Cancer is one of the most life threatening diseases and serious public health problems in both developing and developed countries. Due to the toxic and adverse side effects of synthetic drugs as well as failure of conventional treatments to fulfill their objectives (tumor control), herbal medicine has made a comeback to improve the fulfillment of present and future health needs (Islam et al., 2009).

In the last few years, there was an increased attention directed towards the association of non nutritive component in foods and protection against chronic diseases including some forms of cancer and ageing. Chemoprevention is a means of cancer control by the use of natural or synthetic agents to interrupt the process of carcinogenesis and to prevent or delay tumor growth (Parimalakrishnan et al., 2009).

Plants possess efficient antioxidant defense systems to scavenge the ROS and protect from destructive reactions. A regulated balance between oxygen radical production and their destruction is required to maintain metabolic efficiency and functions under both optimal and stress conditions. Many plant-derived products possess antioxidant activity (Powlowski et al., 2008).

The results and discussion pertaining to the present study entitled “Antioxidative and antitumorigenic efficacy of the protein fraction of Cynodon dactylon (PFC) and its characterisation by in silico methods” are elaborated in this chapter under 5 phases.

IN VITRO STUDIES

PHASE I

4.1 Selection and characterisation of PFC
4.1.1 Protein content of the dialysates of *Cynodon dactylon* in 10 – 100% saturation of recrystallized ammonium sulphate

4.1.2 Characterisation of the selected PFC by native PAGE and SDS PAGE

**PHASE II**

4.2 Antioxidative and antitumorigenic efficacy of PFC

4.2.1 Free radical scavenging activity of PFC

4.2.1.1 DPPH radical scavenging activity of PFC

4.2.1.2 Nitricoxide radical scavenging activity of PFC

4.2.1.3 H₂O₂ scavenging activity of PFC

4.2.2 Antitumorigenic effect of PFC to DLA tumor cells

**IN VIVO STUDIES**

**PHASE III**

4.3 Antioxidative effect of PFC and silymarin in CCl₄ challenged Swiss albino mice

4.3.1 Effect of PFC on the status of the liver

4.3.1.1 Effect of PFC on the Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) activites

4.3.2 Effect of PFC on the activities of hepatic enzymic antioxidants

4.3.2.1 Effect of PFC on superoxide dismutase (SOD) activity

4.3.2.2 Effect of PFC on catalase (CAT) activity

4.3.2.3 Effect of PFC on glutathione peroxidase (GPx) activity

4.3.3 Effect of PFC on the levels of hepatic non enzymic antioxidants

4.3.3.1 Effect of PFC on the levels of vitamin A

4.3.3.2 Effect of PFC on the levels of vitamin C

4.3.3.3 Effect of PFC on the levels of vitamin E

4.3.3.4 Effect of PFC on the levels of reduced glutathione (GSH)
4.3.4 Effect of PFC on the levels of TBARS

PHASE IV

4.4 Antitumorigenic effect of PFC in DLA induced Swiss albino mice

4.4.1 Antitumorigenic effect of PFC

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4.4.2.1 Effect of PFC on superoxide dismutase (SOD) activity

4.4.2.2 Effect of PFC on catalase (CAT) activity

4.4.2.3 Effect of PFC on glutathione peroxidase (GPx) activity

4.4.3 Effect of PFC on the levels of hepatic non enzymic antioxidants

4.4.3.1 Effect of PFC on the levels of vitamin A

4.4.3.2 Effect of PFC on the levels of vitamin C

4.4.3.3 Effect of PFC on the levels of vitamin E

4.4.3.4 Effect of PFC on the levels of reduced glutathione (GSH)

4.4.4 Effect of PFC on the levels of TBARS

4.4.5 Effect of PFC on the histological pattern of liver in DLA induced mice

IN SILICO STUDIES

PHASE V

4.5 Structures and models of PFC

4.5.1 Primary structure of PFC using PROTPARAM tools in ExPASY

4.5.2 Secondary structure of PFC using GOR tool in ExPASY

4.5.3 Molecular models of PFC using template 1KRR

IN VITRO STUDIES

PHASE I

4.1 Selection and characterisation of PFC

4.1.1 Protein content of the dialysates of *Cynodon dactylon* in 10-100% saturation of recrystallised ammonium sulphate
The protein content of dialysates of *Cynodon dactylon* in 10 – 100 % saturation of recrystallised ammonium sulphate was estimated and the results are given in Table 1 and Plate 3.

### TABLE 1
**LEVELS OF PROTEIN IN THE DIALYSETE OF Cynodon dactylon IN TEN TO HUNDRED PERCENT SATURATION OF RECRYSTALLISED AMMONIUM SULPHATE**

<table>
<thead>
<tr>
<th>Ammonium sulphate saturation (%)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of protein (mg / g leaf)</td>
<td>3.33</td>
<td>4.3</td>
<td>9.11</td>
<td>5.64</td>
<td>6.87</td>
<td>8.07</td>
<td>12.94</td>
<td>8.96</td>
<td>4.96</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Values are mean of triplicates

### PLATE 3
**PROTEIN PROFILE OF Cynodon dactylon IN NATIVE PAGE**

Lane 1 to 10: Protein fractions of *Cynodon dactylon* in 10 – 100 percent saturation of recrystallised ammonium sulphate

The protein fraction from 70 per cent saturation of recrystallised ammonium sulphate was found to have the maximum protein content (12.94 mg / g leaf) and hence it was selected for further studies.
4.1.2 Characterisation of the selected PFC by native PAGE and SDS PAGE

The selected PFC was characterised by electrophoretic separation and the results are shown in Plate 4.

PLATE 4

PROTEIN BANDS OF PFC OF 70% SATURATION OF RECRYSTALLISED AMMONIUM SULPHATE IN NATIVE PAGE AND SDS PAGE

A- Protein bands of Molecular markers; B- Protein band of PFC in Native PAGE; C- Protein band of PFC in SDS PAGE

Each fraction showed a number of major and minor bands indicating several proteins. Of the several bands obtained in 70 per cent saturation of ammonium sulphate, the major band alone was eluted. In order to find out the presence of subunits in this band, it was further subjected to SDS PAGE. The results showed one major protein indicating the absence of subunits. The molecular weight of this protein was found to be 29 KD when compared to the standard molecular markers.

PHASE II

4.2 Antioxidative and antitumorigenic efficacy of PFC

4.2.1 Free radical scavenging activity of PFC
The efficacies of antioxidants are often associated with their ability to scavenge free radicals. The harmful action of the free radicals can, however, be blocked by antioxidants by scavenging the free radicals (Balakumar et al., 2010). The *in vitro* antioxidative potential of PFC was evaluated through the ability to quench the synthetic DPPH radical, nitric oxide radical and hydrogen peroxide.

### 4.2.1.1 DPPH radical scavenging activity of PFC

The free radical scavenging capacity of PFC was tested by its ability to bleach the stable DPPH. The DPPH (2,2 diphenyl -1- picryl hydrazyl) radical scavenging activity was carried out using different concentrations of PFC (Figure 8). The radical scavenging activity was found to be dose dependent.

**FIGURE 8**

**DPPH SCAVENGING ACTIVITY OF PFC**

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, the absorption strength is decreased and results in decolourisation (yellow colour) with respect
to the number of electron captured (Blois, 1958). More the decolourisation, more is the reducing ability.

The DPPH radical scavenging has been generally used as a means of quickly evaluating the antioxidant activities of compounds. DPPH radical is considered to be a model of lipophilic radical. Scavenging action of the compounds mainly depends on the number of position of hydroxyl group. In this model, scavenging activity is attributed to hydrogen donating ability of antioxidant (Philips et al., 2010).

Decolourisation of DPPH by PFC might be due its antiradical activity. This dose dependent antiradical activity confirms the antioxidant role of PFC. Our results are in tune with the investigations of Arokiaraj et al. (2008) who reported that the methanol extracts of *Pterocarpus santalinns* exhibited significant DPPH radical inhibition. The aqueous extracts of *Aphanamixis polystachya* Bark exhibited the dose dependent DPPH radical scavenging activity (Krishnaraju et al., 2009). Free radical scavenging activity of methanolic extracts of leaves, root and stem bark of *Aegle marmelos* were evaluated using DPPH method and highest free radical scavenging activity were observed in leaves (Siddique et al., 2010).

Dose dependent DPPH scavenging effects were also observed in aqueous extract of *Iris germanica*, hydroalcoholic extract of *Cystisus scoparius* and in enzymatic digests of *Ishige okamurae* by Nadraroglu et al., (2006), Sundararajan et al. (2006) and Heo and Jeon (2008) respectively.

The DPPH radical scavenging activity of the ethanol and aqueous extracts of aerial parts of *Varthemia iphionoides* Boiss (Al- Dabbas et al., 2006), aqueous extract of the herb *Thymus fallax* (Ozgen et al., 2006), *D. hamiltonii* roots and *Choerospondias axillaries* fruits (Wang et al., 2008), the oils and aqueous extracts of leaves and flowers of *Bidens pilosa* (Deba et al., 2008) goes in accordance with our findings.

### 4.2.1.2 Nitric oxide radical scavenging activity of PFC
Nitric oxide (NO) exhibits numerous physiological properties. The interaction of NO with other radicals leads to the formation of more hazardous radicals such as peroxynitrite anion and hydroxyl radical. NO generated from sodium nitroprusside in aqueous solution at physiological pH, interacted with oxygen to produce nitrite ions and induce mutagenic reaction (Yin et al., 2007). Nitric oxide can react with super oxide to form the peroxy nitrate anion which is a potential oxidant that can decompose to produce OH⁻ and NO⁻ (Pacher et al., 2007). Nitric oxide scavenging activity of PFC was found to be dose dependent and showed an EC₅₀ value of 40 µg (Figure 9). This scavenging effect might be due to the antioxidant activity of PFC which competes with the O₂ to react with the NO and thus inhibits generation of nitrite.

**FIGURE 9**

**NITRIC OXIDE SCAVENGING ACTIVITY OF PFC**

Values are mean of triplicates

From the present study, it is very clear that the PFC have the property to counteract the effect of NO formation due to its antioxidative effect and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in vivo.
The NO is produced by macrophages. NO radical can be converted into peroxynitrites, which will cause cellular oxidative stress by diverse chemical reaction in a biological system including nitration of tyrosine residues of proteins, triggering lipid peroxidation, activation of aconites, inhibition of mitochondrial electron transport and oxidation of biological thiol compounds (Taylor, 2001).

To support our findings, it has been reported by Quershi et al., (2010) that ethanolic extracts of *L.ciliate* leaves exhibited dose dependent radical scavenging activity against NO free radicals.

Similar dose dependent inhibition of NO generation were observed by Saha et al. (2008) in aqueous extracts of *Hibiscus mutabilis, Leucas aspera, Ixora coccinea* and *Polyalthia longifolia* plants and by Sonawane et al. (2010) in aqueous extracts of *Tephrosia purpurea*.L.

### 4.2.1.3 H$_2$O$_2$ scavenging activity of PFC

Scavenging of H$_2$O$_2$ demonstrated the oxidizing and reducing effect, by reverting the hydroxyl radical generated by H$_2$O$_2$. Hydroxyl radical can cause sugar fragmentation, base loss and breakage of DNA strand. It is the major ROS that cause lipid peroxidation and enormous biological damage (Manian et al., 2008). PFC was capable of scavenging H$_2$O$_2$ in a concentration dependent manner (Figure 10) with an EC$_{50}$ value of 20 µg.

The scavenging of H$_2$O$_2$ by PFC may be attributed to its antioxidative potential, which can donate electrons to H$_2$O$_2$ thus neutralizing it to water. H$_2$O$_2$ scavenging effects reflect the control of H$_2$O$_2$ that is allowed to accumulate in the cell. H$_2$O$_2$ scavenging ability of PFC indicates its ability to prevent the accumulation of H$_2$O$_2$ in the cell by the oxidation of essential thiol groups. Thus, the H$_2$O$_2$ scavenging capacity of PFC implicated its potential antioxidative effect. Ebrahimzadeh et al., (2009) also showed the dose dependent DPPH, NO and H$_2$O$_2$ scavenging activities of methanolic extract of *Ornithogalum sinteisii*. 
Manikandan et al. (2009) also showed that the protective effect of ethylacetate, methanolic and ethanolic extracts of Azadirachta indica leaf fractions by scavenging O\textsuperscript{-}, OH and NO radicals. Methanolic extracts of the plants Cassia spectabilis and Cassia fistula were identified as potential novel sources of free radical scavenging agents (Nehru et al., 2008).

**FIGURE 10**

**HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF PFC**

![Graph showing scavenging activity of hydrogen peroxide](image)

Values are mean of triplicates

Thus, free radical scavenging activity in the above three *in vitro* models demonstrated the antioxidative role of PFC at lowest concentration i.e. EC\textsubscript{50} of 40 µg, 40 µg and 20 µg for DPPH, NO and H\textsubscript{2}O\textsubscript{2} respectively. From the present study it is evident that the PFC has promising antioxidant activity against DPPH, NO and H\textsubscript{2}O\textsubscript{2} free radicals.

In line with our reports, Quershi *et al.*, 2010 also reported the strong antioxidant activity of ethanolic extract of Leucas ciliata against DPPH and NO radicals. The aqueous extract of Glycosmis pentaphylla exhibited a strong free radical scavenging activity by the DPPH, NO and H\textsubscript{2}O\textsubscript{2} scavenging assays and prevented the oxidative stress related degenerative diseases (Gupta *et al.*, 2011). Free radical scavenging effect of PFC was considered as most effective,
with its proton donating capacity. This implies that PFC may be useful as an antioxidant for treating the radical related pathological damages.

4.2.2 Antitumorigenic effect of PFC to DLA tumor cells

*In vitro* cytotoxic studies were carried out by trypan blue exclusion method to evaluate the antitumor potential of PFC to DLA tumor cells. Incubation of DLA tumor cells with PFC produced a concentration dependent cytotoxic effect (Figure 11) which was indicated by the increase in number of dead cells with increasing concentrations of PFC (Plate 5). The extract killed 50 percent of DLA tumor cells at a concentration of 40µg. This concentration was designated as fifty percent effective concentration ($EC_{50}$) and was used in *in vivo* studies.

**FIGURE 11**

CYTOTOXIC EFFECT OF PFC TO DLA TUMOR CELLS

Sunila and Kuttan, 2004 also reported the cytotoxic effect of alcoholic extract of fruits of *Piper longum* to Daltons Lymphomo Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC). Ethanolic extract of *Indigofera aspalathides* was also found to be cytotoxic against EAC tumor model in mice (Rajkapoor *et al.*, 2007). The antitumor activity and chemopreventive potential of four
Ayurvedic herbs viz. *Curcuma longa* L., *Ocimum sanctum* L., *Tinospora cordifolia* (Wild) and *Zizyphus mauritiana* Lam. were reported in DLA tumor induced Swiss albino mice (Adhvaryu *et al*., 2008). *In vitro* antioxidant potential of *P. longifolia* extract was also determined owing to the role of reactive oxygen species in (DLA) tumor initiation and progression (Manjula *et al*., 2010). The *in vitro* cytotoxic activity of the aqueous extract of four varieties of *Allium cepa* (onion) and *Allium sativum* (garlic) to melanoma cells were reported by Shrivastava and Ganesh (2010). The aqueous extract of *Areca catechu* was also found to be cytotoxic to cancer cell lines (Chetan *et al*., 2010).

**PLATE 5**

**CYTOTOXIC EFFECT OF PFC TO DLA TUMOR CELLS**

![Image of cytotoxic effect of PFC to DLA tumor cells]

A - Live Cells; B - Dead cells

Protein extract of the seeds of *A. precatorius* showed antitumor activity against Yeshida sarcoma in rats and mice (Siddiqi *et al*., 2001). The lower DLA cell viability observed in the present study might be due to the cytotoxic effect of PFC to DLA cells.

**PHASE III**

4.3 Antioxidative effect of PFC and silymarin in CCl₄ challenged Swiss albino mice

Free radical mediated process has been implicated in the pathogenesis of most of the diseases. Free radical scavenging effect as well as the
cytoprotective action of medicinal plants appears to be related to the enhancement of endogenous and also exogenous antioxidants in addition to the inhibition of lipid peroxidation. Hence, to know the promising antioxidative potential of PFC, in *in vivo* conditions, the present study has been focused on the activities of the liver marker enzymes and activities of enzymic antioxidants, levels of non enzymic antioxidants and levels of TBARS in the Swiss albino mice challenged with the free radical inducer, CCl₄ compared to the standard antioxidant silymarin (SMN).

### 4.3.1 Effect of PFC on the status of the liver

To evaluate the status of the liver by the administration of EC₅₀ of the PFC (40 μg) the liver marker enzymes were analyzed in the serum. When the liver cell membrane is damaged, a variety of hepatic enzymes (SGOT & SGPT) normally located in the cytosol are released into the blood stream. Hence their estimation in the serum is an useful quantitative marker to follow the extent and type of cellular damage.

#### 4.3.1.1 Effect of PFC on serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities

The activities of SGOT and SGPT were found to be significantly increased in CCl₄ challenged animals when compared to all the other groups. Co-administration of PFC to CCl₄ induced mice showed a significant decrease in the activities of SGOT and SGPT when compared to CCl₄ alone induced mice. These results are depicted in the Table 2.

In the present study, it was seen that the free radical inducer CCl₄ affects the cellular permeability of hepatocytes, leading to elevated levels of serum biochemical parameters SGOT and SGPT. Administration of PFC + CCl₄ significantly decreased the activities of serum liver marker enzymes when compared to CCl₄ treated mice (Figure 12). This decrease might be due to the
protection of the CCl₄ induced cell membrane disturbances by PFC. These findings indicated that PFC maintained the structural integrity of the liver individually as well as in the presence of the free radical inducer CCl₄.

**TABLE 2**

**ACTIVITIES OF THE LIVER MARKER ENZYMES IN THE SERUM OF CONTROL AND EXPERIMENTAL GROUPS OF MICE**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>SGOT a (U/L)</th>
<th>SGPT b (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBS</td>
<td>35.49</td>
<td>34.69</td>
</tr>
<tr>
<td>2</td>
<td>PO</td>
<td>35.52</td>
<td>34.79</td>
</tr>
<tr>
<td>3</td>
<td>PFC</td>
<td>35.59</td>
<td>35.12</td>
</tr>
<tr>
<td>4</td>
<td>SMN</td>
<td>35.59</td>
<td>35.8</td>
</tr>
<tr>
<td>5</td>
<td>CCl₄</td>
<td>121.25</td>
<td>110.19</td>
</tr>
<tr>
<td>6</td>
<td>SMN + CCl₄</td>
<td>78.49</td>
<td>70.79</td>
</tr>
<tr>
<td>7</td>
<td>PFC + CCl₄</td>
<td>82.56</td>
<td>77.72</td>
</tr>
<tr>
<td></td>
<td>CD (0.05)</td>
<td>0.2427</td>
<td>1.7078</td>
</tr>
</tbody>
</table>

Values are mean of six mice in each group

| a | micromole of pyruvate formed / minute |
| b | micromole of phenol formed / minute |

**FIGURE 12**

**EFFECT OF PFC ON THE ACTIVITIES OF SGOT AND SGPT IN CCl₄ INDUCED SWISS ALBINO MICE**
The leaves of *Balanites roxburghii* markedly prevented the damage in CCl$_4$ induced animals (Thirupathy *et al*., 2009). Sandhu *et al*.(2010) also reported a significant decrease in the activities of liver marker enzymes by a protein isolated from the leaves of the herb *Cajanus acutifolius* linn.

Thus, it is clear that PFC has the potent protective activity against CCl$_4$ induced free radical toxicity in mice and maintained the normal functioning of the liver. The stabilization of hepatic marker enzyme activities by PFC is an indication of the improvement of the functional status of the liver cells.

In tune with our results significant hepatoprotective activities of aqueous extract of *A. mammelos* were also reported in CCl$_4$ induced liver damage in mice against silymarin (Kalaivani *et al*., 2009). These results are in accordance with Young *et al*.(2007) who reported that the *Piper betal* leaf extract significantly inhibited the elevated SGOT and SGPT activities caused by CCl$_4$ intoxication thereby protected the liver from cellular damage and with Joshi *et al* (2008) who reported on the hepatoprotective and antioxidant effect of aqueous extract of *Angelica gauca* Edgew root in CCl$_4$ induced rats.

### 4.3.2 Effect of PFC on the activities of hepatic enzymic antioxidants

Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against degenerative diseases. Our body has developed several endogenous antioxidant systems to deal with the production of free radicals. These systems may be divided into enzymic and non enzymic groups. The enzymic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD catalyzes the breaking down of a free radical called superoxide, which plays a major role in lipid peroxidation, into oxygen and hydrogen peroxide, which is further decomposed into water and oxygen by CAT. One molecule of CAT can convert approximately 6 million molecules of H$_2$O$_2$ to water and O$_2$ per minute (Ferombi...
et al., 2000). GPx is also used by the body to consume free peroxide in the cells (Kashyap et al., 2005).

The activities of these enzymic antioxidants were assessed and the results are shown in the Table 3.

**TABLE 3**

**ACTIVITIES OF HEPATIC ENZYMIC ANTIOXIDANTS IN CONTROL AND EXPERIMENTAL GROUPS OF MICE**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>SOD (U @/mg protein)</th>
<th>CAT (U #/mg protein)</th>
<th>GPx (U ^/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBS</td>
<td>4.37</td>
<td>5.73</td>
<td>100.83</td>
</tr>
<tr>
<td>2</td>
<td>PO</td>
<td>4.45</td>
<td>5.91</td>
<td>101.64</td>
</tr>
<tr>
<td>3</td>
<td>PFC</td>
<td>6.17</td>
<td>8.06</td>
<td>113.49</td>
</tr>
<tr>
<td>4</td>
<td>SMN</td>
<td>6.10</td>
<td>6.12</td>
<td>116.88</td>
</tr>
<tr>
<td>5</td>
<td>CCl₄</td>
<td>2.46</td>
<td>2.92</td>
<td>95.03</td>
</tr>
<tr>
<td>6</td>
<td>SMN+CCl₄</td>
<td>6.23</td>
<td>14.90</td>
<td>117.23</td>
</tr>
<tr>
<td>7</td>
<td>PFC +CCl₄</td>
<td>6.49</td>
<td>17.17</td>
<td>121.07</td>
</tr>
<tr>
<td>8</td>
<td>CD (0.05)</td>
<td>1.37</td>
<td>2.05</td>
<td>4.85</td>
</tr>
</tbody>
</table>

Values are mean of six mice in each group
@ - 1 Unit is defined as the amount of enzyme that gives 50% inhibition of the extent of NBT reduction/min.
# - 1 Unit is defined as the amount of enzyme required to decrease the absorbance by 0.05 units at 240nm
^ - 1 Unit is defined as the nano moles of GSH oxidized / min

**4.3.2.1 Effect of PFC on superoxide dismutase (SOD) activity**

Table 3 shows the SOD activity in the control and experimental groups of mice administrated with PFC. Activity of SOD was found to be decreased in CCl₄ alone induced mice when compared to all other groups of mice. Co-administration of PFC to CCl₄ induced mice showed the increased levels in par with that of other experimental groups. Increase in SOD activity leads to production of free radicals and thus enhance the natural defence system.
Superoxide radicals are one of the most important reactive O$_2$ free radicals constantly produced in living cells. Superoxide is inactivated by SOD, the only enzyme known to use free radical as a substrate. SOD scavenges the superoxide ions produced as cellular by products. SOD is a major defense for aerobic cells in combating the toxic effects of superoxide radicals (Kerksick and Willoughby, 2005).

**FIGURE 13**

**EFFECT OF PFC ON SUPEROXIDE DISMUTASE (SOD) IN CCl$_4$ INDUCED SWISS ALBINO MICE**

The free radical scavenging activity of SOD is effective only when there is an increase in GPx activity since SOD generates H$_2$O$_2$ as a metabolite, which is more toxic than oxidative radicals and has been removed by GPx.

The SOD activity in CCl$_4$ group was found to be significantly lower than that of all other experimental groups. Significant increase in SOD activity of PFC + CCl$_4$ group was found to be similar to that of silymarin + CCl$_4$ treated group (Figure 13) indicating that the co-administration of PFC and silymarin to CCl$_4$ induced mice combats the effects of free radical inducer.

In line with our reports, Padmavathi *et al.* (2005) also observed an increase in SOD and CAT activities and increased levels of GSH in *Withania*
root fed mice. Koneri et al. (2008) have shown that the ethanolic extract of roots of *Momordica cymbalaria* as well as silymarin increased the levels of antioxidant markers like GSH, SOD and CAT in CCl₄ induced rats. Sundararajan et al. (2006) have shown that the administration of hydrochloric extract of *Cystisus scoparius* enhanced the activities of CAT and SOD in the liver of rats. Ahmed et al., (2004) also showed significant increase in SOD activity in mice treated with aqueous extracts of *Hibiscus cannabinus* leaves. Palanivel et al., (2008) have also reported a significant increase of hepatic SOD activity thereby reducing reactive free radical induced oxidative damage to liver in the rats treated with alcoholic extracts of *Pisonia aculeate* L.

### 4.3.2.2 Effect of PFC on catalase (CAT) ACTIVITY

The CAT activity in CCl₄ alone treated group was found to be significantly lower than that found in controls and experimental groups. CAT activity in PFC treated group was found to be significantly increased when compared to vehicle control group. In PFC + CCl₄ administered group the enzyme activity was found to be more significant than that of silymarin + CCl₄ treated group (Table 3).

**FIGURE 14**

**EFFECT OF PFC ON CATALASE (CAT) IN CCl₄ INDUCED SWISS ALBINO MICE**

![Graph showing the effect of PFC on catalase activity in CCl₄ induced Swiss albino mice.](image)
The observed decline in CAT activity in the CCl₄ induced animals is mostly due to the increased levels of ROS generated in them. However the administration of PFC reverted the CAT activity significantly (Figure 14).

A study by Ng et al. (2005) showed significant increase in CAT activity by the administration of Rose (Rosa rugosa) flower extract to 6-month old Swiss albino mice. Another study by Manna et al., (2006) also showed increased level of CAT in CCl₄ induced mice treated with aqueous extract of Terminalia arjuna.

Singh et al., (2006) have reported a significant increase in the levels of enzymic antioxidants (CAT, SOD and GST) in the liver of Swiss albino mice administered with 80 percent hydroalcoholic extract of Andrographicus paniclata and aerial roots of Tinospora cordifolia. In another study, the administration of Triphala fruit powder showed significant increase in CAT activity in rats treated with it (Srikumar et al., 2006).

4.3.2.3 Effect of PFC on glutathione peroxidase (GPx) activity

The selenium dependent enzyme GPx is also one of the most essential antioxidant defense systems. This enzyme acts in conjunction with the tripeptide glutathione (GSH), one of the major non enzymic antioxidant by protecting the cellular function against the high levels of oxidative stress (Leelavinothan and Rosalin, 2005).

In the present study, activity of GPx in liver was significantly decreased in CCl₄ treated group as compared to control mice. The intraperitoneal administration of PFC to CCl₄ induced mice afforded a significant increase in hepatic GPx activity (Table 3).

The low levels of GPx observed in CCl₄ treated animals are due to the increased generation of free radicals caused by the disturbance in the cellular membrane rigidity. The activity was found to be reversed by the
co-administration of PFC which shows that PFC might have destroyed the free radical generated by CCl₄ (Figure 15).

**FIGURE 15**

EFFECT OF PFC ON GLUTATHIONE PEROXIDASE (GPx) IN CCl₄ INDUCED SWISS ALBINO MICE

Concomitant increase in GPx and SOD activity observed in PFC + CCl₄ and silymarin + CCl₄ was online with the report of Bhattacharya et al. (2001).

*Trianthema decanda* root extract administration to CCl₄ treated Wister rats improved the antioxidant status by increasing the activities of liver CAT, SOD, GPx and GR (Balamurugan and Muthuswamy, 2008). Administration of extracts of *P. aculeata* and silymarin were found to enhance the hepatic activities of GPx, GST, SOD and CAT in CCl₄ intoxicated rats (Palanivel et al., 2008).

**4.3.3 Effect of PFC on the levels of hepatic non enzymic antioxidants.**

The enzymic antioxidants are inactivated by free radicals, and hence the presence of non enzymic antioxidant is presumably essential for the removal of these radicals. The results of the non enzymic antioxidants vitamin A, vitamin C,
vitamin E and GSH in the liver of controls and experimental groups of mice are depicted in the Table 4.

**TABLE 4**

**LEVELS OF HEPATIC NON ENZYMIC ANTIOXIDANTS IN CONTROLS AND EXPERIMENTAL GROUPS OF MICE**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Vitamin A (µg/ g tissue)</th>
<th>Vitamin C (mg/g tissue)</th>
<th>Vitamin E (µg/g tissue)</th>
<th>GSH (nmoles/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBS</td>
<td>6.83</td>
<td>1.08</td>
<td>4.07</td>
<td>3.29</td>
</tr>
<tr>
<td>2</td>
<td>PO</td>
<td>6.98</td>
<td>1.55</td>
<td>4.21</td>
<td>5.01</td>
</tr>
<tr>
<td>3</td>
<td>PFC</td>
<td>19.75</td>
<td>3.46</td>
<td>4.96</td>
<td>7.82</td>
</tr>
<tr>
<td>4</td>
<td>SMN</td>
<td>12.22</td>
<td>1.69</td>
<td>4.96</td>
<td>5.48</td>
</tr>
<tr>
<td>5</td>
<td>CCl₄</td>
<td>3.6</td>
<td>0.47</td>
<td>2.14</td>
<td>1.59</td>
</tr>
<tr>
<td>6</td>
<td>SMN + CCl₄</td>
<td>9.71</td>
<td>4.08</td>
<td>5.19</td>
<td>5.31</td>
</tr>
<tr>
<td>7</td>
<td>PFC + CCl₄</td>
<td>16.3</td>
<td>3.64</td>
<td>4.95</td>
<td>8.16</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>3.54</td>
<td>1.16</td>
<td>2.65</td>
<td>2.31</td>
</tr>
</tbody>
</table>

Values are mean of six mice in each group

4.3.3.1 **Effect of PFC on the levels of vitamin A**

The level of vitamin A in the PFC treated group was found to be significantly increased when compared with the control and CCl₄ treated groups (Table 4). In the present study, the administration of PFC plus CCl₄ and silymarin plus CCl₄ significantly increased the level of vitamin A in the liver of mice when compared to their respective control groups.

Vitamin A is known to be an important natural antioxidant capable of counteracting oxygen free radicals and exerts a protective antioxidant effect (Wang et al., 2008). PFC showed more significant effect than that of silymarin.

The results of the present study revealed that the levels of the lipid soluble vitamin A was decreased in the free radical induced group of animals which was found to be significantly increased in PFC alone supplemented mice (Figure
The decreased vitamin A level observed in CCl₄ induced mice might be due to the liberation of lipid peroxide. Increased levels of vitamin A in PFC + CCl₄ treated mice might be due to decreased levels of lipid peroxides. Esmaeili et al. (2009) in S. sahendica showed significant dose dependent increase in the levels of non enzymic antioxidants GSH, vitamin C, E and A. Similar increased levels of vitamin A in plasma and liver of albino rats fed with normal quantity of protein in diet were reported by Karar and Manvalan (2002).

FIGURE 16
EFFECT OF PFC ON VITAMIN A IN CCl₄ INDUCED SWISS ALBINO MICE

4.3.3.2 Effect of PFC on the levels of vitamin C

The levels of vitamin C in the liver of mice administered with PFC were found to be significantly increased when compared to its control and silymarin groups of mice. The levels of vitamin C in the liver of mice administered with PFC plus CCl₄ and silymarin plus CCl₄ were found to be significantly increased when compared to the CCl₄ and control groups (Table 4).

Ascorbate is an excellent non enzymic antioxidant and scavenges free radical and oxidants (Nonaka, 1991). Its antioxidant activity might be through
the scavenging of a variety of free radicals and oxidants, \textit{in vitro}, including superoxide radical (O$_2^-$), peroxyl radicals, hydrogen peroxide, hypochlorous acid, singlet oxygen, oxidant air pollutants and oxidants that leak from activated neutrophils and macrophages. Ascorbate also acts indirectly to prevent lipid peroxidation and contributes to the regeneration of membrane bound oxidised vitamin E (Romieu \textit{et al.}, 2008).

**FIGURE 17**

**EFFECT OF PFC ON VITAMIN C IN CCl$_4$ INDUCED SWISS ALBINO MICE**

Availability of vitamin C is a determined factor in controlling and potentiating many aspects of host resistance to cancer. Vitamin C can protect cell membranes and lipoprotein particles from the oxidative damage by regenerating the antioxidant form of vitamin E. Vitamin C and E act synergistically in scavenging a wide variety of ROS. The significant increase of vitamin C in PFC and PFC + CCl$_4$ treated group when compared to control and silymarin groups of mice might be due to the potent antioxidant effect of PFC (Figure 17). Narendhirakannan \textit{et al.} (2001) reported that administration of \textit{Cleome gynandra} L. leaf extract significantly increased the levels of vitamin C in arthritis induced rats. Kumar \textit{et al.} (2005) also reported a significant increase
in the levels of vitamin C, vitamin E and reduced glutathione in the liver of Wistar albino rats after treating them with organic extract of *Careya arborea*.

4.3.3.3 Effect of PFC on the levels of vitamin E

Administration of PFC was found to increase the vitamin E content when compared to its vehicle control. The administration of PFC + CCl₄ and silymarin + CCl₄ significantly increased the level of vitamin E in the liver of mice when compared to the control groups. This is indicated in Table 4.

![Figure 18: Effect of PFC on Vitamin E in CCl₄-induced Swiss Albino Mice](image)

Vitamin E is a powerful biological antioxidant which is considered to be the major membrane bound antioxidant protecting the cell against lipid peroxidation and act as inhibitors of carcinogenesis. Vitamin E is one of the most important free radical scavenging chain breaking antioxidant within the biomembrane (Parks and Traber 2000). The decreased vitamin E levels in free radical induced animals observed in the present study might be due to the excessive utilization of this antioxidant for quenching enormous free radicals that are generated (Figure 18).
Skrzydlewska et al. (2002) also reported a significant reduction in the levels of vitamin C, E and A caused by the alcohol intoxication in the liver and serum of rats whereas the administration of the green tea reverted significantly by enhancing their levels. A significant increase in the levels of vitamins E and A in the liver and kidney of rats induced with ammonium metavanadate toxicity by the prior treatment with green tea (Soussi et al., 2006) and a similar kind of increase in hepatic antioxidative vitamins shown by methanolic extract of Careya arborea Roxb and silymarin in rats with CCl₄ induced liver damage in a study by Sambathkumar et al. (2005) goes in accordance with our results.

4.3.3.4 Effect of PFC on the levels of reduced glutathione (GSH)

The hepatic levels of reduced glutathione is found to be significantly increased in mice administered with PFC and PFC plus CCl₄ when compared to vehicle controls and CCl₄ alone administered mice (Table 4). Induction in GSH levels in PFC administered group was found to be more significant than that of silymarin administered group.

One of the most abundant intracellular thiols - GSH, along with GST protect the cells from the lethal effects of toxic and carcinogenic compounds. Induction of these GSH and GST have been evaluated as a means for determining the potency of many anticarcinogenic substances (Singh et al., 2006). The low level of GSH in free radical induced group of animals might be due to the over utilization of GSH to combat the free radicals, which have been neutralized by the protective action exerted by PFC (Figure 19).

**FIGURE 19**

EFFECT OF PFC ON REDUCED GLUTHIONE (GSH) IN CCl₄ INDUCED SWISS ALBINO MICE

![Graph showing the effect of PFC on reduced glutathione (GSH) in CCl₄ induced Swiss albino mice](image)
Treatment of leaf extract of *Ardisia compressa* showed increased level of reduced glutathione in the hepatocyte of rats (de Mejia and Mares, 2002). In another study, administration of henna leaf extract showed significant elevation of reduced glutathione in the liver and extrahepatic organs (fore stomach, kidney and lung) of Swiss albino mice treated with it (Dasgupta *et al.*, 2003).

### 4.3.4 Effect of PFC on the levels of TBARS

Table 5 shows the levels of TBARS in hepatic tissues of controls and experimental groups of Swiss albino mice challenged with free radical inducer CCl$_4$. Increased levels of TBARS were seen in CCl$_4$ alone treated group when compared to all other experimental groups. Co-administration of PFC to CCl$_4$ induced mice showed significant decreased levels of TBARS when compared to silymarin and CCl$_4$ treated mice.

**TABLE 5**

**LEVELS OF TBARS IN THE LIVER OF CONTROLS AND EXPERIMENTAL GROUPS OF MICE**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>TBARS (nmoles of MDA / mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBS</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>PO</td>
<td>1.86</td>
</tr>
<tr>
<td>3</td>
<td>PFC</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>SMN</td>
<td>0.82</td>
</tr>
<tr>
<td>5</td>
<td>CCl$_4$</td>
<td>7.01</td>
</tr>
<tr>
<td>6</td>
<td>SMN + CCl$_4$</td>
<td>5.04</td>
</tr>
<tr>
<td>7</td>
<td>PFC + CCl$_4$</td>
<td>4.12</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>0.585</td>
</tr>
</tbody>
</table>

Values are mean of six mice in each group

Lipid peroxidation is a biological pathway concerning peroxidation of cell membrane phospholipids and Poly Unsaturated Fatty Acids (PUFA) by reactive...
free oxygen radicals. It is an index of oxidative stress leading to deterioration of biological systems. The extent of lipid peroxidation is measured through Thio Barbituric Acid Reactive Substances (TBARS), a pro-oxidant factor that determines the oxidative damage (Govindarajan et al., 2006).

In the present study, TBARS content of the liver of PFC plus CCl₄ treated mice was found to be significantly decreased when compared to CCl₄ group. This decrease in TBARS level indicated the inhibition of lipid peroxidation and enhancement of antioxidative defense mechanisms to prevent the formation of excessive free radicals (Figure 20). This decreased level of TBARS in intraperitoneal administration of PFC to free radical induced group is probably due to the decreased susceptibility of hepatic tissues to free radical attacks.

**FIGURE 20**

**EFFECT OF PFC ON TBARS IN CCl₄ INDUCED SWISS ALBINO MICE**

The aqueous extract of *Conopus didymus* were found to have significant protective effect against LPO induced by CCl₄ in mice (Mantana et al., 2005). Lakshmi et al., 2005 have shown that the ethylacetate extracts of *Plentus florida* and *Phellinus rimosus* significantly inhibited the formation of lipid peroxide and
indicated the membrane protective effect. These reports also supported our findings.

Sambathkumar et al., (2005) has reported that the methanolic extract of Careya arborea Roxb stem bark and silymarin produced a significant hepatoprotective effect in Wistar albino rats by decreasing the rate of lipid peroxidation. Treatment with ethanolic extract of Pongamia pinnata leaves showed a significant reduction in the levels of lipid peroxides in liver and kidney of ammonium chloride induced hyperammonemic rats (Essa and Subramanian, 2006). Administration of ethanolic extract of Boerhaavia diffusa reduced the LPO rate in liver and kidney of ethanol induced hepatotoxic rats (Devaki et al., 2005).

Sing et al., (2001) has reported a significant decrease in the levels of lipid peroxide in the liver of Swiss albino mice treated with 80 percent hydroalcoholic extract of Adrographic panicula when compared to control groups.

The study of the above enzyme activities and the levels of non enzymic antioxidants and TBARS have been found to be of great value in the assessment of clinical and normal functioning of liver damage in the experimental animals treated with PFC against the free radical inducer CCl₄. The above results indicated a high degree (5 fold) of protection against free radical induced by CCl₄. The bio active PFC might be responsible for this free radical scavenging activity.

The free radicals produced in vivo from CCl₄ attack the cell membrane and alters the structure and function of celluar membrane (Mondal et al., 2005). The PFC prevents the liver damage caused by free radical inducer by increasing activities of hepatic enzymic antioxidants and the levels of non enzymic antioxidants and also by decreasing the rate of lipid peroxidation.

The above findings generated an interest to investigate the antitumor potential of PFC in DLA bearing animal under different stages of tumorigenesis.
such as initiation, post initiation (promotion) and for the entire period of tumorigenesis.

IN VIVO STUDIES

PHASE IV

4.4 ANTITUMORIGENIC EFFECT OF PFC IN DLA INDUCED SWISS ALBINO MICE

The antitumorigenic potential of the protein fraction was assessed by evaluating the increase in life span, the activity of enzymic antioxidants, the levels of non enzymic antioxidants and TBARS in the Swiss albino mice administrated with 40 µg of PFC (EC$_{50}$) at different stages of tumor induction (initiation, post initiation and entire period of tumorigenesis).

4.4.1. Antitumorigenic effect of PFC

The reliable criteria for judging the antitumorigenic effect lies in the prolongation of life span of the animals under the study. In vivo studies were carried out in DLA transplanted Swiss albino mice, to follow the antitumorigenic activity in terms of increase in life span (ILS) of DLA tumor bearing mice treated with PFC (Table 6).

Results showed that all the DLA control group animals died within 19 - 20 days with an average life span of 19.8 days after the tumor induction, whereas, all PFC treated tumor bearing animals survived until they were sacrificed for analysis (90 days). Moreover, no toxic symptoms were observed in these group of animals. The percentage increase in life span of PFC administered to tumor induced mice was found to be 354 per cent indicating its in vivo antitumorigenic potential against DLA. Thus, in vivo antitumorigenic potential of PFC could be attributed to its in vitro antioxidant potential as shown in phase II and III. Plant based antioxidants showed cytotoxicity towards tumor cells and antitumor activity in experimental animals. Methanolic extracts of
Phyllanthus polyphyllus possessed significant antitumor and cytotoxic activity on EAC and human cancer cell lines (Rajkapoor et al., 2007).

This result agrees with the previous report of Sivakumar et al. (2010) on the effect of the methanolic extract of Triumfetta rhomboidea in DLA bearing
<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Average number of mice that survived after transplantation of DLA tumor cells</th>
<th>Average life span</th>
<th>% ILS = T-C/ C x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>1.</td>
<td>DLA</td>
<td>6/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>2.</td>
<td>DLA + PFC</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
</tbody>
</table>

DLA - 1 x 10^6 cells / 100 µl of PBS
PFC - 40 µg / 100 µl of PBS
C = Average life span of DLA control mice
T = Average life span of DLA + PFC treated mice
Swiss albino mice by an increase in life span by 20 days. Administration of alcoholic extracts of *Piper longum* as well as *Piperine* were found to inhibit the solid tumor development and a similar increase in the life span of mice bearing EAC tumors (Sunila and Kuttan, 2004). Stephen and Vijayammal (2000) reported 150 per cent ILS of mice transplanted with both DLA and EAC cells using *Tylophora asthmatica*.

Rajeshkumar *et al.* (2008) have reported that the aqueous extract of *Phyllanthus amarus* increased the life span of DLA bearing mice and reduced the volume of transplanted solid tumors. Methanolic extract of *Careya arborea* bark was effective in inhibiting the tumor growth in DLA induced ascites and solid tumors (Natesan *et al.*, 2007). 30-70 per cent ILS of Swiss albino mice transplanted with DLA for different medicinal plants such as *Dillenia pentagya*, *Ageratum conyzoides* and *Potentilla fulgens* were reported by Rosangkima and Prasad (2004). The *in vivo* antitumor activity of methanol extract of *Hypericum hookerianum* against the DLA model increased the survival rate of animals indicating its potent anticancer properties (Santhoshkumar *et al.*, 2008).

### 4.4.2 Effect of PFC on the activities of hepatic enzymic antioxidants

Oxygen free radicals are reactive species which are constantly generated in living cells as a part of normal metabolism. An excess production of ROS is harmful to cells, which is likely to exert toxic effects in the cells involved in the pathogenesis of certain diseases and ageing. To scavenge and neutralize these free radicals, the cells are endowed with the antioxidative defense system of enzymes such as SOD, CAT and GPx. Liver is the main organ responsible for drug metabolism. So, the activities of enzymic antioxidants - SOD, CAT and GPx, in the liver of control and experimental groups of mice were evaluated and are indicated in Table 7.
# TABLE 7

**ACTIVITIES OF HEPATIC ENZYMIC ANTIOXIDANTS IN CONTROLS AND DLA INDUCED TUMORIGENESIS**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>SOD (U@/mg protein)</th>
<th>CAT (U#/mg protein)</th>
<th>GPx (U^/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 days</td>
<td>90 days</td>
<td>15 days</td>
</tr>
<tr>
<td>1</td>
<td>PBS</td>
<td>4.05</td>
<td>6.02</td>
<td>5.73</td>
</tr>
<tr>
<td>2</td>
<td>PFC</td>
<td>4.49</td>
<td>12.18</td>
<td>17.17</td>
</tr>
<tr>
<td>3</td>
<td>PFC + DLA (I)</td>
<td>6.03</td>
<td>8.30</td>
<td>6.07</td>
</tr>
<tr>
<td>4</td>
<td>PFC + DLA (P)</td>
<td>5.23</td>
<td>10.03</td>
<td>6.17</td>
</tr>
<tr>
<td>5</td>
<td>PFC + DLA (E)</td>
<td>4.37</td>
<td>10.55</td>
<td>7.12</td>
</tr>
<tr>
<td>6</td>
<td>DLA</td>
<td>3.06</td>
<td>-</td>
<td>5.17</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>0.2709</td>
<td>0.1048</td>
<td>0.1588</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>0.1302</td>
<td></td>
<td>0.131</td>
</tr>
</tbody>
</table>

Values are mean of six mice

<table>
<thead>
<tr>
<th>I – Initiation</th>
<th>P – Post initiation</th>
<th>E – Entire period</th>
</tr>
</thead>
</table>

@ -1 Unit is defined as the amount of enzyme that gives 50% inhibition of the extent of NBT reduction / min.

# -1 Unit is defined as the amount of enzyme required to decrease the absorbance by 0.05 units at 240nm

^ -1 Unit is defined as the nano moles of GSH oxidized / min
4.4.2.1 Effect of PFC on superoxide dismutase (SOD) activity

The present study showed that the PFC administration caused a significant increase in hepatic SOD activity when compared to PBS control on 15 and 90 days of treatment periods (Table 7). The DLA induced mice showed significant low level of SOD activity when compared to all the other control and experimental groups.

The SOD, an enzymic antioxidant catalytically scavenges the superoxide radical and provides the first line of defense against free radical damage. SOD is one of the crucial components in the antioxidant defense system through the involvement in the reduction of ROS and peroxides produced in living organism as well as in detoxification of certain compounds of exogenous origin, thus playing a primary role in the maintenance of balanced redox status (Alia et al., 2003).

**FIGURE 21**

**EFFECT OF PFC ON SUPEROXIDE DISMUTASE (SOD) IN THE LIVER OF CONTROLS AND DLA INDUCED TUMORIGENESIS**

A significant increase in the SOD activity that was observed in the liver of mice administered with PFC, clearly indicated that the protein fraction reduces DLA induced oxidative damage to liver in all the three tumorigenesis protocol.
(initiation, post initiation and entire period). Comparing both treatment periods, the activity of all the groups was found to be more significant in 90 days of treatment period (Figure 21). These effects could have a potential role in the detoxification and elimination of DLA induced toxicity from the body leading to the tumor preventing efficacy.

In line with these reports, Gupta et al.,(2004) also showed increase in SOD activity after the administration of methanol extract of Bauhina racemosa in rats with hepato cellular carcinoma. The aqueous extracts of Eucalyptus globulus were found to elevate the activities of SOD in rat liver (Arise et al., 2009).

4.4.2.2 Effect of PFC on catalase (CAT) activity

The DLA induced mice showed a significant decrease in the enzyme activity on 15 days of treatment period. PFC administration showed more significant CAT activity compared to controls on 15 and 90 days of treatment periods. The activity of CAT was significantly increased in liver of mice treated with PFC in the initiation, postinitiation and entire period of tumorigenesis on 15 days treatment when compared to DLA induced mice and also when 15 days and 90 days periods when compared to control mice (Table 7).

The CAT is a hemeprotein localized in the peroxisomes or the microperoxisomes. It is a key component of the antioxidant defense system. CAT is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in liver. The present study revealed a significant increase in the CAT activity in the liver of mice administered with PFC when compared to control groups on 15 and 90 days of treatment periods (Figure 22).

The CAT reduces the H2O2 produced by a dismutation reaction and prevents the generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisomes. Therefore, the reduction in
activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and $\text{H}_2\text{O}_2$ (Lenson, 2008). CAT decomposes $\text{H}_2\text{O}_2$ and protects the tissue from highly reactive hydroxyl $\text{H}_2\text{O}_2$ and other radicals induced by carcinogens. Inhibition of these protective mechanisms resulted in enhanced sensitivity to free radical induced cellular damage (Palanivel et al., 2008).

**FIGURE 22**

**EFFECT OF PFC ON CATALASE (CAT) IN THE LIVER OF CONTROLS AND DLA INDUCED TUMORIGENESIS**

Low levels of CAT observed in DLA tumor inoculated mice are regarded as markers of malignant transformation (Gupta et al., 2004). The activity of CAT was found to be increased significantly in all the groups on 90 days treatment period when compared to 15 days period, thereby protecting the tissues from free radical induced damage. The increased activity of CAT was found to be more significant than that of control mice. The significant elevation of SOD and CAT in the animals by the administration of PFC to DLA induced in all the 3 stages of tumorigenesis in the present study confirms the protective effect exerted by PFC. Our findings were supported by Selvandrian et al., (2001) who reported that the oral administration of *piperine* found to increase the activities of enzymic antioxidants in the lung cancer bearing Swiss albino mice. There
was significant increase in the level of catalase in the liver of rats treated with *Tridax procubens* extract (Ravikumar et al., 2005).

### 4.4.2.3 Effect of PFC on glutathione peroxidase (GPx) activity

In the present study, increase in hepatic GPx activity was noticed in mice treated with PFC when compared to that of control groups in the 15 and 90 days of treatment periods as shown in Table 7. The activity of GPx was found to be increased significantly in PFC administered mice when compared to PBS, the vehicle control. The present study showed a reduction in the activity of GPx in the liver of DLA tumor induced mice on 15 days of treatment period.

The enzyme activity in the initiation, post initiation and entire period of tumorigenesis was found to be increased significantly when compared to DLA control on 15 days of treatment period. Compared to 15 days treatment period, the 90 days treatment period showed significant increase in the activity of GPx in all the experimental groups (Figure 23).

**FIGURE 23**

**EFFECT OF PFC ON GLUTATHIONE PEROXIDASE (GPx) IN THE LIVER OF CONTROLS AND DLA INDUCED TUMORIGENESIS**

The GPx is also considered to be an important \( \text{H}_2\text{O}_2 \) removing enzyme in mammalian cells and is more important than catalase for removing \( \text{H}_2\text{O}_2 \). GPx is
involved in the defense mechanism against oxidative damage. It reduces the \( \text{H}_2\text{O}_2 \) and hydroperoxide levels.

In the present study, the decreased activity observed in DLA control group may be due to the excessive production of lipid hydroperoxides in cancer condition. However GPx was found to be increased significantly by the co-administration of PFC to both treatment periods. This proves that it offers protection to the cellular and subcellular membranes from the peroxidative damage by eliminating hydrogen peroxide and lipid peroxide. The administration of methanolic extract of *Bauhinia racemosa* elevated the activities SOD, CAT and GSH in tumor bearing Swiss albino mice (Gupta *et al.*, 2004). Rizk and Ibrahim (2008) observed that the intake of diallyl sulfide from garlic reverted the decrease in liver enzymic antioxidant activities in rats subjected to carcinogenesis by N-nitrosodiethylamine.

Meena *et al.*(2008), have shown increase of antioxidants GPx, SOD and GSH by extracts of *Sargassum polycystum* in liver of rats with D galactosamine induced hepatitis. A study by Arivazhagan *et al.*, (2000) showed increased levels of GPx when treated with garlic and neem extracts to male Wistar rats. Chakrabarty *et al.* (2001) showed that the administration of aqueous extract of the herb *Desmotrichum fimbriatum* increased peroxidase titre in the hepatic cells of normal mice. Sivalokanathan *et al.*, (2007), observed a significant decrease in the activity of GPx in liver and kidney of hepatocellular carcinoma bearing animals.

### 4.4.3 Effect of PFC on the levels of hepatic non enzymic antioxidants

Antioxidants are important substances with the ability to protect the body from damage caused by free radical induced oxidative stress. A variety of free radicals scavenging antioxidants exist within the body. The levels of non enzymic antioxidants vitamin A, vitamin C, vitamin E and reduced glutathione (GSH) observed in the liver of experimental groups of mice are indicated in Table 8.
### TABLE 8

**LEVELS OF HEPATIC NON ENZYMIC ANTIOXIDANTS IN CONTROLS AND DLA INDUCED TUMORIGENESIS**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Vitamin A (µg / g tissue)</th>
<th>Vitamin C (mg / g tissue)</th>
<th>Vitamin E (µg / g tissue)</th>
<th>GSH (nmoles / g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 days</td>
<td>90 days</td>
<td>15 days</td>
<td>90 days</td>
</tr>
<tr>
<td>1</td>
<td>PBS</td>
<td>4.05</td>
<td>4.65</td>
<td>3.62</td>
<td>4.17</td>
</tr>
<tr>
<td>2</td>
<td>PFC</td>
<td>12.05</td>
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</table>

Values are mean of six mice

I – Initiation ; P – Post initiation ; E – Entire period
4.4.3.1. Effect of PFC on the levels of vitamin A

The levels of vitamin A was found to be increased in the PFC treated group when compared with the control groups on 15 and 90 days of treatment periods. The DLA induced mice showed a decreased level of vitamin A on 15 days of treatment period (Table 8). The level of vitamin A was found to be increased significantly in the mice grouped under initiation, postinitiation and entire period of tumorigenesis when compared to DLA control on 15 days of treatment period and a similar trend was noticed in 90 days treatment period when compared to 15 days treatment periods.

**FIGURE 24**

**EFFECT OF PFC ON VITAMIN A IN LIVER OF CONTROLS AND DLA INDUCED TUMORIGENESIS**

Vitamin A and its derivatives are lipophilic compounds that can inhibit free radical reactions by reducing ROO− thereby limiting oxidative membrane damage and alteration of membrane structure integrity (Stahl and Sies, 2007). The level of vitamin A was found to be increased significantly in the mice grouped under initiation, post initiation and entire period of tumorigenesis when compared to DLA control on 15 days of treatment period. Compared to 15 days
of treatment the levels of vitamin A in all the experimental groups were found to be increased significantly on 90 days of treatment period (Figure 24). The increase was found to be more significant in the entire period of tumorigenesis protocol which was followed by post initiation and initiation and indicated the protective effect of PFC in the tumor bearing animals.

Bhaya and Saini (2008) reported that the supplementation of Aloe vera to irradiated mice, lowered lipid peroxidation in liver, which was due to the enhancement of concentrations of antioxidants vitamin A, C and E. Khanum et al. (2000) reported that increase in vitamin A by curry leaves has been associated with a decreased risk of dimethyl hydrazine carcinogen induced toxicity in rats.

4.4.3.2 Effect of PFC on the levels of vitamin C

The levels of vitamin C in the liver of experimental groups of mice are shown in Table 8. The levels of vitamin C in the liver of mice administered with PFC showed significant increase when compared to that of the control groups.

The level of vitamin C was found to be increased significantly in the mice grouped under initiation, post initiation and entire period of tumorigenesis when compared to DLA control on 15 days of treatment period. Compared to 15 days of treatment the level of vitamin C in all the experimental groups was found to be increased significantly on 90 days of treatment period.

This significant increased levels of vitamin C observed due to the prolonged administration of PFC in the entire period of tumorigenesis protocol which was followed by initiation and post initiation exerted its protective effect in the tumor burden (Figure 25).

Vitamin C is a water soluble antioxidant that scavenges reactive O₂ metabolites generated during the metabolism of carcinogen thus protecting genetic material from the initiation and promotion stages of carcinogenesis.
Ascorbic acid has also been reported to preserve the intracellular concentration of GSH likely helps maintain nitric oxide levels and potentiate its vasoactive effects (Nonaka, 1991).

**FIGURE 25**

**EFFECT OF PFC ON VITAMIN C IN THE LIVER OF CONTROLS AND DLA INDUCED TUMORIGENESIS**

The enhanced levels of the vitamins (C and E) observed in the study reported by Manju *et al.* (2005), may be due to the increased levels of GSH in the tissues and also due to an active rate of cell proliferation (Kuralko and Pence 1992). A decreased level of vitamin C was observed in cancer bearing animals by Sivalokanathan *et al.* (2007) which may be due to the utilization of antioxidant to scavenge free radicals, goes in accordance with our results.

**4.4.3.3. Effect of PFC on the levels of vitamin E**

The administration of PFC afforded a significant increase in the levels of vitamin E in the liver of mice when compared to that of the control group on 15 and 90 days of treatment periods. This is indicated in Table 8. The level of vitamin E was found to be significantly decreased in DLA induced mice on 15 days of treatment period. On 15 and 90 days treatment periods the level of
vitamin E were increased significantly in initiation, postinitiation and entire period of tumorigenesis.

Among lipid soluble antioxidants, α-tocopherol (Vitamin E) plays a central role in antioxidant defense system as it controls radical induced lipoprotein lipid peroxidation (Ramesh et al., 2006).

**FIGURE 26**

**EFFECT OF PFC ON VITAMIN E IN THE LIVER OF CONTROLS AND DLA INDUCED TUMORIGENESIS**

Vitamin E is a powerful lipid soluble antioxidant and a free radical scavenger that inhibits LPO by terminating the chain reaction initiated in membrane lipids. Vitamin E is a chain breaking antioxidant by donating the labile hydrogen atom from phenolic hydroxyl groups to inhibit the formation of lipid peroxyl and alcoxyl radical intermediates of LPO. In the present study, the level of vitamin E was found to be increased in initiation, post initiation and entire period of tumorigenesis groups in 15 days of treatment period when compared to DLA control. The level was found to be increased in 90 days in all the treatment groups when compared to 15 days of treatment period (Figure 26). PFC prevent free radical induced tissue damage by preventing the formation of radicals and scavenging them or by promoting their decomposition.
Decreased vitamin E content was observed by Sivalokanathan et al. (2007) in cancer bearing animal and are due to the excessive utilization of vitamin E for quenching enormous free radicals. Besides vitamin E has potent antioxidant activity due to its ability to penetrate to a precise site into the membrane which may be the important feature of protection against highly reactive radicals.

Manju and Nalini (2005) have reported a significant increase in the levels of non enzymic antioxidants when compared to the control groups in male Wistar rats (at post initiation stages of carcinogenesis) treated with natural dietary component ginger (Zingiber officinale Rosc) which has antioxidant and anticarcinogenic properties. A similar effect was reported by Selvendraian et al. (2001) in Swiss albino mice which were treated with Piperine when compared to the lung cancer bearing animals.

4.4.3.4 Effect of PFC on the levels of reduced glutathione (GSH)

The hepatic levels of GSH in mice administered with PFC showed a significant increase in comparison with the control on 15 and 90 days of treatment periods. The levels of GSH in liver of experimental groups of mice are shown in Table 8.

The level of GSH in DLA induced mice was found to be significantly decreased where as levels were found to be significantly increased in the initiation, post initiation and entire period of tumorigenesis group of mice on 15 days of treatment periods. 90 days of treatment period also showed significant increase in the initiation, postinitiation and entire period of tumorigenesis when compared to 15 days of treatment period.

GSH acts on multiple levels of the defence systems. The thiol groups of GSH participate in the protection against deleterious effects of ROS evolved during biological imbalance in tumorigenesis.
The GSH levels can be increased due to an adaptive mechanism to slight oxidative stress through an increase in its synthesis; however, severe oxidative stress may suppress GSH levels due to the loss of adaptive mechanism and oxidation of GSH to GSSG. Decreased GSH level may be due to its increased utilization during the burst of ROS production in protecting ‘SH’ group containing proteins from LPO (Halliwell et al., 1995).

The significant enhancement of GSH levels indicated the possible elimination of the excess reactive oxygen species produced by DLA which in turn might protect cells from damage. Jagadeesh et al. (2010) have reported on the increased levels of GSH in rats treated with ethanol extract of Anorphophallus paeonifolius tuber against 7, 12-dimethyl benz (a) anthracene (DMBA) induced mammary tumours.

**FIGURE 27**

**EFFECT OF PFC ON REDUCED GLUTATHIONE (GSH) IN THE LIVER OF CONTROL AND DLA INDUCED TUMORIGENESIS**

Simalr significant increase levels of GSH were reported by the administration of S. anacardium leaves extract to mice (Sujatha and Sachdanandam, 2004).
Significant depressed levels of non enzymic antioxidants could be due to the increased utilization of these antioxidants in compacting the oxidative stress due to tumour burden.

GSH is the major cytosolic low molecular weight sulfhydryl compound that acts as a cellular reducing agent and a protective agent against numerous toxic substances including most inorganic pollutants through the SH group. It plays a role in coordinating the body’s antioxidant defense processes. Depletion of GSH renders the cell more susceptible to oxidative stress. The non enzymic antioxidant glutathione is one of the most abundant tripeptides present in the liver. Its function is mainly concerned with the removal of free radical species such as H$_2$O$_2$, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate for GPx and GST (Prakash et al., 2001). In consistence with this report, the PFC enhanced the levels of GSH in the liver of all experimental mice. Compared to 15 days, 90 days treatment showed significant increase in the levels of GSH in all the experimental groups indicating its efficacy in all tumorigenesis protocol (Figure 27).

4.4.4 Effect of PFC on the levels of TBARS

Table 9 shows the levels of TBARS in the liver of the Swiss albino mice during the initiation, post initiation and entire period of tumorigenesis protocol. Significant increase in the TBARS level was observed in the DLA induced mice. PFC administration in the initiation, post initiation and entire experimental period significantly reduced the levels of TBARS when compared to that of DLA control, which is due to the inhibition of lipid peroxidation by free radicals.
TABLE 9
LEVELS OF HEPATIC TBARS IN THE CONTROLS AND DLA INDUCED TUMORIGENESIS IN MICE

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<th>Groups</th>
<th>Treatments</th>
<th>TBARS (nmoles of MDA / mg protein)</th>
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<tr>
<td></td>
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<td>15 days</td>
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<tr>
<td>1</td>
<td>PBS</td>
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<tr>
<td>2</td>
<td>PFC</td>
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<tr>
<td>3</td>
<td>PFC + DLA (I)</td>
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<td>4</td>
<td>PFC + DLA (P)</td>
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<tr>
<td>5</td>
<td>PFC + DLA (E)</td>
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<tr>
<td>6</td>
<td>DLA</td>
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<td>CD (0.05)</td>
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</table>

Values are mean of six mice

I – Initiation; P – Post initiation; E – Entire period

Tumor cells produces more peroxides. They proliferate actively after inoculation of tumors. Administration of PFC to the DLA induced mice showed significant decrease in the levels of TBARS when compared to DLA induced mice in 15 days of treatment period. In the entire period of tumorigenesis protocol the maximum decrease in the level of TBARS was noticed which was followed by post initiation and initiation (Figure 28). The observed decrease in the levels of lipid peroxides might be due to the antilipoperoxidative property of PFC suggesting that PFC could inhibit the lipid peroxide produced by the tumor burden.
An increase in MDA level suggestive of enhanced LPO and failure of antioxidant defense mechanism leading to tissue damage in cancer bearing animals was also been reported by Sivaloganathan et al. (2007). It has been reported that *O. Sanctum* have the ability to scavenge free radicals mediated events in neoplastic development (Karthikeyan et al., 1999).

Administration of *Scoparia dulcis* plant extract to diabetic rats showed a significant decrease in lipid peroxides formation in brain suggesting its role in protection against lipid peroxidation induced membrane damage (Latha and Pari, 2004). Ahmed et al., (2004) also reported that methanolic extract of *Hibiscus cannabinus* showed decreased lipid peroxidation in stress-induced rats. Isoproterenol administered rats showed a significant increase in the heart lipid peroxide level (Suchalatha et al., 2004).

From the above data, it is clear that the free radical scavenging effect of PFC is directly related to the decrease in the levels of TBARS. The
enhancement of hepatic enzymic antioxidants SOD, CAT, GPx, and non enzymic antioxidants vitamin A, C, E and GSH by PFC might be a major mechanism by which it counteracts the deleterious effects of DLA tumorigenesis. Thus, the above results obtained clearly showed that the PFC protects against the oxidative stress by enhancing the activities of enzymic antioxidants, increasing the levels of non enzymic antioxidants and antilipid peroxidative role by decreasing the TBARS levels.

Further the PFC treatment was effective in inhibiting tumor growth in animals. The observed activity might be due to its cytotoxic and antioxidant properties. Chemopreventive effect of PFC might be due to the defense that can combat the oxidative stress produced by reactive oxygen species in tumorigenesis period. Since, PFC have shown no toxic effect at the tested doses in the initiation, post inititaion and entire period of tumorigenesis it could well be applied in cancer chemoprevention to reduce the risk of cancer. The PFC merits further investigation in dry lab to elucidate the exact mechanism of action.

4.4.5 Effect of PFC on the histological pattern of liver in DLA induced mice.

The histological investigation showed significant pathological changes in the normal cellular architecture of the DLA induced mice liver sections.

Plate 6 illustrates the architecture of the section of the liver of all the experimental groups.

A. PBS control:

Portal tract and central vein were found normal .No ballooning degeneration was found in the lobules.

B. PFC:
Portal tract and central vein were found normal. No ballooning degeneration was found in the lobules.

PLATE 6

HISTOLOGICAL STATUS OF MICE LIVER OF CONTROL AND DLA INDUCED TUMORIGENESIS

A - PBS Control  
B - PFC  
C - DLA  
D - Initiation  
E - Post- initiation  
F - Entire period
C. DLA :
   Dialated central vein was found. The portal tract was found enlarged and few bile ducts in some tract filled with bile were observed. Anisonucleosis in hepatocyte zone with scattered enlarged nuclei and spotty neurons with collection of inflammatory cells- (neutrophils, mononuclear cells) with scattered neutrophils in sinusoids were observed.

D. INITIATION :
   Portal tract central vein were found normal. Focal fatty change of hepatocytes, enlarged vesicular nuclei in some cells and few binucleated cells with balooning degeneration of some cells were also observed.

E. POST INITIATION :
   The central vein was found normal. Few plasma cells found in the portal tract and balooning degeneration in all zones and some binucleated cells observed in the lobules.

F. ENTIRE PERIOD :
   The central vein and portal tract were found normal. Balooning degeneration and patchy necrosis were found in the lobules.

   These changes were absent in the PFC induced mice indicating the protective effects of PFC to combat against the carcinogenic effects of DLA tumor cells.

**IN SILICO STUDIES**

**PHASE V**

**4.5 Structures and model of PFC**

   Knowing the antitumorigenic activity of PFC the fundamental structural units were characterized by an automated Edman degradation sequence analysis (Edman and Begg, 1967).
Bio-informatics provides short cut methods to predict the structure and properties of a newly identified protein. Newly purified protein is put into various physico chemical tests in the laboratory to find out its function X-ray crystallography, circular dichroism spectroscopy, optical rotatory dispersion, nuclear magnetic resonance are some of the powerful tools employed in determination of structure and function of proteins.

There are a number of programs available in ExPASY tools server. (Expert Protein Analysis). These tools make use of the annotated protein database namely SWISS-PROT. The ExPASY tools assist in analysis and identification of proteins isolated through 2D gel electrophoresis and also help in the prediction of physical properties of other known proteins.

4.5.1 Primary structure analysis of PFC using protparam tools in ExPASY

The sequence (primary structure) of the protein is as follows.

```
MVVALLAVVALSKGEGGVIMQHCKLACEAPIAAGYRLEKWYPKERAVLLASTALFGNN
FGQTWSPSGFYGGSDASGFTYGGSAGFVNELERVTVAQRAAIGVVPVKSGWRSV
SGGWPNNCRPDGQNLDGQGAWGGLSCLCAGAIVLPVRDEGPLILDGGPSRTDVDP
DPPKPLKPFWEWLMIFDPRQQRFTAEEIVSRLAQQRSGGKRFEFMFREPAGALKRWDTS
VDVTPKIEQYAKLPAGDGPTAKPNISVGGMALCAMKQTMVKVGPDSD
```

Submission of the sequence to the Bioinformatic tools- PROTPARAM

MVVALLAVVA LSKGEGGVIM QHCKLACEAP IAAGYRLEKW
YPKERAVLLA STALFGNNFGQTWSPSGFY GSDASGFTYGG
GSAGFVNELE RTVSAQRAA IGVPVKSGW RSVSGGWPP
NCRPDGQNL DGQGAWGGLS CLCAGAIVLP VRDEGPLILD
GGPSRTDVDP DPPKPLKPFWEWLMIFDPRQ RRFTAEEIVS
RLAQRSGGKR RFEMFREPAGA LKRWDTSVDT VPKIEQYAKL
PAGDGPTAKPNISVGGMALCAMKQTMVKVGPDSD

Number of amino acids : 275
Bibliography

Antioxidative and Antitumorigenic efficacy of Protein fraction of Cynodon dactylon and its Characterisation by in silico methods

Molecular weight : 29252.5
Theoretical pI : 9.14
Total number of negatively charged residues (Asp + Glu) : 26
Total number of positively charged residues (Arg + Lys) : 33

Atomic composition:

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</table>

Formula : C_{1299}H_{2045}N_{367}O_{377}S_{13}
Total number of atoms : 4101

4.5.2 Secondary structure of PFC using GOR tool in expasy

The primary structure of proteins is the aminoacid sequence. The secondary structure is, coiling of the chain or buckling into a pleated sheet, which are called as alpha helix and beta sheet. The coiling and pleating give stability to peptide chain. Most of the side chains in the main chain are hydrophobic and the backbone of the main chain is hydrophilic. In order to achieve stability and to avoid repulsive interaction, the polypeptide chain twists and pleats.

The alpha helical region within a protein is called alpha structure and similarly beta pleat as beta structure

In the present study, the sequence was submitted to GOR.

GOR4 result for Cynodon dactylon
Bibliography

Antioxidative and Antitumorigenic efficacy of Protein fraction of Cynodon dactylon and its Characterisation by *in silico* methods

MVVALAVVALSKGEGGIVMQHCKLACEAPIAGYRLEKWYPKERAFLAALFGNN
FGQTWSPSGIFYG

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4.5.3 Molecular model of PFC using the template 1 KRR

In the present study, to predict the image the structure of the protein sequence was subjected to CPH model - Automated neural network based protein modeling server.

Prediction results and images of PFC

Plate 7. CPH model of PFC

The PDB file format was viewed in the Rasmol. The various images viewed are given below.

Molecular space model
Bibliography

Antioxidative and Antitumorigenic efficacy of Protein fraction of *Cynodon dactylon* and its Characterisation by *in silico* methods

Plate 8. Molecular model of PFC

**Ball and stick model**

Plate 9. Ball and stick model of PFC

The above models of the *Cynodon dactylon* protein using the template of 1KRR was done in the remote modeling using the CPH server. The suggested models of PFC by CPH server predicted the cellular detoxification role which goes in accordance with the template 1 KRR.

From the findings of *in silico* analysis, it can be postulated that PFC possess the detoxicifying role against DLA tumor cells which might be responsible for the antitumorigenic activity. Further investigations are required to follow the mechanism at the cellular level.
Antioxidative and Antitumorigenic efficacy of Protein fraction of Cynodon dactylon and its Characterisation by in silico methods