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PROTECTIVE ROLE OF β-CAROTENE FROM *CHLOROCOCCUM HUMICOLA* AGAINST REACTIVE OXYGEN SPECIES AND LIPID PEROXIDATION IN B(A)P INDUCED TOXICITY

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Abstract- Background: The mechanism of Benzo(a)Pyrene toxicity is complicated which alters the physiological system and the production of its by products have severe adverse effects. It is important to verify the mechanism of B(a)P induced damage in order to perform cancer chemotherapy with natural substances. Dietary intake of β-carotene plays a beneficial role in lowering oxidative stress, highly accumulated in fresh water green algae *Chlorococcum humicola*.
Methods: The β-carotene was extracted, purified from *C. humicola* by column chromatographic method, the structure was confirmed by UV-VIS, NMR and MASS spectrophotometric analysis. Four groups of Swiss albino mice were treated as control, B(a)P, β-carotene, B(a)P+β-carotene respectively for a period of 30 days. Production of Reactive Oxygen species (ROS) and Total antioxidant capacity (TAC) were assessed in the blood sample. Lipid peroxidation and the antioxidant status were assessed in various tissue samples. Histopathology of liver was observed.
Results: The levels of ROS and lipidperoxidation increased in blood and in collected tissues respectively show the severity of the tissue damage. The TAC, enzymic antioxidants (CAT, SOD and GPx) and non enzymic antioxidants (GSH, Vit A, Vit C, and Vit E) showed significant changes compared with the control mice. The histopathology of liver also confirms the protection of β-carotene from the cellular damage.
Conclusion: The overall findings demonstrate the animals post treated with β-carotene from *C. humicola* may prevent toxicity induced by carcinogenic chemical and hence aid in establishing the chemo preventive effect. Therefore *C.humicola* can be further extended to exploits its possible application for various health benefits as neutraceuticals and food additives.
Key words: Beta carotene, Benzo(a)Pyrene, Reactive oxygen species, Lipid peroxidation, Total antioxidant capacity, Enzymic antioxidants, Non enzymic antioxidants

INTRODUCTION

Damage to DNA by Reactive Oxygen Species (ROS) has been widely accepted as a major cause of cancer. There is growing awareness that oxidative stress plays a role in various clinical conditions such as malignant diseases, diabetes, atherosclerosis, chronic inflammation, viral infection, and ischemia-reperfusion injury. ROS can cause oxidative DNA and protein damage, damage to tumour suppressor genes and enhanced expression of proto-oncogenes [1]. In both experimental animals and possibly in humans, cancers of the skin, lung, colon, breast, prostate, cervix, bladder and oesophagus arise in epithelial tissues and acquire the ability to grow and invade through the basement membrane [2]. It is widely recognized that carcinogenesis in mouse skin and presumably in human skin and other tissues is a multistage process comprised of initiation, promotion and progression [3].

Using experimental carcinogenesis models, it has become well established that oxidative stress plays a causative role during carcinogenesis, specifically in tumour promotion. Consistent with the involvement of oxidative stress in cancer induction and its subsequent development [4], efforts are being made to identify naturally occurring antioxidants which could prevent, slow and/or reverse cancer induction and its subsequent development [5],[6]. The cellular antioxidant systems helps to minimize ROS induced tissue injury. These include enzymic antioxidants and non enzymic antioxidants [7]. Carotenoids are naturally occurring compounds in plants, but only a limited number of carotenoids are found in human plasma and tissues. The major carotenoids are β-carotene, lutein, α-carotene, zeaxanthin, cryptoxanthin and lycopene. A number of carotenoids are precursors

of retinol and retinoids, but carotenoids also have several other functions in humans, including protecting against oxidation by quenching singlet oxygen [8]. Beta-carotene is an excellent antioxidant and free radical scavenger. A large number of epidemiological studies suggested that the antioxidant nutrients, especially beta carotene, have a protective effect against genetic damage and the development of cancer induced by carcinogenic chemicals [9]. In addition, β-carotene reacts chemically with peroxyl radicals to produce epoxide and apocarotenal products [10]. A low level of carotenoids is associated with poor cognitive performance, and higher plasma carotene levels are associated with better memory performance in elderly people [11]. On the basis of intake, carotenoids have been postulated to play a protective role in angina pectoris, cardiovascular disease and cancer [12,13], particularly chemopreventive agent against cancer disease in various organs like lung, stomach, colon, breast and prostate [14]. There are intervention studies used β-carotene supplements rather than a mixture of carotenoids as present in fruits and vegetables. The positive effects on health postulated for the carotenoids have been attributed largely to their antioxidant actions. However, studies linking higher carotenoid intakes to better antioxidant defence and to a decrease in oxidative damage in the body are very few [15].

In the search for feasible new sources of natural antioxidants like β- carotene, algae and microalgae have been suggested as possible raw materials for carotenoids and numerous health benefits have been associated with their use. Algae and microalgae are potentially a great source of natural compounds that could be used as ingredients for preparing functional foods. Different compounds with antibacterial, antiviral and antifungal activity can be found in these types of organisms, along with compounds with antioxidant activity [16,17]. Therefore, the main target of the present study is to investigate the protective effect of β-carotene from green algae, *Chlorococcum humicola* in B(a)P induced tissue damage using animal model.

MATERIALS AND METHODS

Algal Source:

Fresh water, unicellular, nonmotile green algae *C. humicola* was obtained from the culture collected from the Department of Plant Biotechnology, Vivekantha Institute, Chennai, India.

Culture Conditions:

Algal culturing was carried out with 100 ml Bold's basal medium [18] supplemented with sterile compressed air and kept under fluorescent light (20 µmol m- 2s-1) with 16 h light period and at 25 ± 2 ºC temperature.

Carotenoid Extraction:

Algal sample (1 g dry wt.) was extracted with ethanol until all the pigments removed, filtered through a sintered glass filter (porosity 3; pore size 20-30 µ). An equal volume of diethyl ether was added to the combined ethanol extracts, followed by the addition of water droplets until two layers were formed. The ethereal epiphase, containing all the pigments, were washed free from ethanol with water, and the solvent removed. The residue was then saponified with equal volume of 10% methanolic KOH and kept in overnight in the room temperature at dark, after which the carotenoid solution was washed with water to remove the alkali (pH: 7.0) dried over Na2SO4. The unsaponifiable residue was dissolved in a little ether and then in 10 ml of petroleum ether (b.p.40-60ºC). This extraction was used for further analysis

Total carotenoid estimation:

The total caroteoids were estimated spectrophotometrically at 450 nm [20].

β-Carotene separation:

Beta carotene was separated by Open Column Chromatographic (OCC) method with neutral alumina column deactivated with methanol using petroleum ether as mobile phase [19].

β-Carotene quantification:

The collected β-Carotene fraction was quantified spectrophotometrically at 436 nm [21].

Structural analysis:

UV-VIS, NMR and GC-MS spectroscopic studies were carried out for the structural elucidation. The spectrum was recorded with UV-VIS spectrophotometer (Shimadzu UV 200-2500). For proton NMR 5mg of the purified lyophilized sample was dissolved in chloroform and the NMR spectrum was recorded in JEOL AL 300 NMR Spectrophotometer. The chemical shifts were recorded in ppm at MHZ using microtube. The GC-MS spectra were recorded on a shimadzu R-5300.

Animals Experiments:

Animals: Swiss albino mice weighing 20-25 g were used for the animal experiments were purchased from Mohamed Sathak AJ College of Pharmacy, Chennai, India. Animals were grouped and housed in polyacrylic cages (six animals per cage) and fed on standard pellet and given water ad libitum. Animals were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All experiments were performed in accordance with the guidelines for research with experimental animals and animal ethical clearance was obtained from the institutional ethical committee. (Reg.No:991/C/06/CPCSEA)

Assessment of the Oral E50 for β-carotene: Preliminary investigation was carried out to calculate the median Effective Dose (ED50) for the β-carotene fractions to albino mice. Six groups of mice, each of six individuals (n = 6) were used for each the specified dose. Doses were prepared with different concentration in corn oil. Oral dosing was done by a special syringe that has a needle equipped with a ball tip. Mortality counts of animals were recorded after

fifteen days of treatment. The ED50 values were calculated according to the statistical method [22].

Animal treatment: The mice belonging to the Group I (controls) were treated with corn oil given orally by gavages (0.1 ml). The mice belonging to the Group II were treated with benzo(a)pyrene [B(a)P] dissolved in corn oil (0.1 ml) and given by gavages in eight doses (1 mg per dose) twice per week for 4 weeks. Group III was orally administered with purified β-carotene dissolved in corn oil for a period of 30 days. The mice belonging to the Group IV [B (a) P + purified β-carotene] were treated with in the same way as mice in the second group. Food intake and body weight were monitored throughout the experimental period. Blood samples were collected every week from tail vein and used for the assay of TAC and ROS production. At the end of treatment, animals were killed by cervical dislocation after deep anaesthesia with diethyl ether and the tissue samples of lung, liver, stomach and kidney were immediately dissected out, trimmed of excess fat and weighed.

Tissue analysis: 10% tissues homogenate was prepared in 0.1M phosphate buffer (pH 7.0) and centrifuged at 9000 rpm for 15 min at 4^{o}C. The supernatant obtained from all the organs were used for further analysis.

Lipid peroxidation (LPO):

The estimation of lipid peroxidation was done by the spectrophotometric method [23]. 0.1 ml of the tissue homogenate was incubated in the medium containing 150mM KCL (0.1ml) at 37^{0}C for one hour and at the end added 1ml of 20% TCA. After thorough mixing 2ml of 0.67% TBA was added and placed in the boiling water bath for 15 mins, cooled. The absorbance of the clear supernatant was measured against reference blank at 535nm.

Reactive Oxygen Species (ROS):

Activated white blood cells are the important source of ROS, which may impose on oxidative changes to plasma constituents and neighbouring cells, such as circumulating red blood cells [24]. The level of intracellular accumulation of ROS was determined by the alteration of fluorescence resulting from oxidation of 2V, 7V-dichlorofluorescein diacetate (DCFH-DA) [25,26, [27]. In the presence of ROS, such as superoxide anion radical (O2_), hydrogen peroxide (H2O2), hydroxyl radical (OH), and singlet oxygen (1O2), DCFH is rapidly oxidized to highly fluorescent 2V–7V-dichlorofluorescein (DCF). Thus, the DCF assay, which detects all of the oxidizing species, provides a global approach to evaluating the production of ROS. DCFH-DA was dissolved in DMSO to a final concentration of 20 mM before use. Activated white blood cells are important sources of ROS. White blood cells were separated and then incubated with 10 AM DCFH-DA at 37^{0}C for 30 min, the excess DCFH-DA was washed with RPMI-1640 medium. The intensity of fluorescence was recorded using a fluorescence spectrophotometer, with an excitation filter of 485 nm and an emission filter 535 nm. The ROS level was calculated as a ratio: ROS=mean intensity of exposed cells/mean intensity of unexposed cells.

Total antioxidant capacity (TAC):

Total antioxidant capacity was measured using a kit test [28]. This assay ensures the ferric-reduction ability of plasma. The stable colour of the Fe2+-o-phenanthroline complexes due to the overall reducing agents in plasma reduced Fe3+ to Fe2+, which reacted with substrate o-phenanthroline and was measured at 520 nm. The final result of TAC was expressed as 1 ml of blood led absorbency (OD520 nm) value increase 0.01/min at 37^{0}C as one unit (U/ml). In theory, the total antioxidant capacity is a sum of the activities of the various antioxidative substances [29]. This method has been used frequently for TAC determination in scientific research and gives comparable results [28].

Tissue protein:

Protein in tissues was determined by the colorimetric method [30], using bovine serum albumin as the standard at 660nm Values were expressed as mg/g tissue.

Antioxidants:

Catalase (CAT): The activity of catalase was assayed colorimetrically at 620 nm [31]. The reaction mixture contained 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml tissue homogenate and 0.4ml of 2M H2O2. The reaction was stopped by the addition of 2.0ml of dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid reagent were mixed in 1:3 ratio). The activity was expressed as units/mg protein.

Superoxide Dismutase (SOD): Superoxide dismutase in the tissues was assessed based on the inhibition of formation of nitroblue tertarzolium (NBT) [32]. A single unit of enzyme expressed as 50% inhibition of NBT reduction per minute/mg of protein.

Glutathione peroxidise (GPx): Glutathione peroxidise activity was measured by the colorimetric method [33]. A known amount of enzyme preparation was allowed to react with hydrogen peroxide in the presence of GSH for a specified time period. Then the remaining GSH was measured using the Ellman's reagent. The activity was expressed as units/mg protein.

Reduced Glutathione (GSH): Reduced glutathione was determined in all the tissue samples [34]. 1 ml of supernatant was treated with 0.5ml of Ellman's reagent (19.8mg of 5,5'dithio bis nitrobenzoic acid in 100ml of 0.1% sodium citrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm in spectrophotometer. The values are expressed as nmol/g tissue.

Vitamin A (Vit A): For the tissue vitamin A estimation [35] the tissue homogenate was saponified with equal volume of 0.1N ethanolic KOH at 60^{0}C for 20 mins, cooled, shaken vigorously with equal volume of N-hexane then centrifuged, 0.8 ml of hexane layer was evaporated 60^{0}C in a stem oxygen free nitrogen. The residue was taken up in 0.5ml of chloroform, 1 drop of acetic acid, followed by 1.0ml of trifluoro acetic acid, shaken well and the absorbance measured at 620nm after 30 secs. The units were expressed as µg/g tissue.

Vitamin C (Vit C): Vitamin C in tissues was estimated by the colorimetric method [36]. To 0.5ml of tissue homogenate, 1.5ml of 6% TCA was added and centrifuged. To 0.5ml of supernatant, 0.5ml of DNPH reagent (2% DNPH and 4% thiourea in 9N sulphuric acid) was added and incubated for 3h at room temperature. After incubation, 2.5ml of 85% sulphuric acid was added and colour developed was read at 530nm after 30min. The values are expressed as µg/g tissue.

Vitamin E (Vit E): Vitamin E from the collected tissues was extracted from tissue by addition of 1.6ml ethanol and 2.0ml petroleum ether and centrifuged. The supernatant was separated and evaporated on air. To the residue, 0.2ml of 0.2% 2,2' dipyridyl, 0.2ml of 0.5% ferric chloride was added and kept in dark for 5 mins. Anintense red colour layer obtained on addition of 4ml butanol was read at 520nm. The values are expressed as µg/g tissue [37].

Histopathology:

Histopathology was carried out to check the influence of the β-carotene on B(a)P on liver cells [38].

Statistical Analysis:

All data were analyzed with SPSS 12 student software. Hypothesis testing methods included two way analysis of variance (ANOVA) followed by least significant difference (LSD) test. The values are expressed as mean ± S.D. P-values of less than 0.05 were considered to indicate statistically significant.

RESULTS AND DISCUSSION

Structural analysis (Fig 1 and 2)

Of the total extracted carotenoids, the chromatographic purification of β-carotene was found to be 52.4% in the selected algal sample *C. humicola*. The purified compound showed absorption maxima at 462nm in the VIS range (Fig 1). The characteristic signals in 13C NMR was observed. The 13C NMR spectrum showed the signals concentrated in the given particular range are the characteristic of β-carotene. The mass spectrum fragmentation of the compound under investigation shown in. It reveals the presence of peak at m/z of 536 as molecular weight of the molecule. (Fig 2)

Assessment of the Oral ED50 for β-carotene:

Results showed that, the oral ED50 value for purified β-carotene was found to be 15mg/kg body weight.

Lipid peroxidation:

The lipid peroxidation, measured as MDA in various organs of mice was shown in Table I. Tissue MDA was significantly increased ($P<0.001$) following B(a)P administration compared with control. These adverse changes were reversed to normal values in β-carotene and to some extent in combination with B(a)P treated mice.

Reactive Oxygen Species and Total Antioxidant Capacity

After treated with B(a)P, the ROS production in white blood cells of mice increased significantly up to 1.85 fold of control, meanwhile, TAC decreased 32% from 300 U/ml. Increase of ROS production and decrease of TAC was slowly reverted in mice after treated with β-carotene during the period of 30 days treatment, ROS production in white blood cells of mice decreased significantly ($P<0.001$), simultaneously TAC was improved. (Table II, III, Fig. 3)

Enzymic antioxidants

The activities of enzymic antioxidants CAT, SOD and GPx were found to be significantly ($p<0.05$) decreased in cancer induced in mice while comparing with the control group in all the four assessed tissues. The improvement in the enzymic antioxidant status was observed in β-carotene treatment alone and in combination with B(a)P. (Fig.4).

Non enzymic antioxidants

In the present study a decreased level of non enzymic antioxidants Vit A, Vit C, Vit E and GSH was observed in B(a)P treated animals and these decrease was reverted in case of β-carotene fed animals alone and in combination in the selected tissue samples. (Fig:5,6)

Histopathology

Plate 1 demonstrates the cellular changes produced in the liver of mice treated with β-carotene alone and in combination with B(a)P. Normal liver section showed the normal cellular pattern with few lymphatic collections. Mice treated with B(a)P had liver nuclear changes like hyperchromatism, pleomorphism and mitotic activity in the central field compared to the normal cells. Mice treated with β-carotene had the normal liver cells and mice treated with β-carotene and B(a)P maintains the normal cellular pattern with minimum vascular degeneration and necrosis.

DISCUSSION

There are clear links between human cancers and diet, dietary risk factors rank higher than tobacco usage and much higher than pollution or occupational hazards in their association with cancer deaths. In addition to avoidance of carcinogenic agents, regular intake of chemopreventive compounds is a promising approach for reducing cancer incidence. A number of substances naturally occurring in foodstuffs, particularly antioxidant compounds in plant products, have shown promise as potential chemopreventive agents [39]. Among these phytonutrients, the yellow, orange and red carotenoid pigments have recently

sparked much interest. In epidemiological studies, vegetable and fruit consumption has consistently been associated with reduced incidence of various cancers, and dietary carotenoid intake from these sources has similarly been correlated with reduced cancer risk [40]. However, several recent large-scale intervention trials failed to find any chemopreventive effect of long-term supplementation with β-carotene, the most abundant dietary carotenoid. Several naturally occurring carotenoids other than β-carotene have exhibited anticancer activity, and are being considered further as potential chemopreventive agents [41, 42].

Benzo (a) Pyrene [B(a)P], the polycyclic aromatic hydrocarbon (PAH) is an environmental carcinogen which promotes the lipid peroxidation and ROS production, through that it causes the cellular dysfunction [43]. The active metabolites of B(a)P are 3-OH-B(a)P, 6-quinone B(a)P, 9-OH-B(a)P, B(a)P-1,6-quinone, trace amounts of B(a)P-4,5-dihydrodiol, and B(a)P-7,8-dihydrodiol are primarily produced in liver and then bio transformed into various organs and causes cellular toxicity [44].

In the present investigation it was proved that, an increase in lipid peroxidation indicates serious damage to cell membranes, inhibition of several enzymes and cellular dysfunction. A number of ROS are generated during normal aerobic metabolism, such as superoxide, hydrogen peroxide and the hydroxyl radical. In addition, singlet oxygen can be generated through photochemical events, and lipid peroxidation can lead to peroxyl radical formation [45]. These oxidants collectively contribute to aging and degenerative diseases such as cancer through oxidation of DNA, proteins and lipids [46, 47].

Reactive Oxygen Species alters cytokine expression through nuclear transcriptional factors but also affect adhesion molecules [48]. ROS contribute towards increased transendothelial and transepithelial permeability [49]. The increase of transepithelial permeability allows toxins to permeate through the barrier, which leads to inflammation [50]. ROS produced by the B(a)P induction may possibly cause the above events, leading to the B(a)P induced tissue damage.

Beta carotene significantly reduced the membrane lipid peroxides and ROS production in toxicity induced animals. Antioxidant compounds can decrease those effects, and thus carcinogenesis, both by decreasing oxidative damage to DNA and by decreasing oxidant stimulated cell division [51]. The antioxidant enzymes may reduce the carcinogen-DNA interaction by providing a large nucleophilic pool for the electrophilic carcinogens. Exogenous dietary antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and carotenoids play important roles in reducing oxidative damage as well, and their serum levels have the potential to be manipulated [52].

In malignancy it is well known that SOD, CAT, GPx plays an important role as protective enzymes against LPO in tissues [53]. The results showed significant changes in the enzymic antioxidants that proves the preventive role of β-carotene against cancer. The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap peroxyl radicals [54]. The quenching activity of a carotenoid mainly depends on the number of conjugated double bonds of the molecule and is influenced to a lesser extent by carotenoid end groups [55]. β-Carotene is a scavenger of peroxyl radicals, especially at low oxygen tension. This activity may be also exhibited by others carotenoids. The interactions of carotenoids with peroxyl radicals may precede via an unstable β-carotene radical adduct [56].

The non enzymic antioxidants GSH, Vit A, Vit C and Vit E, play an excellent role in preventing the cells from oxidative threats. The decreased non enzymic antioxidants might due to excess utilization of these antioxidants by cancer cells. The vitamins A, C and E also exist in inter convertible forms and participate in neutralizing free radicals [57, 58].

In the present study a decrease in the activities of the blood TAC and the tissue antioxidants, was observed in the B(a)P treated mice. Since oxidative stress preceded the decrease in the activities of antioxidant enzymes. It is proposed that the decrease of the above contribute to the chemical toxicity of B(a)P [59]. The results of current data, is suggested that oxidative stress produced is due to B(a)P induced toxicity which effectively depletes cellular antioxidants in the system.

The findings have focused on elucidating the mechanisms by which β-carotene may modify tumour incidence and development. It is known that β-carotene is converted to retinoids, and the hormone-like effects of these compounds may exert potent influences on cell differentiation, proliferation and development [60]. Moreover, it may enhance gap junction communication, restricting clonal expansion of initiated cells. It may also influence carcinogenesis by interfering with the metabolic pathways involved in the metabolization of chemical carcinogens [40].

Many epidemiologic studies have associated high carotenoid intake with a decrease in the incidence of chronic disease [14]. Multiple possibilities exist - certain carotenoids, can be converted to retinoids, can modulate the enzymatic activities of lipoxygenases, can have antioxidants properties which are seen with vitamin A, can activate the expression of genes which encode the message for production of a protein, which is an integral component of the gap junctions required for cell to cell communication. Such gene activation is not associated with antioxidant capacity and is independent of pro-vitamin A activity [61].

In plants and algae, carotenoids serve both photosynthetic and photoprotective roles, in animals, carotenoids are effective chain-breaking antioxidants

and singlet oxygen quenchers, and some also serve as precursors for retinoids. Some carotenoids also appear to have effects on cell communication and proliferation in animals. Because animals cannot synthesize carotenoids de novo, they must obtain them from dietary sources [62].

CONCLUSION

Regular intake of chemopreventive compounds is a promising approach for reducing cancer incidence. A number of substances naturally occurring in foodstuffs, particularly antioxidant compounds in plant products, have shown promise as potential chemopreventive agents. Among these phytonutrients, the yellow, orange and red carotenoid pigments have recently sparked much interest. In epidemiological studies, vegetable and fruit consumption has consistently been associated with reduced incidence of various cancers, and dietary carotenoid intake from these sources has similarly been correlated with reduced cancer risk. β-carotene significantly reduced the membrane lipid peroxides, ROS production and improves the blood and cellular antioxidant levels there by acting against the toxicity. β-carotene, the most abundant dietary carotenoid from *C.humicola* being considered further as potential chemopreventive agents.

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

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Table 1: Effects of B(a)P and β-carotene alone and in combination, on MDA production in different organs by the end of 30 days treatments, values are significant at p<0.001. Values are mean±SD of six animals

Levels of Lipid peroxidation

	Lipid Peroxidation (nmoles of MDA/mg protein)			
Treatment	Lung	Liver	Stomach	Intestine
Control	1.05±0.09	1.36±0.12	1.60±0.13	1.32±0.12
B(a)P	2.38±0.11	3.60±0.14	2.83±0.13	3.12±0.14
β-carotene	0.68±0.04	0.84±0.08	0.76±0.06	0.79±0.06
B(a)P+β-carotene	1.10±0.10	1.12±0.10	1.32±0.12	1.24±0.10

Table 2. ROS production of white blood cells in mice was stimulated by B(a)P and inhibited by β-carotene. The white blood cells were collected at each week interval and used for assays of ROS at once. The level of blood ROS were significant (P<0.001) in β-carotene treatment. Values are mean±SD of six animals.

Levels of Reactive Oxygen Species

	ROS Production			
Treatment	1st Week	2nd Week	3rd Week	4th Week
Control	1.05±0.05	1.01±0.04	1.03±0.05	1.05±0.05
B(a)P	1.26±0.05	1.57±0.07	2.61±0.10	2.85±0.12
β-carotene	0.98±0.03	1.05±0.05	1.00±0.04	1.01±0.04
B(a)P+β-carotene	1.15±0.05	1.35±0.06	1.64±0.07	1.83±0.08

Table 3. Total antioxidant capacity (TAC) of blood in mice was inhibited by B(a)P and increased by β-Carotene. The TAC was measured by the alteration of the ferric-reduction ability of blood. The data are significant at P<0.001 with control. Values are mean±SD of six animals

Levels of Total Antioxidant Capacity

	TAC (Units/ml blood)			
Treatment	1st Week	2nd Week	3rd Week	4th Week
Control	299.33±4.87	302.00±4.98	301.50±4.89	302.33±4.92
B(a)P	263.67±4.32	255.33±4.43	236.83±4.48	205.00±4.49
β-carotene	308.16±5.60	312.83±5.87	313.67±5.98	308.00±5.57
B(a)P+β-carotene	267.00±5.09	271.67±5.13	273.67±5.15	284.67±5.25

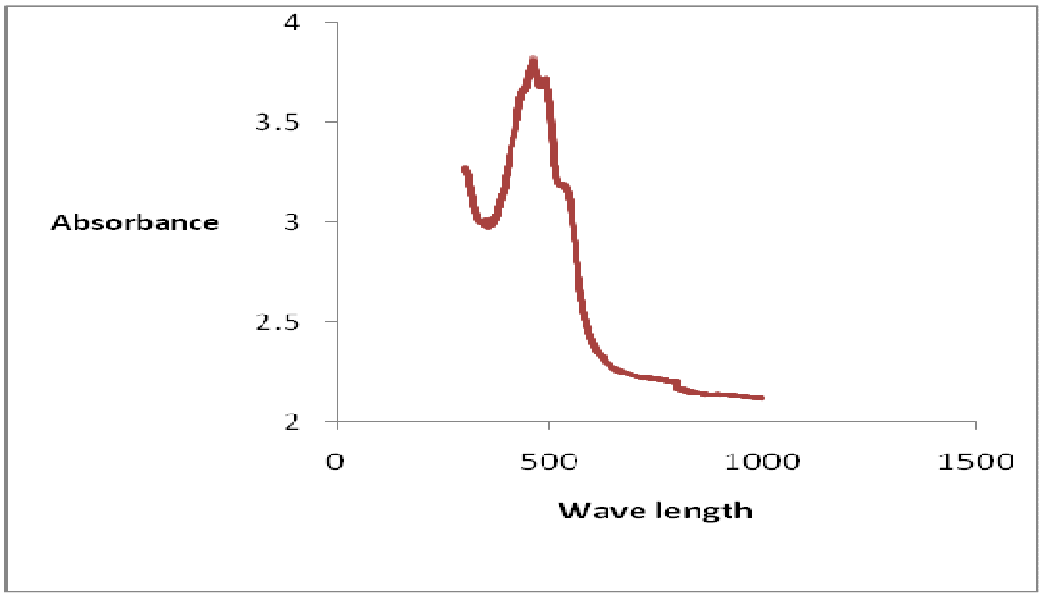
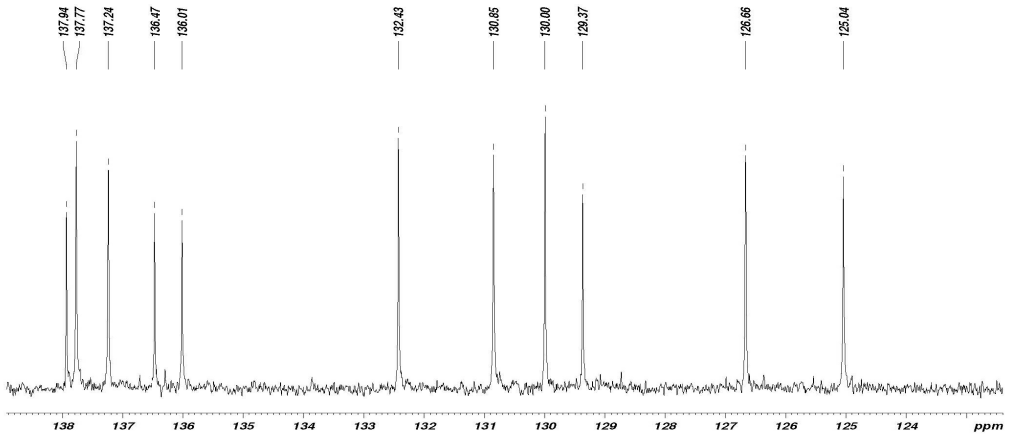


Fig 1. The visible absorption spectra of purified β-carotene from *C.humicola* was taken in UV-VIS spectrophotometer which shows the λmax at 462nm.



Sp2 Region of β-carotene from C. humicola

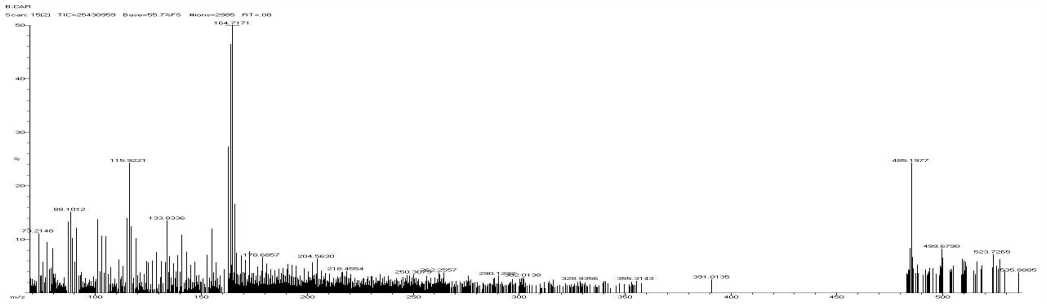
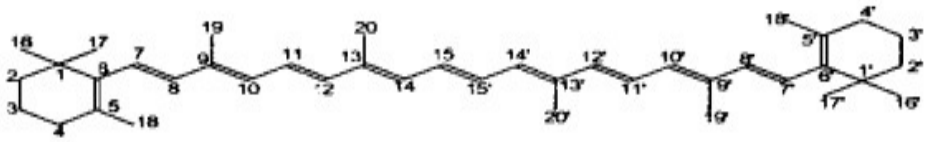


Fig 2. NMR and MASS spectrum of β-carotene from C.humicola.



β-carotene

Percentage production of ROS and TAC

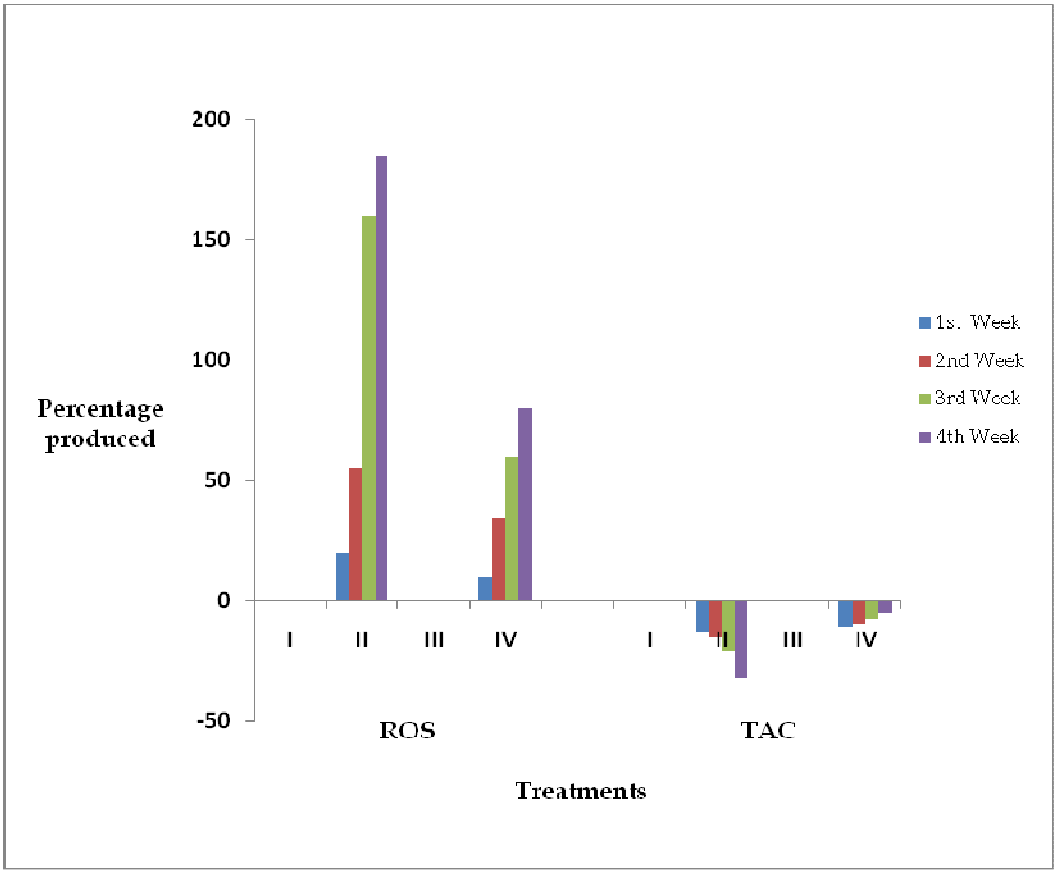
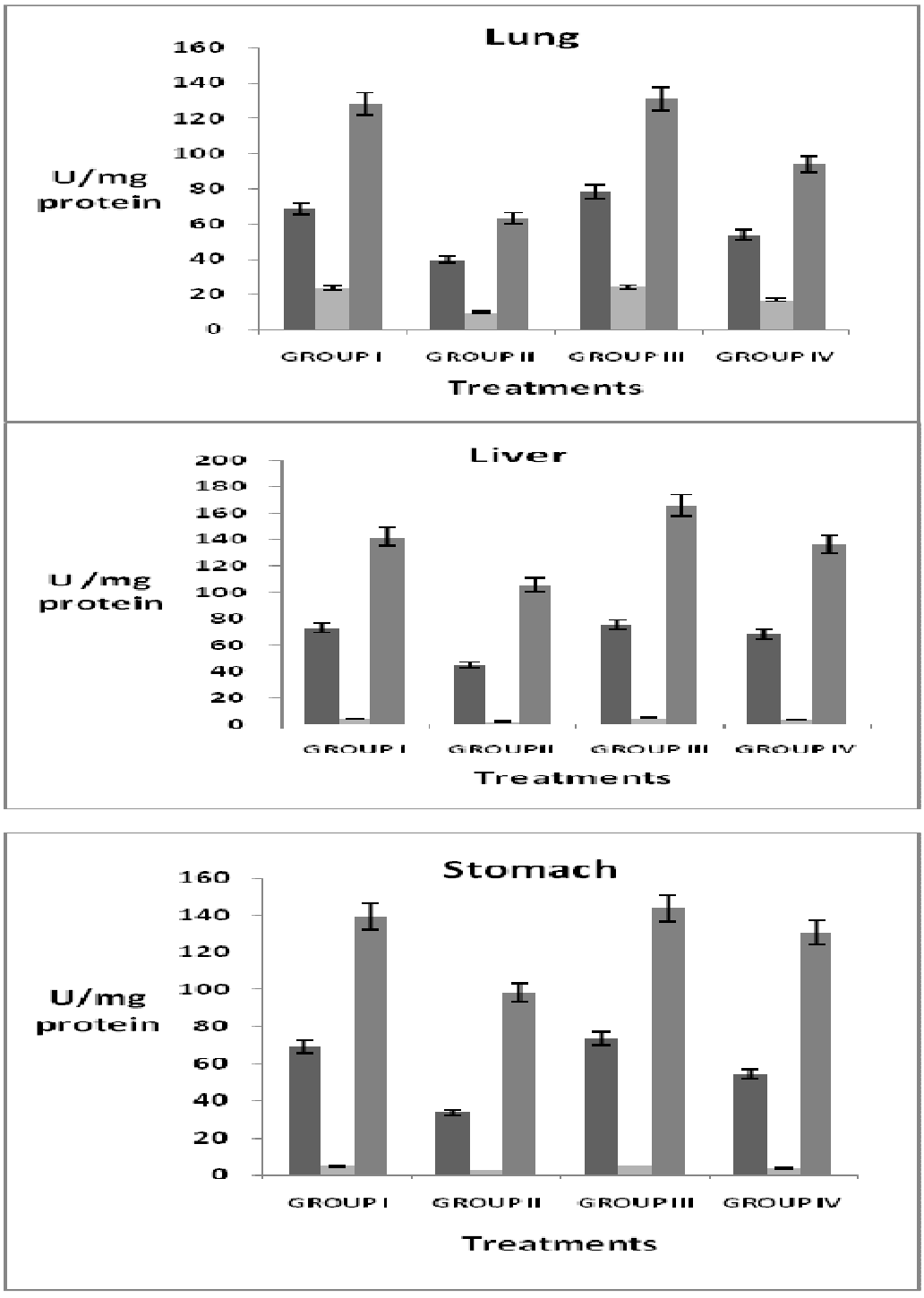


Fig 3 ROS production of white blood cells in mice was stimulated by B(a)P and inhibited by β-carotene and that improves the TAC production by 30 days treatment period.

Enzymic antioxidants in various tissues



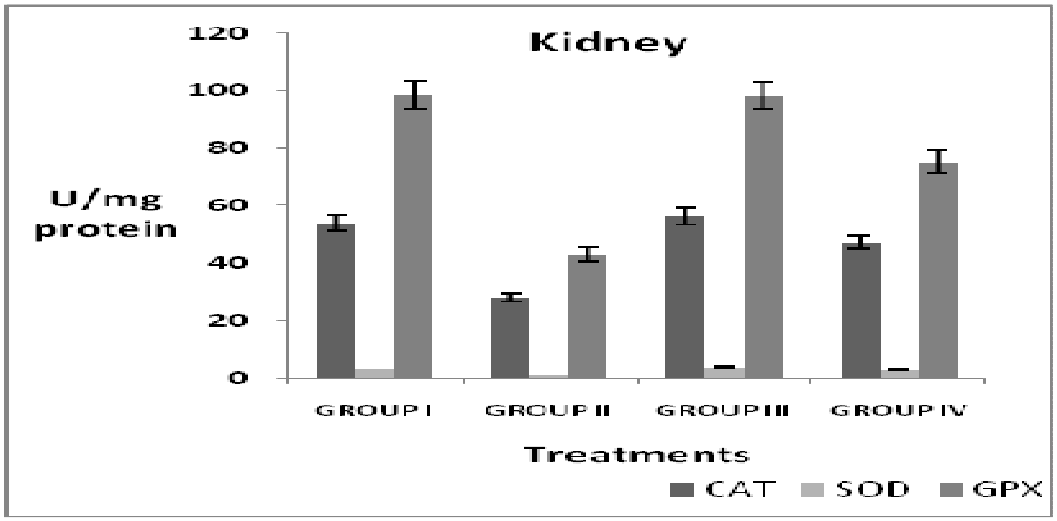
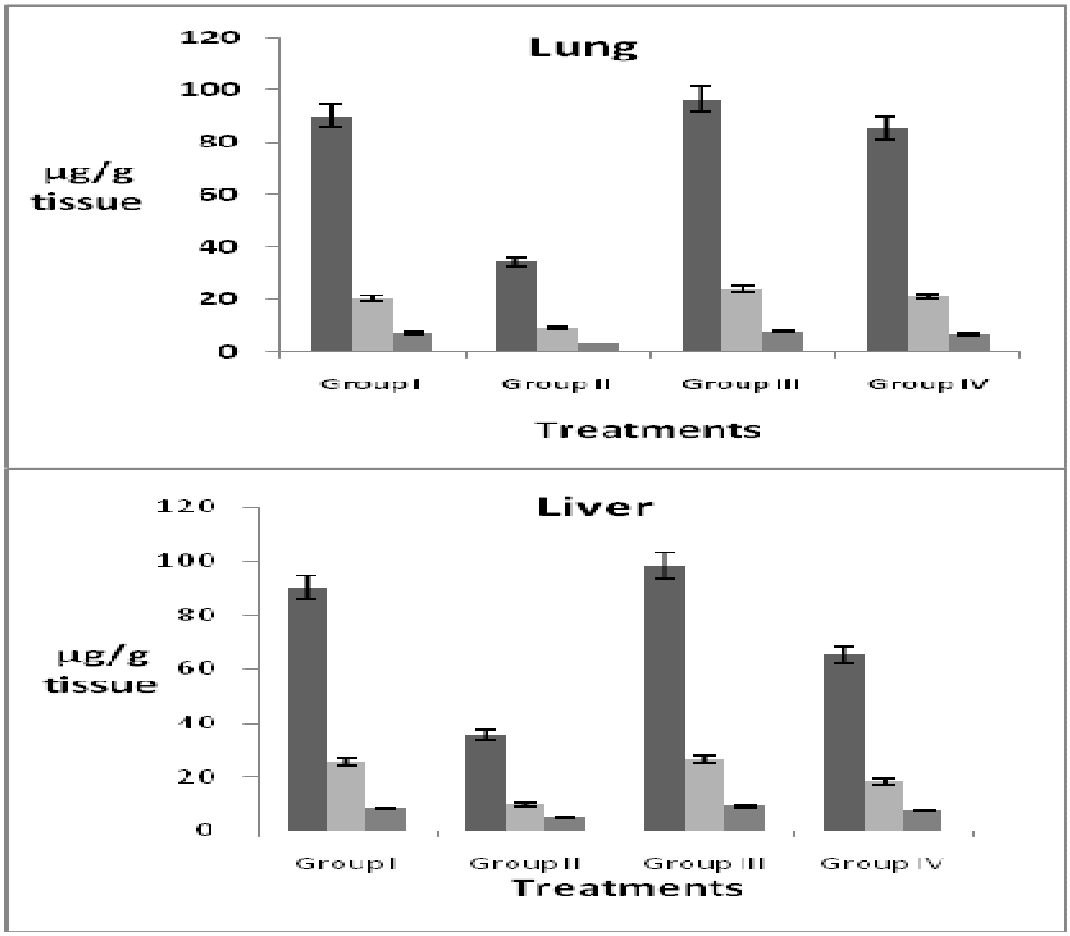


Fig 4: Alterations in enzymic antioxidants on the treatment of β-carotene in B(a)P induced mice. The CAT, SOD and GPx levels in the different organs lung, liver, stomach and kidney. The activity was significantly (P<0.05) improved in β-carotene treated mice. Values are mean±SD of six animals.

Non enzymic antioxidants in various tissues



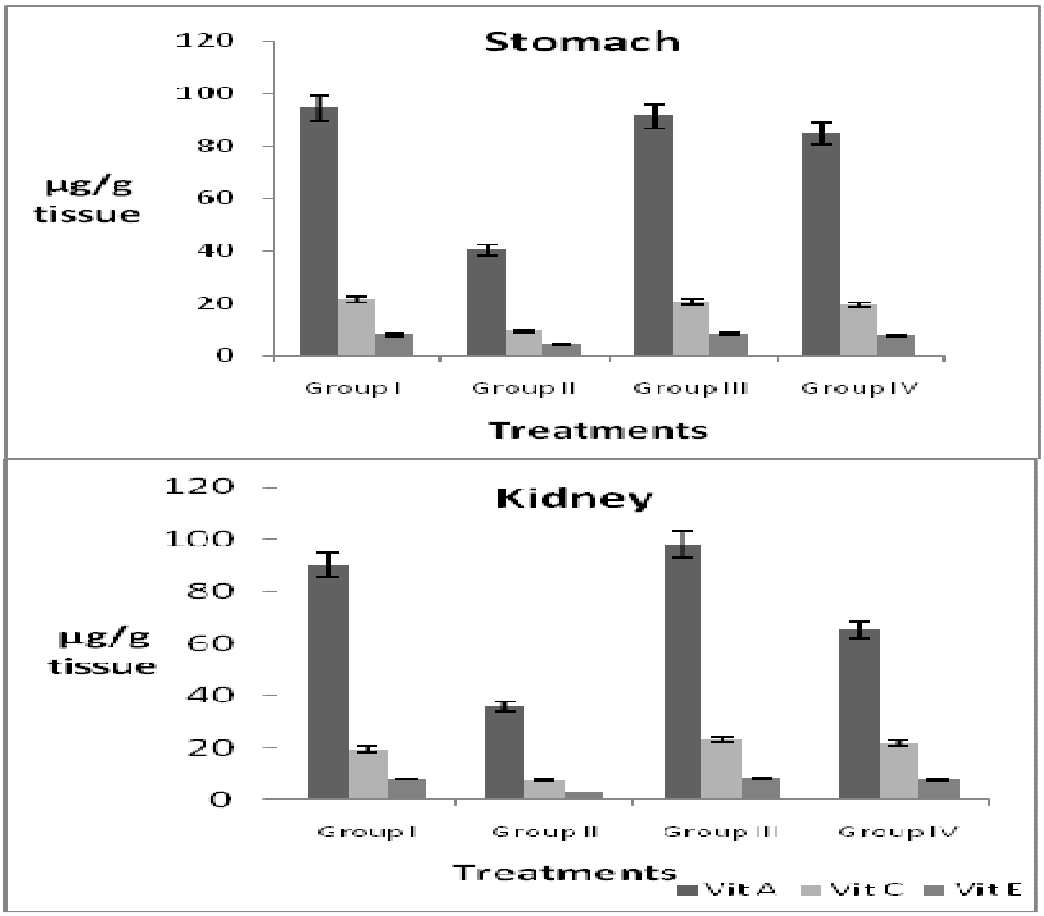
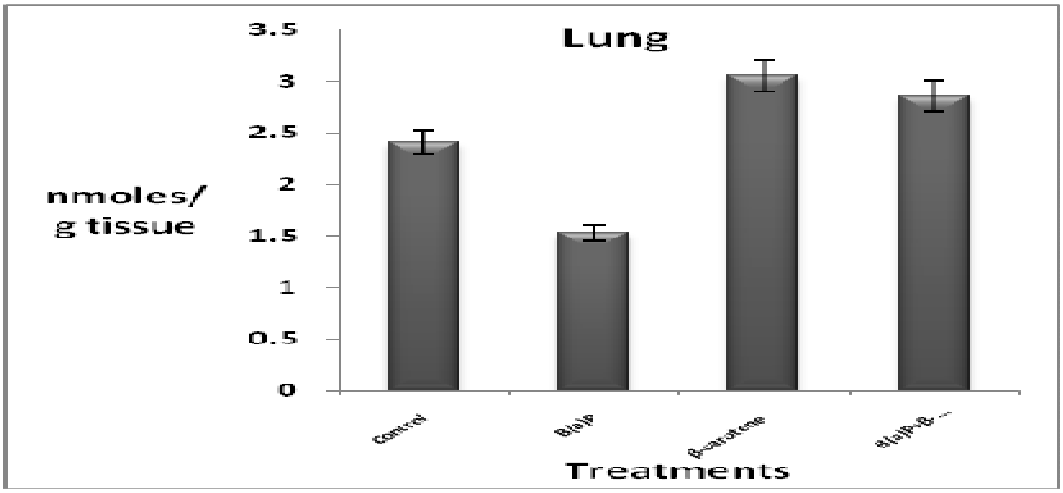


Fig 5: Alterations in nonenzymic antioxidants on the treatment of β-carotene in B(a)P induced mice. The Vit A, Vit C and Vit E levels in the different organs lung, liver, stomach and kidney. The activity was significantly ($P<0.05$) improved in β-carotene treated mice. Values are mean±SD of six animals.

Glutathione level in various tissues



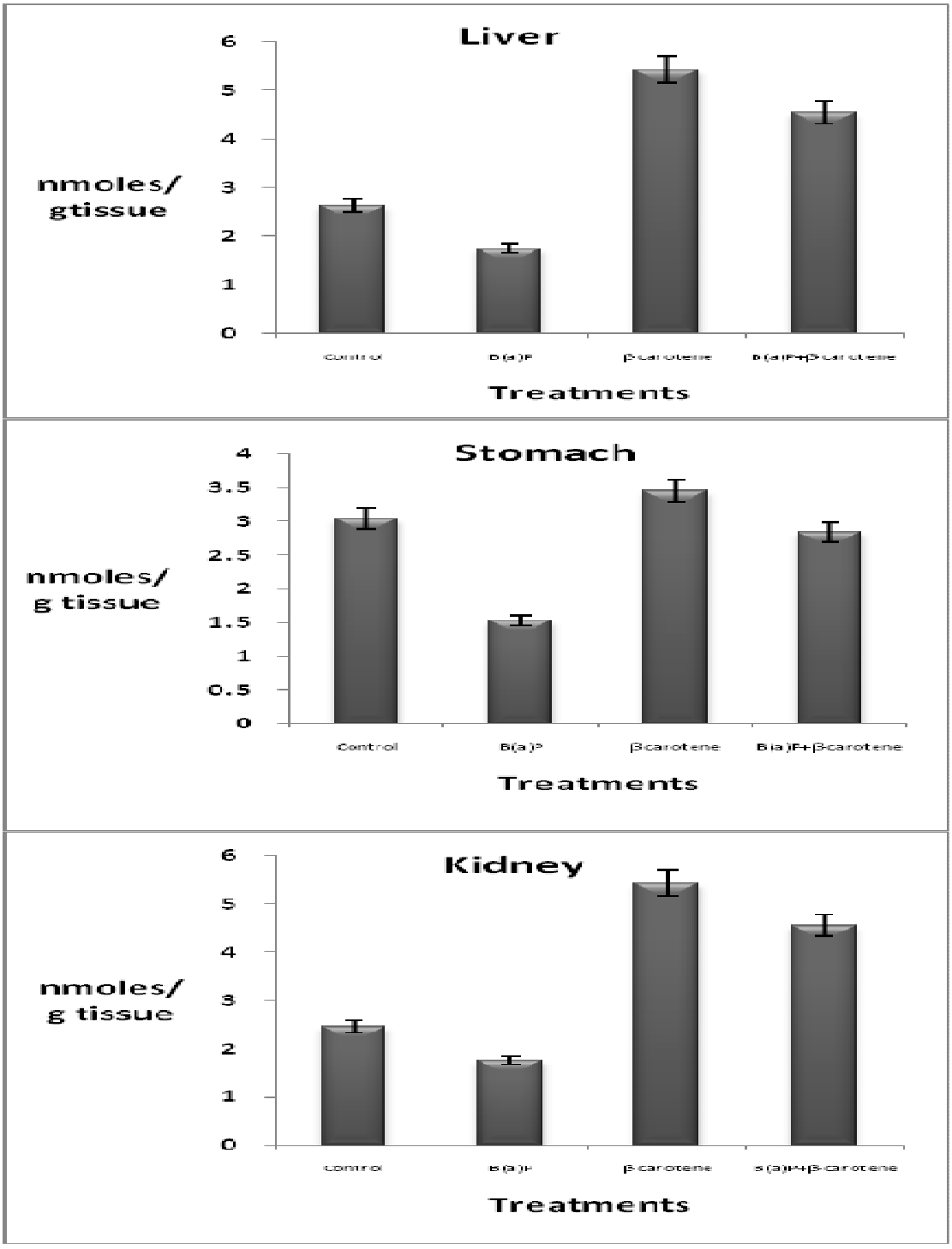


Fig 6: Changes in GSH level shows decreased in the toxicity induced groups which was significantly ($p<0.05$) increased by the treatment of β-carotene in all the tested tissues lung, liver, stomach and kidney. Values are mean±SD of six animals.

PLATE 1

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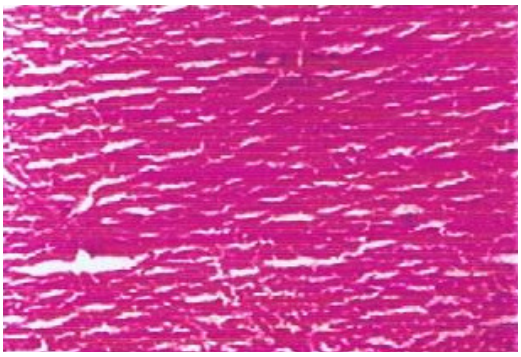
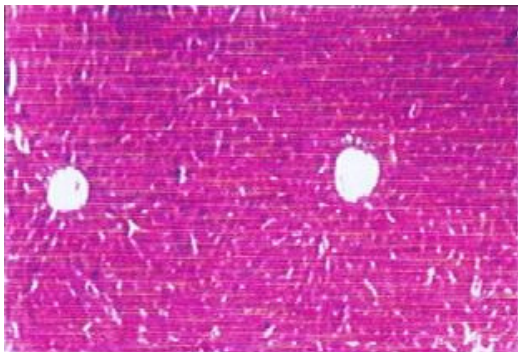


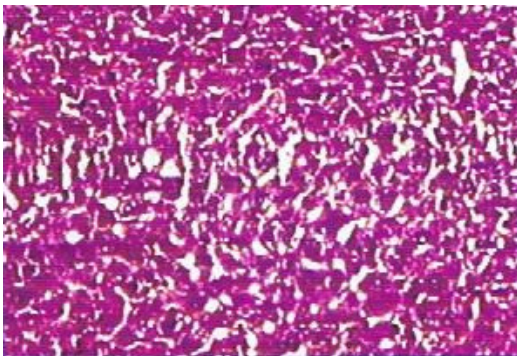
Control

Normal liver architecture



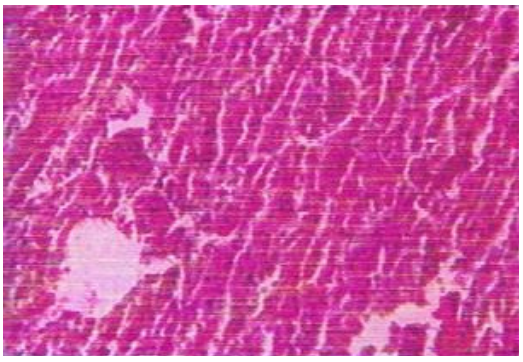
B(a)P

Hyperchromatism, Pleomorphism and Mitotic activity



β-carotene

Normal liver architecture



β-carotene +B(a)P

Mild vascular degeneration necrosis.

Plate:1 The protective effect of β-carotene against the toxic agent B(a)P on liver cells at the end of 30 days treatment period, the control liver cells showed the normal cellular pattern, while the B(a)P showed the necrosis and cellular damage. On β-carotene treatment the cells gets regenerated from the oxidative damage and protect the liver cells.