE

TREATMENT GROUPS	DAYS AFTER INDUCTION (x/y)									
	5	10	15	20	25	30	35	40	45	50
DLA control	4/4	4/4	4/4	4/4	1/4	0/4	0/4	0/4	0/4	0/4
DLA + BBL extract	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	0/4
DLA + RBL extract	4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4	0/4
Untreated control	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
BBL extract alone	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
RBL extract alone	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4

x/y: x = number of live animals y = total number of animals

TABLE 35

EFFECT OF *Solanum nigrum* LEAVES ON MEAN SURVIVAL TIME AND PERCENT INCREASE IN LIFE SPAN

TREATMENTS	MEAN SURVIVAL PERIOD (DAYS)	INCREASE IN LIFE SPAN	
		PERCENT LIFE SPAN	% INCREASE IN LIFE SPAN
DLA	24.25 ± 0.50	100.00	-
DLA+BBL	45.25 ± 1.50*	184.69	84.69
DLA+RBL	39.75 ± 2.22*	162.24	62.24

Mean ± SD n = 4 LSD (5%) = 2.515 * Statistically significant ($p<0.05$) compared to DLA group

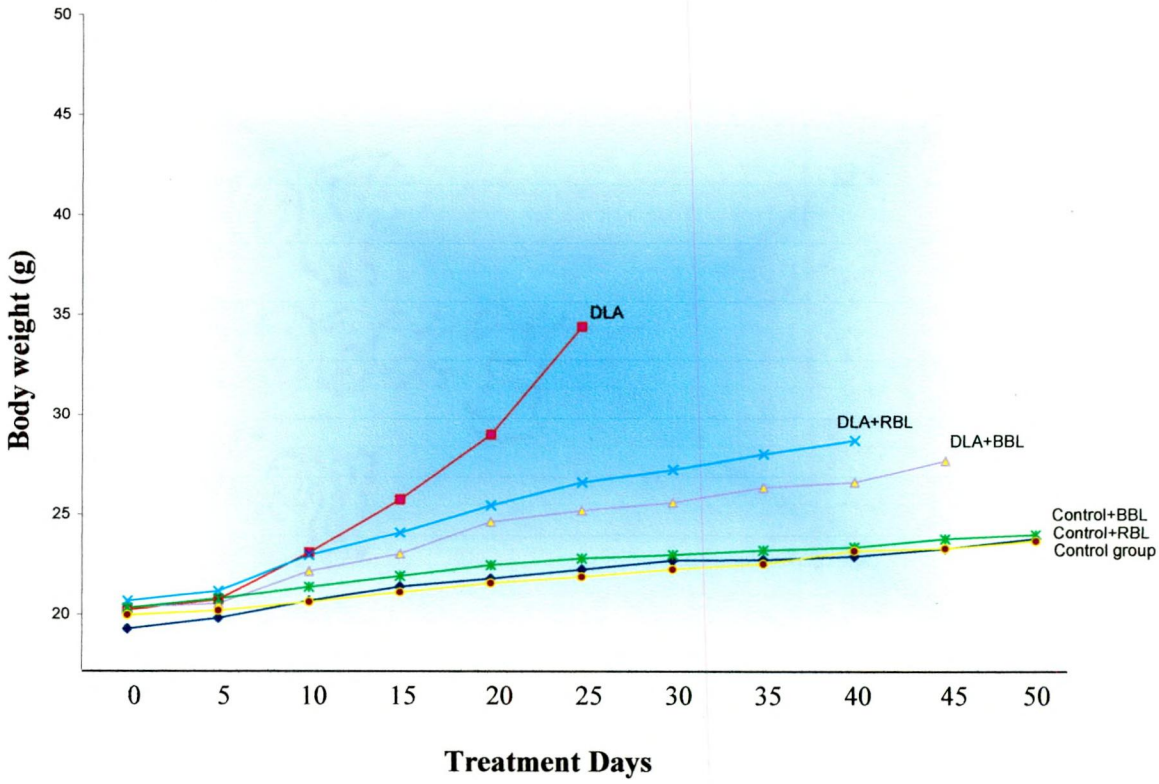
Comparatively, the mean survival time was found to be more in BBL treated DLA tumour bearing mice than the RBL treated group. The control groups treated with both the extracts survived the entire experimental period and even after that for a prolonged period when compared to the DLA tumour induced animals.

BODY WEIGHT

The body weight of the experimental mice were recorded both in the control, DLA tumour induced and extract treated groups from the day of the DLA tumour inducement and on every fifth day during the treatment period. The results obtained are presented in Figure 14.

FIGURE 14

EFFECT OF *Solanum nigrum* LEAVES ON THE BODY WEIGHT OF THE DLA TUMOUR INDUCED MICE



Following the tumour transplantation, an increase in the belly size and body weight with sluggish movement was observed from the fifth to sixth day onwards. There was a significant increase in the body weight in the DLA tumour induced mice. Simultaneous treatment with BBL and RBL extracts tremendously reduced the body weight. Better effect was observed in BBL treated than the RBL treated groups. There was no significant change in the body weight of the mice in normal group and there was not much difference in the body weight of the control mice treated with BBL and RBL extracts.

HEMATOLOGICAL PARAMETERS

Another experimental set up, each group containing six mice, was terminated on 24th day. The animals were sacrificed by cervical dislocation and the blood was collected immediately by cardiac puncture. Hematological parameters,

namely hemoglobin content, total RBC count, total and differential WBC count were determined in the whole blood. The results are presented in Tables 36, 37 and 38 respectively and in Figure 15.

TABLE 36

EFFECT OF *Solanum nigrum* LEAVES ON HEMOGLOBIN LEVEL IN DLA TUMOR INDUCED MICE

TREATMENTS	Hb LEVEL (g/dl)	
	CONTROL	DLA INDUCED
No extract	15.31 ± 0.79	5.99 ± 0.37[a]
BBL extract	15.12 ± 0.44	8.89 ± 0.50[a,b,c]
RBL extract	14.90 ± 0.53	7.23 ± 0.32[a,b]

Values are mean ± SD (n=6) LSD (5%) = 0.607
a – Statisfically significant ($p<0.05$) compared to untreated control group
b – Statisfically significant ($p<0.05$) compared to DLA tumour induced group
c – Statisfically significant ($p<0.05$) compared to RBL extract treated group

TABLE 37

EFFECT OF *Solanum nigrum* LEAVES ON RED BLOOD CELL COUNT IN DLA TUMOR INDUCED MICE

TREATMENTS	Total RBC Count ($1x10^6/mm^3$)	
	CONTROL	DLA INDUCED
No extract	5.24 ± 0.44	2.00 ± 0.230[a]
BBL extract	6.18 ± 0.36	3.03 ± 0.151[a,b,c]
RBL extract	4.93 ± 0.10	2.34 ± 0.104[a,b]

Values are mean ± SD (n=6) LSD (5%) = 0.312
a – Statistically significant ($p<0.05$) compared to untreated control
b – Statistically significant ($p<0.05$) compared to DLA tumour induced group
c – Statistically significant ($p<0.05$) compared to RBL extract treated group

TABLE 38

EFFECT OF *Solanum nigrum* LEAVES ON TOTAL WBC COUNT IN DLA TUMOUR INDUCED MICE

TREATMENTS	WBC COUNT ($1x10^3/mm^3$)	
	CONTROL	DLA INDUCED GROUP
No extract	7.57 ± 0.391	18.97 ± 0.624[a]
BBL extract	8.49 ± 0.393[a]	14.56 ± 0.715[a,b]
RBL extract	7.58 ± 0.147	15.48 ± 0.663[a]

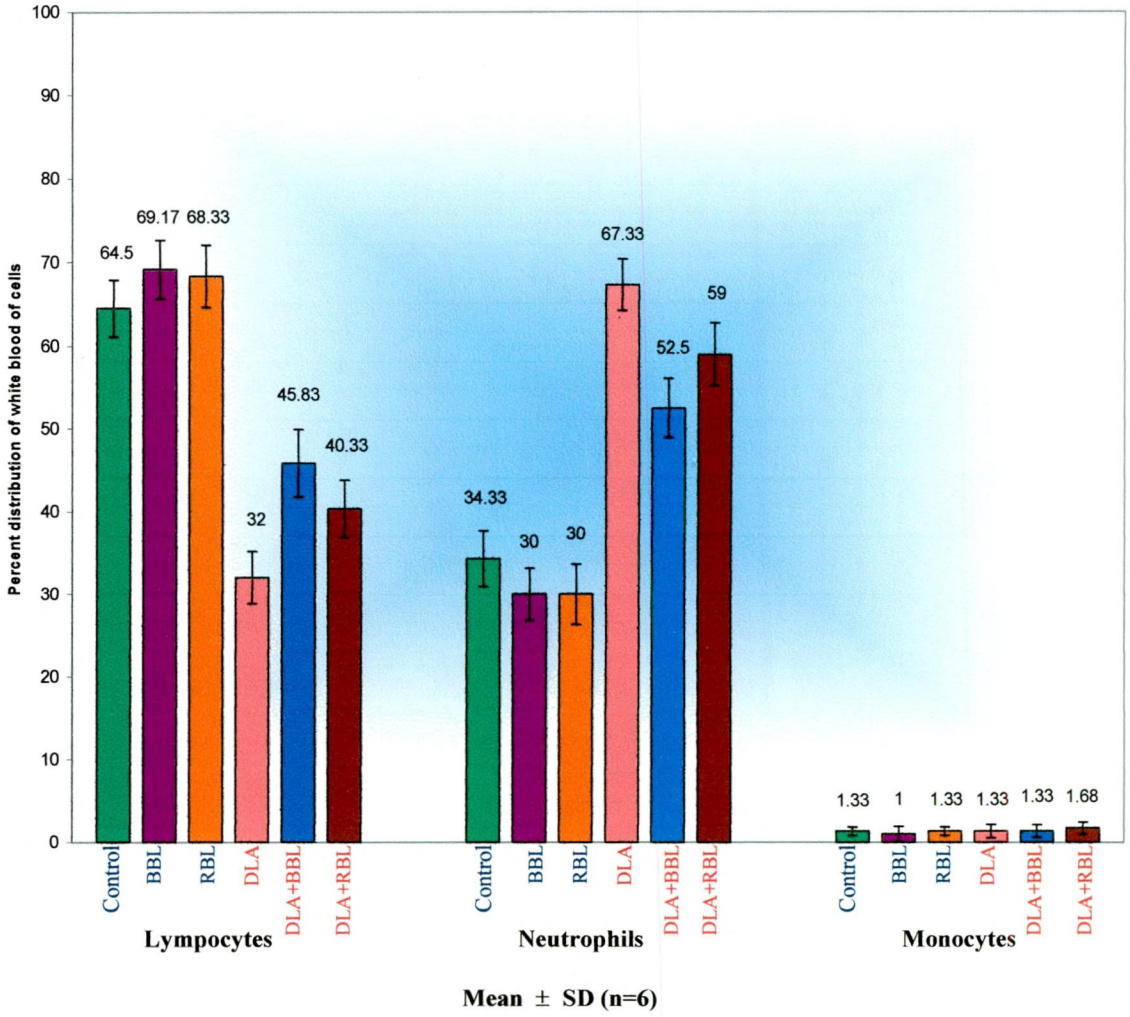
Values are mean ± SD (n=6) LSD (5%) = 0.622

a – Statistically significant ($p<0.05$) compared to untreated control

b – Statistically significant ($p<0.05$) compared to DLA tumour induced group

FIGURE 15

EFFECT OF *Solanum nigrum* LEAVES ON DIFFERENTIAL WBC COUNT IN DLA TUMOUR INDUCED MICE



The DLA tumour bearing group showed a significant ($p<0.05$) reduction in hemoglobin content and total RBC count when compared to control group. DLA tumour induced animals treated with BBL and RBL extracts exhibited significantly higher levels as compared to untreated DLA tumor induced group. BBL extract treatment was found to have a better effect than the treatment with RBL extract.

A significant ($p<0.05$) increase in WBC count was observed in DLA tumour bearing animals. There was a change in the level of differential count of WBC in the DLA mice when compared to the normal. Administration of BBL and RBL extracts reversed the level significantly towards normal. The effect was more pronounced in BBL extract treated group than RBL extract treated groups.

LIPID PEROXIDATION

Lipid peroxidation levels are depicted in Table 39.

TABLE 39

EFFECT OF *Solanum nigrum* LEAVES ON HEPATIC LIPID PEROXIDATION LEVEL IN DLA TUMOUR INDUCED MICE

TREATMENTS	LIPID PEROXIDATION (nmoles of MDA formed / g tissue)	
	CONTROL	DLA INDUCED GROUP
No extract	15.50 ± 1.17	32.27 ± 0.41[a]
BBL extract	13.14 ± 0.30	21.92 ± 0.55[a,b,c]
RBL extract	14.19 ± 0.54	24.48 ± 0.45[a,b]

Values are mean ± SD (n=6) LSD (5%) = 0.751
a – Statistically significant ($p<0.05$) compared to untreated control
b – Statistically significant ($p<0.05$) compared to DLA tumour induced group
c – Statistically significant ($p<0.05$) compared to RBL extract treated group

The development of DLA tumor caused a significant ($p<0.05$) elevation in LPO level in hepatic tissues while treatment with BBL and RBL extracts reduced the level, but the levels did not reach those of the control group.

PHASE IV

PRELIMINARY PHYTHOCHEMICAL SCREENING OF TWO VARIETIES OF *Solanum nigrum* LEAVES

As a first step of analysing the chemical nature of the compound responsible for maximum antioxidant and anticancer activities in the leaves of both the varieties, a preliminary phytochemical screening was done in the different fractions of the leaves, i.e., the fractions extracted sequentially into solvents of increasing polarity (petroleum ether, benzene, ethylacetate, methanol and water). The results obtained for the various qualitative confirmatory tests for alkaloids, phenolics and flavonoids.

The different solvent extracts of BBL and RBL indicated the presence of the secondary metabolites like alkaloids, phenols and flavonoids, which might be responsible for both the antioxidant and anticancer effects.

TLC ANALYSIS OF THE ALKALOID AND PHENOLIC FRACTIONS

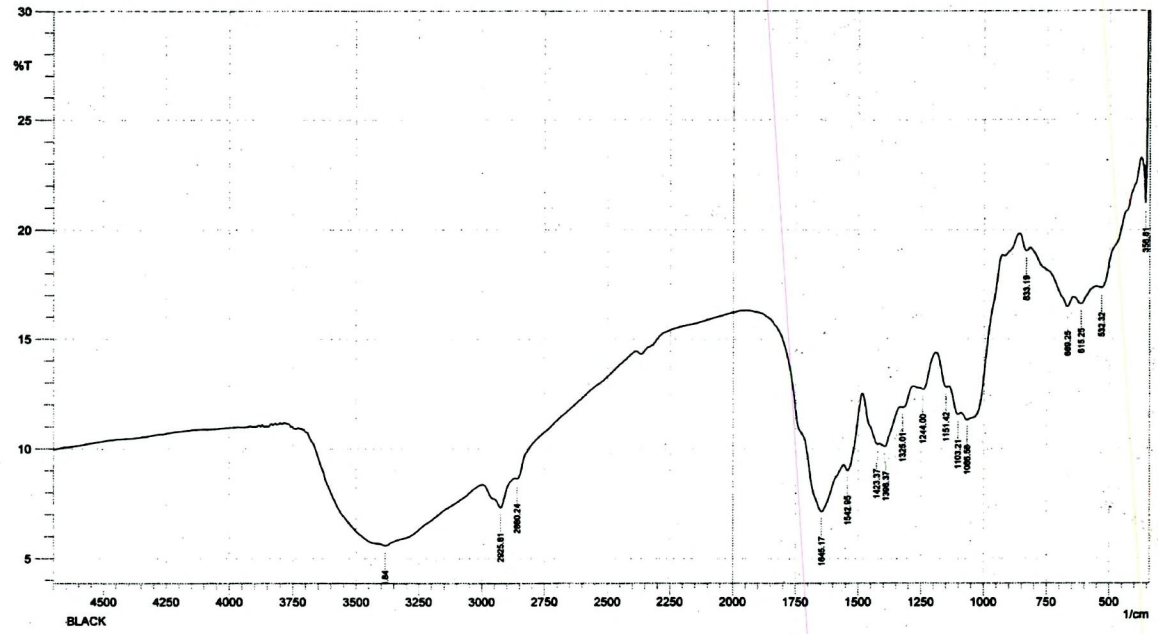
The *Solanum nigrum* leaves (BBL) showed the presence of a spot for alkaloids having the R_f value of 0.52 and the phenolics showed the presence of two spots having the R_f values of 0.84 and 0.92. The other variety RBL indicated the presence of a single spot for alkaloids with R_f value of 0.78 and for phenolics two spots having R_f values of 0.78 and 0.84.

FT-IR SPECTRAL STUDIES

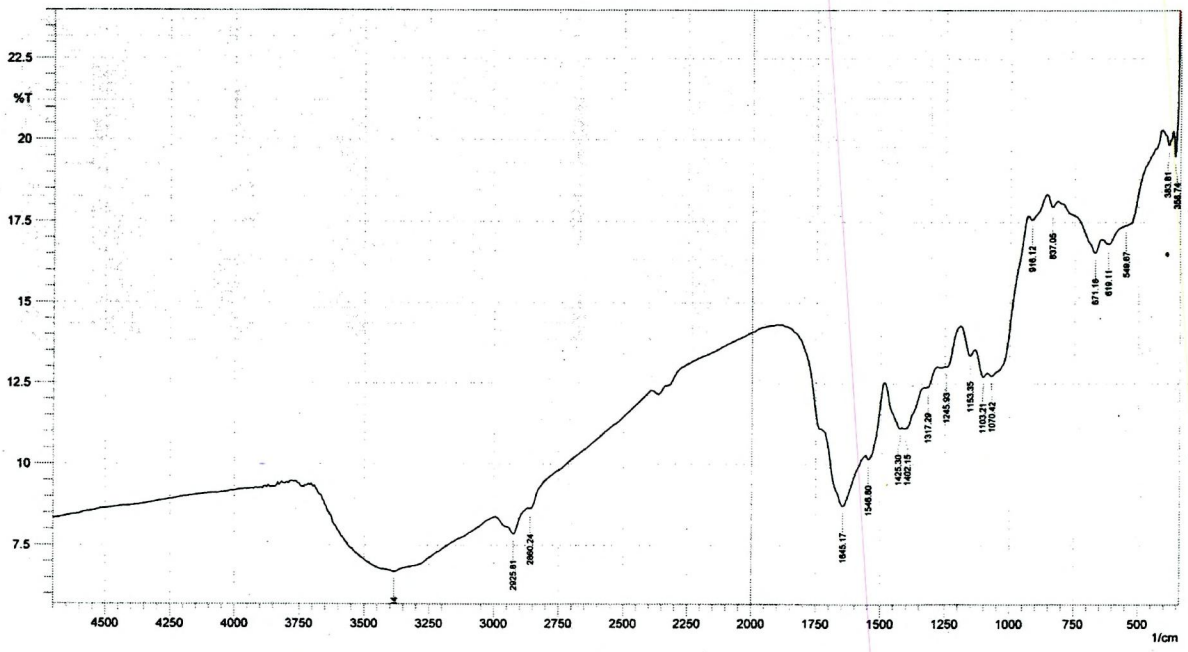
The FT-IR spectra of the crude dried powder of leaves of the two varieties of *Solanum nigrum,* the BBL and RBL are shown in figure 16.The IR spectrum reveals bands with region 1500-900cm^{-1} and 900-650 cm^{-1}. The band at 2925cm^{-1} may be due to the CH_3-asymmetric stretching of aromatics or = CH group. The band at 2860cm^{-1} may be due to the CH_3-symmetric stretching (R-CH_3). The peak at 1647cm^{-1} may be due to the C=O stretching for α, β unsaturated ketones. Both extracts gave similar IR spectra.

FIGURE – 16

FT-IR SPECTRA OF *Solanum nigrum* LEAVES



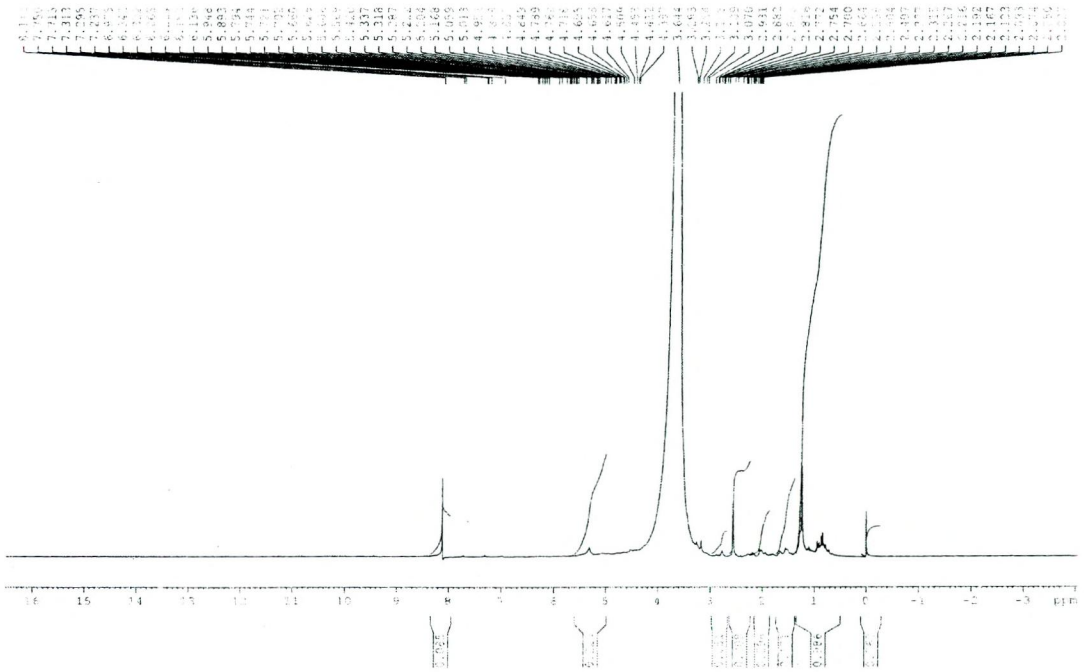
BBL



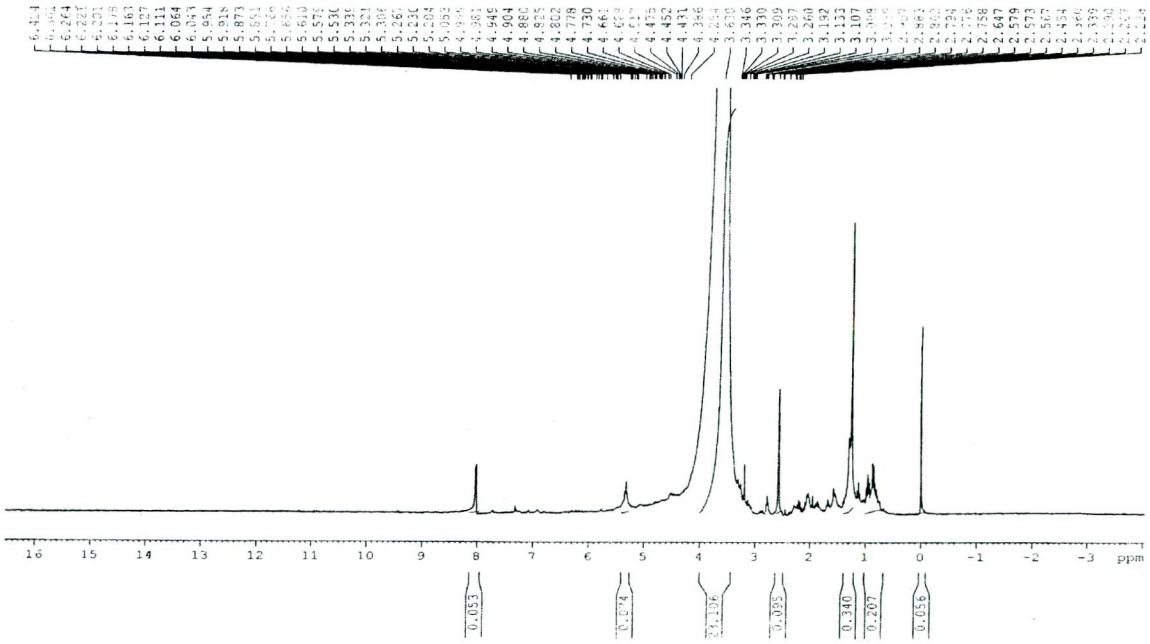
RBL

FIGURE – 17

^{1}H-NMR SPECTRA OF *Solanum nigrum* LEAVES



BBL

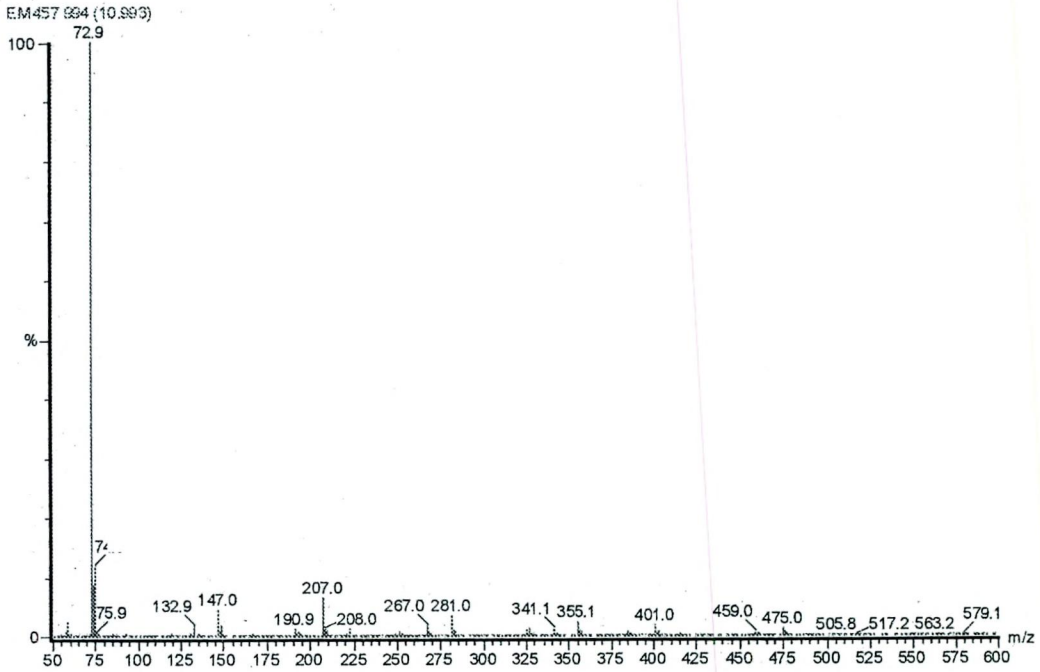


RBL

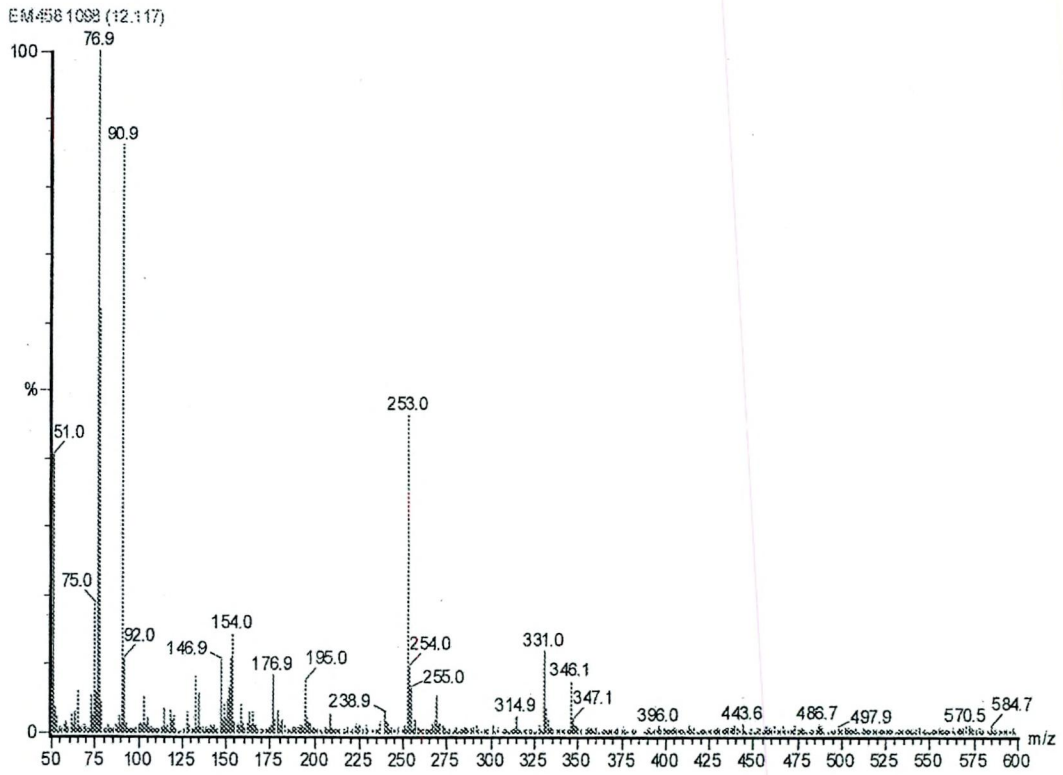
FIGURE - 18

GC – MS SPECTRA OF *Solanum nigrum* LEAVES

(FRAGMANTATION PATTERN OF PEAKS)



BBL



RBL

NMR SPECTRAL STUDIES

The ^{1}H-NMR spectra of the crude powder of BBL and RBL are exhibited in figure 17. The high frequency peaks (above δ6) are aromatic i.e. δ8.114 and frequency peaks at δ5.204 denotes alkenes (may be aromatic alkene) and frequency peak at 2.267-2.216 may be due to (-Ph-Ar- group). Ph Ar (Ar=aryl (CH_3x) x=PhAR) and the frequency peak at δ1.562 denotes double bond. The NMR spectra shows a sharp peak at δ8.2 and multiplets in the aromatic region, probably indicating the presence of flavonoidal compounds. It also suggested the presence of terpenoidal or steroidal glycosides as shown by the absorption in the aliphatic region and those due to the sugar protons. The IR and NMR spectra of the samples revealed a similar absorption patterns for both the leaves, suggesting similar chemical composition in both the extracts.

GC-MS SPECTRAL STUDIES

The GC-MS spectra of both varieties, the BBL and RBL, are presented in figure 18.

In the mass spectrum of an organic molecule, each peak corresponds to an ion of a particular isotopic composition and its m/z value is calculated from the isotopic mass. In the mass spectrum of BBL, the base peaks correspond to m/z of 72.9 and 327 in the various fragments. The mass spectrum of RBL reveals base peaks at 55 and 76.9. The molecular ions of aromatic hydrocarbons are always abundant and have stable molecular ions where doubly or triply charged ions are possible. The m/z produced at 77 denotes a phenyl cation and the base peaks corresponding to the presence of phenyl rings are seen in the mass spectra of both BBL and RBL.

The GC-MS banding pattern of the leaves were more or less similar to each other and they indicate that the composition of both varieties might be the same but the quantity of these phytochemicals might be varying.

It is evident from the results that these plants can be exploited in the preparation of drugs which can combat free radicals mediated disorders and diseases.

The outcome of the results is discussed in the next chapter with reference to relevant published literature.