Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Modulation of lipid peroxidation and antioxidant status upon administration of ´Shemamruthaa´ in 7,12−dimethylbenz[a]anthracene induced mammary carcinoma bearing rats

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ARTICLE INFO

ABSTRACT

Objective: To investigate the therapeutic efficacy of a Shemamruthaa (SM), (combination of *Hibiscus rosasinensis* (*H. rosasinensis*) flowers, fruits of *Phyllanthus emblica* (*P. emblica*) and pure honey in definite ratio), against lipid peroxidation (LPO) and antioxidant status in experimentally induced mammary carcinoma rats. **Methods:** Adult female Sprague−Dawley rats were used for the study and were divided into four groups. Group I control animals received standard pellet diet and water *ad libitum*. Group II rats were induced with 7,12−dimethyl benz[a]anthracene (DMBA) (25 mg in 1 mL olive oil) by gastric intubation, whereas another set of DMBA−induced rats were treated with SM (400 mg/kg body weight/d) in olive oil orally by gastric intubation for 14 d after 3 months of induction period (group III). Group IV rats served as SM−treated control animals. At the end of the experimental period, the rats were anaesthetised and sacrificed and used for biochemical measures and histology studies. **Results:** The LPO was increased and antioxidant levels were decreased in the serum, liver and mammary tissues of cancer−induced rats. The administration of SM drug significantly ($P<0.05$) decreased LPO and reversed the status of antioxidants to near normal level in cancer−bearing animals. **Conclusions:** The results obtained indicate the additive and synergistic action of constituents' plants in the SM drug against oxidative damage and its protective role in DMBA induced mammary cancer.

1. Introduction

Cancer is a major public health problem, with significant associated death and disability. Breast cancer is the most common malignant disease affecting women worldwide, with 1 290 000 new cancer cases and 519 000 deaths annually[1]. It accounts for about one fourth of all cancers in Indian women and about half of all cancer related deaths[2]. The etiology of breast cancer is multifactorial and the risk factors include early menarche, late menopause, nuliparity, and late age at first birth, postmenopausal obesity, extended use of oral contraceptives, hormone replacement therapy, family history and previous benign breast disease. In addition to this, the common risk factor in the development of breast cancer is the increased lifetime exposure to endogenous or exogenous estrogens and it has been reported that a number of genes including BRCA1 and BRCA2, HER−2/neu, p53 and modulation of expression of Bax and Bcl−2 have been linked in the event of breast cancer susceptibility and development[3].

The human body is constantly subjected to significant oxidative stress as a result of a misbalance between antioxidative protective systems and the formation of reactive oxygen species (ROS) including free radicals. Experimental investigations as well as clinical and epidemiological findings has provided evidence supporting the role of reactive oxygen species (ROS) such as singlet oxygen ($1O_2$), superoxide anions ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) in the etiology of cancer, cardiovascular diseases, aging Parkinson's disease, etc[4]. Medicinal plants and herbs are a promising and diverse source of natural antioxidants. They provide protection without causing any side effects and therefore, development of drugs from plant products is desired. Many plant extracts and plant products have been identified as good protectors against the free radicals by triggering antioxidant gene expression. For that account natural antioxidants from plant

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sources have been viewed as promising therapeutic drugs[5]. *Hibiscus rosa−sinensis* (*H. rosa−sinensis*) L. (family: Malvaceae), commonly known as China rose is a potent herb in traditional system of medicine[6]. It is being used against cough, fever, dysentery, venereal diseases and cancerous swellings[7]. Investigations of the pharmacological properties have shown immense antioxidant[8], anticancer[9], antidiabetic[10], hepatoprotective[11] and cardioprotective[12] activities in various animal models. The constituents present in the extract include flavonoids, cyanidine, quercetin, hibiscetin, glycosides, riboflavin, niacin, carotene, taraxeryl acetate, b−sitosterol, campesterol, stigmasterol, ergosterol, citric, tartaric and oxalic acids, cyclopropenoids and anthocyanin pigments[7,13].

The fruits of *Emblica officinalis* (*E. officinalis*) Gaertn. (*Phyllanthus emblica* (*P. emblica*) L.; family: Euphorbiaceae) are principal constituents of many Ayurvedic preparations. The fruit extract has many pharmacological activities: it inhibits clastogenicity and mutagenicity induced by heavy metals and protects against radiations[14], possesses antidiabetic, cytoprotective and immunomodulating[15], anticancer and antioxidant activities[16]. Phytochemical investigation of the plant revealed the presence of tannins, ellagic acid flavonoids like quercetin, hydrolysable tannins (Emblicannin−A, Emblicannin−B, Punigluconin, Pedunculagin), Gallo−ellagitannoids, flavonoid (rutin), trigalloyl glucose and phyllemblic acid[14].

Considering the potential of several crude extracts, when used in formulation enhances the beneficial effects through synergistic amplification and offers advantage over a single isolated ingredient, the present study was carried out to investigate the antioxidant activity of Shemamruthaa [SM] (combination of *H. rosasinensis* flowers, fruits of *P. emblica* and pure honey in definite ratio) against DMBA induced breast cancer in experimental rats.

2. Materials and methods

2.1. Drug and Chemicals

The flowers of *H. rosasinensis* and deseeded *E. officinalis* fruits were air dried under shade, pulverized to fine powder using a cutting mill and mixed with pure honey in definite ratio. Butylated hydroxy toluene (BHT), 1,1 diphenyl−2−picryl hydrazyl (DPPH), 7,12−Dimethylbenz(a)anthracene (DMBA), 5′adenosine monophosphate (AMP), thiobarbituric acid (TBA), malondialdehyde (MDA), gallic acid and quercetin were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used in the study were of high purity and analytical grade.

2.2. Extraction of phenolic compounds

Total phenolic compounds were extracted from the peel and the pulp as described by Makkar[17]. The drug SM (2 g) was extracted twice with 50% cold aqueous methanol (10 mL). The extracts were combined, made up to 20 mL with 50% aqueous methanol, centrifuged at 3 000 rpm for 10 min and transferred into small sample bottles for analysis.

2.3. Phytochemical analysis

Qualitative analysis on phytochemical constituents of the SM extract was carried out using standard methods[18].

2.4. DPPH radical−scavenging activity

The free radical scavenging activity was determined by the method of Shimada *et al* and Yang *et al*[19,20]. The methanolic extracts were dissolved in methanol to prepare various sample solutions at 5, 10, 20, 40, 60, 80 and 100 μ g/mL. Each extract solution (2 mL) was mixed with 1ml of methanolic solution containing DPPH radicals, with a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and maintained for 30 min in dark. The absorbance was measured at 517 nm. The absorbance of the control was obtained by replacing the extract with methanol. Quercetin and BHA were used as standard reference. The scavenging activity was calculated using the formula, DPPH scavenging activity (%) = [(A_{517} of control− A_{517} of sample)/A_{517} of control] $\times$100.

2.5. Animals and diet

Adult female rats of Sprague−Dawley strain weighing (190 $\pm$10) g were provided from Central Animal House facility, University of Madras, Taramani Campus, Chennai−600 113, Tamil Nadu, India. The animals were maintained under standard conditions of humidity, temperature (25$\pm$2) ℃ and light (12 h light/dark). They were fed with standard rat pellet diet and water ad libitum. The study has got the approval from the Institutional Animal Ethical Committee (IAEC), regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests (Animal Welfare Division), Government of India (No. 01/030/2010).

2.6. Experimental design

The animals were randomly divided into four groups of six animals each: group I, control animals; group II, breast cancer induced in overnight−fasted animals by a single dose of DMBA in olive oil (25 mg/kg body weight) by gastric intubation; group III, breast cancer−induced animals (as in Group II) treated with SM drug (400 mg/kg body weight/day) orally by gastric intubation for 14 d; group IV, SM−treated control (400 mg/kg body wt) administered to control animals for 14 d by gastric intubation.

2.7. Experimental procedure

After the experimental period (90 d of induction + 14 d treatment), the animals were sacrificed. Blood was collected and serum was separated for the assays. The liver and mammary tissue were dissected out and washed with ice−cold 0.9% NaCl solution. A 10% homogenate was prepared in 0.01 M Tris HCl buffer, pH 7.4 by means of a Potter Elvehjem

homogenizer with a Teflon pestle.

2.8. Tumor volume

During the experimental period, the animals were weighed, explored by inspection and palpation and the two major and perpendicular diameters of each tumour were measured with a vernier calliper. From these data, latency time and tumour volume were studied. Latency time was analysed by means of the average time for to first tumour development. Tumour volume was measured as described by Escrich et al using the formula[21]:

v= 4/3 π (d1/2) × (d2/2)2 where d1 and d2 are the two diameter of the tumour (d1>d2).

2.9. Biochemical assays

Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH). TBARS were assayed in the mammarygland by the method of Ohkawa et al[22]. Lipid hydroperoxides were estimated by the method of Jiang et al[23]. Protein oxidation was measured by the method of Levine et al[24] based on the reaction of the carbonyl group with 2,4–dinitrophenylhydrazine to form 2,4–dinitrophenylhydrazone.

The protein content was estimated by the method of Lowry et al[25]. Superoxide dismutase (SOD) activity was measured at absorbance 420 nm using a spectrophotometer as the degree of inhibition of autoxidation of pyrogallol in an alkaline pH according to the method of Marklund and Marklund[26]. Catalase (CAT) activity was assayed by the method of Sinha[27]. The results were expressed in terms of μ mol of H_2O_2 liberated/min/mg protein. The activity of glutathione peroxidase (GPx) was assayed by the method of Rotruck et al[28]. The activity of GPx was expressed as μ mol glutathione oxidized/min/mg protein.

Reduced glutathione was determined by the method of Moron et al[29]. The sample (1.0 mL) was precipitated with 1.0 mL of TCA and centrifuged at 1 200×g for 20 min. To 0.5 mL of supernatant 2.0 mL of 5, 5′–dithiobis–(2–nitrobenzoic acid) (DTNB) was added and the colour developed was read immediately at 412 nm using a spectrophotometer. Vitamin E (α – tocopherol) and Vitamin C (ascorbic acid) levels were estimated by the method of Desai[30] and Omaye et al[31] respectively and expressed as μ g/mg protein.

2.10. Histopathological analysis

Formalin–fixed mammary tumour samples were paraffin embedded, sectioned (3 mm thickness) and placed on glass slides. Paraffin–embedded sections of tissue were deparaffinised, rehydrated with graded alcohol and stained with Harris′ haematoxylin and eosin (Dako, Glostrup, Denmark) in a Leica Autostainer (Wetzlar, Germany).

2.11. Statistical analysis

Values are given as the mean ± SD of six rats. The results were statistically evaluated using Student′s t–test using SPSS 16 (Statistical Package for Social Sciences) software and one–way analysis of variance (ANOVA). Values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Qualitative phytochemical analysis of SM

Qualitative analysis carried out on SM drug showed the presence of phytochemical constituents such as tannins, saponins, flavonoids, terpenoids and alkaloids and the results are summarized in Table 1.

3.2. DPPH radical–scavenging activity

The DPPH radical–scavenging assay is a widely used method for evaluating the ability of plant extracts to scavenge free radicals generated from DPPH reagent. The extent of the reaction depends on the hydrogen–donating ability of the antioxidant. The methanolic extract of SM showed appreciable free radical scavenging activity. As can be seen in Figure 1, the scavenging activity of SM drug tested was concentration dependent and the IC_{50} value of the extract was found to be (29.64±1.36) μ g/mL.

3.3. Effect of SM on body weight and tumour volume changes

The changes in the body weight of experimental animals are shown in Figure 2. There was a decrease in the body weight of tumour–bearing animals. Upon drug administration there was a significant ($P<0.05$) increase in body weight. No changes were observed in group IV animals. The carcinogenic parameter like tumour volume was observed during the investigation period and it is shown in Figure 3. The period of time from carcinogen administration to the palpable detection of the tumour was recorded. The tumour volume increased in both the groups (II and III) during the induction period. Upon administration of the drug SM to cancer induced animals (group III), there was a significant ($P<0.05$) decrease in the tumour volume when compared with group II animals.

3.4. Effect of SM on lipid peroxidation indices

Figure 4a–4c shows the effect of administration of the drug on lipid peroxidation and protein oxidation. The extent of lipid peroxidation was significantly higher in the

Table 1.
Qualitative analysis on phytochemical constituents of Shemamruthaa (SM).

Particular	Alkaloids	Cardiac glycosides	Flavonoids	Phlobatannis	Saponins	Tannins	Terpenoids
SM	+	−	+	−	+	+	+

+: Presence; −: Absence of particular phytochemical constituent.

Table 2.
Effect of 'Shemamruthaa' on activities of enzymatic antioxidants in serum, liver and mammary gland of control and experimental animals.

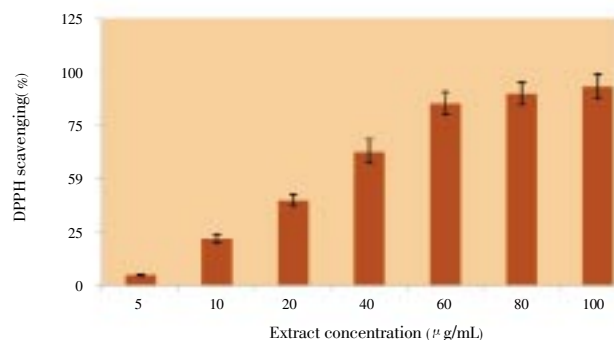| Sample	Parameters	Group I (Control)	Group II (DMBA induced)	Group III (DMBA+SM)	Group IV (SM)
Serum	Superoxide dismutase (unit min^{-1} mg protein^{-1})	14.38 ± 1.26	$8.75 \pm 0.76^{a*}$	$11.82 \pm 0.84^{a\#b\#}$	15.27 ± 0.86
	Catalase (μ mol H$_2$O$_2$ utilised min^{-1} mg protein^{-1})	58.73 ± 4.68	$39.29 \pm 2.41^{a*}$	$46.72 \pm 2.84^{a\#b\#}$	65.36 ± 4.76
	Glutathione peroxidase (μ mol of GSH consumed min^{-1} mg protein^{-1})	8.74 ± 0.64	$6.34 \pm 0.53^{a*}$	$7.26 \pm 0.64^{a\#b\#}$	8.68 ± 0.73
Liver	Superoxide dismutase (unit min^{-1} mg protein^{-1})	7.24 ± 0.71	$4.28 \pm 0.21^{a*}$	$5.17 \pm 0.36^{a*b*}$	7.54 ± 0.64
	Catalase (μ mol H$_2$O$_2$ utilised min^{-1} mg protein^{-1})	56.92 ± 2.36	$34.56 \pm 1.69^{a*}$	$43.48 \pm 1.34^{a*b*}$	56.32 ± 0.87
	Glutathione peroxidase (μ mol of GSH consumed min^{-1} mg protein^{-1})	11.34 ± 0.85	$6.81 \pm 0.74^{a*}$	$8.26 \pm 0.82^{a*b*}$	11.62 ± 0.94
Mammary	Superoxide dismutase (unit min^{-1} mg protein^{-1})	15.82 ± 1.37	$7.26 \pm 0.65^{a*}$	$11.29 \pm 0.78^{a*b*}$	15.86 ± 1.28
	Catalase (μ mol H$_2$O$_2$ utilised min^{-1} mg protein^{-1})	72.56 ± 2.35	$48.36 \pm 1.47^{a*}$	$57.36 \pm 1.54^{a*b*}$	73.61 ± 2.78
	Glutathione peroxidase (μ mol of GSH consumed min^{-1} mg protein^{-1})	13.28 ± 0.69	$6.59 \pm 0.54^{a*}$	$8.64 \pm 0.62^{a*b*}$	13.63 ± 0.75

Values are expressed as mean $\pm$ SD for six animals. (group I, control; group II, tumor–induced; group III, tumor induced + SM treated; group IV, control+ SM). ANOVA followed by Student's *t* test. [a]Groups II and III compared with Group I; [b]Group III compared with Group II. Statistical significance: [*]$P<0.01$; [#]$P<0.05$.

Table 3.
Effect of 'Shemamruthaa' on activities of non–enzymatic antioxidants in serum, liver and mammary gland of control and experimental animals.

Sample	Parameters	Group I (Control)	Group II (DMBA induced)	Group III (DMBA+SM)	Group IV (SM)
Serum	Reduced glutathione (μ g/mg protein)	9.87 ± 0.86	$6.85 \pm 0.57^{a*}$	$7.79 \pm 0.58^{a\#b\#}$	9.62 ± 0.79
	Vitamin C (μ g/mg protein)	2.32 ± 0.32	$1.46 \pm 0.19^{a*}$	$1.94 \pm 0.14^{a*b*}$	2.46 ± 0.18
	Vitamin E (μ g/mg protein)	1.86 ± 0.13	$1.27 \pm 0.08^{a*}$	$1.64 \pm 0.15^{a*b\#}$	1.87 ± 0.17
Liver	Reduced glutathione (μ g/mg protein)	6.88 ± 0.77	$4.26 \pm 0.53^{a*}$	$5.42 \pm 0.24^{a*b*}$	6.84 ± 0.41
	Vitamin C (μ g/mg protein)	3.66 ± 0.27	$2.85 \pm 0.29^{a*}$	$3.12 \pm 0.26^{a*b*}$	3.82 ± 0.29
	Vitamin E (μ g/mg protein)	2.89 ± 0.24	$1.64 \pm 0.14^{a*}$	$2.54 \pm 0.23^{a*b*}$	2.86 ± 0.29
Mammary	Reduced glutathione (μ g/mg protein)	12.82 ± 0.87	$7.36 \pm 0.69^{a*}$	$8.64 \pm 0.72^{a*b*}$	12.63 ± 0.96
	Vitamin C (μ g/mg protein)	3.47 ± 0.24	$1.86 \pm 0.16^{a*}$	$2.68 \pm 0.22^{a*b*}$	3.52 ± 0.26
	Vitamin E (μ g/mg protein)	2.46 ± 0.24	$1.15 \pm 0.13^{a*}$	$2.23 \pm 0.21^{a*b*}$	2.56 ± 0.27

Values are expressed as mean $\pm$ SD for six animals. (group I, control; group II, tumor–induced; group III, tumor induced+SM treated; group IV, control+ SM). ANOVA followed by Student's *t* test. [a]Groups II and III compared with Group I; [b]Group III compared with Group II. NS: Not Significant. Statistical significance: [*]$P<0.01$; [#]$P<0.05$.
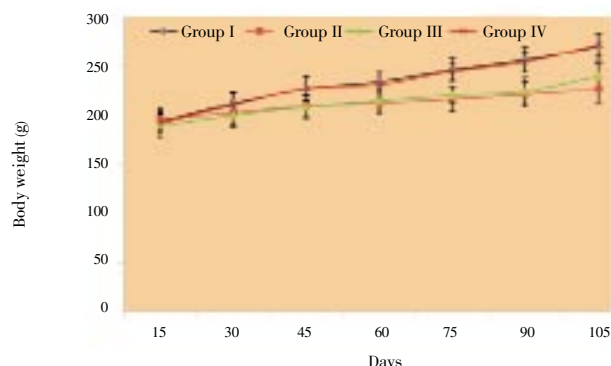
mammary gland of DMBA–administered animals (group I) compared to control. The administration of SM (group III) significantly reduced DMBA–induced changes in lipid and protein oxidation and increased the antioxidant status. No significant changes were found in drug control animals when compared to control (group I).

(group I) animals ($P<0.05$). On drug treatment (group III), the activities of these enzymes were found to be significantly ($P<0.05$) increased when compared with induced (group II) animals. The drug control animals (group IV) did not show any significant changes when compared to control (group I) animals.

Figure 1. DPPH radical scavenging activities of methanolic extracts of *Shemamruthaa*. Each value was presented as the mean $\pm$ SD of three replicate determinations.

Figure 2. Changes in body weight of the rats recorded from the day of tumour induction until the completion of the experimental period (90 d of tumour induction+14 d of SM treatment). Values are expressed as mean $\pm$ SD for six animals. Group I, control; group II, tumor–induced; group III, tumor induced + SM treated; group IV, control +SM.

3.5. Effect of SM on enzymic and non–enzymic antioxidant status

The activities of enzymatic antioxidants, like SOD, CAT and GPx in serum, liver and mammary tissue of control and experimental animals were represented in Table 2. The status of these enzymes was significantly lowered in DMBA–induced (group II) animals when compared to control

The levels of non–enzymic antioxidants, namely, reduced GSH, vitamins C and E in serum, liver and mammary tissue of control and experimental animals were represented in Table 3. In untreated DMBA–induced (group II) animals, the levels of GSH, vitamins C and E were significantly decreased when compared to control (group I) animals. In drug–treated

(group III) animals, the levels of GSH, vitamins C and E ($P<0.05$) were significantly increased when compared with diseased (group II) animals. Drug control (group IV) animals did not show any significant variations when compared to control (group I) animals.

3.6. Effect of SM on histological alterations in mammary gland of experimental rats

Figure 5 shows the histology of breast tissue. Normal rats showed mammary gland skin with underlying fibro fatty tissue containing scattered mammary gland ducts and bundles of skeletal muscles are seen in one area, whereas DMBA−induced rats show parts of a tumour composed of hyperchromatic, pleomorphic, vescicular nuclei and moderate cytoplasm arranged in nests, sheets and acinar structures with numerous mitotic figures (Figure 5a). Mammary carcinoma bearing rats treated with SM show

regression in tumour cells and fibro fatty tissue with few ducts are seen (Figure 5b). The drug control rats' mammary tissue showed normal architecture.
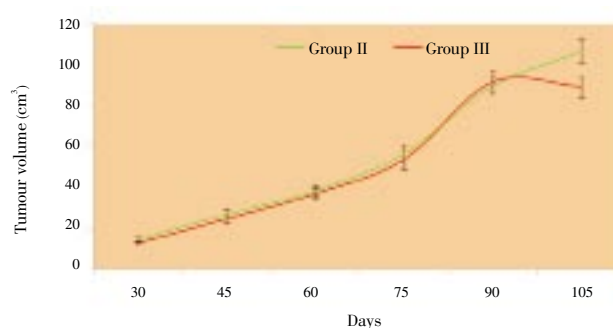

Figure 3. Changes in tumour volume of group II (tumour induced) and group III (tumor induced + SM treated) rats. Values are expressed as mean $\pm$ SD for six animals.
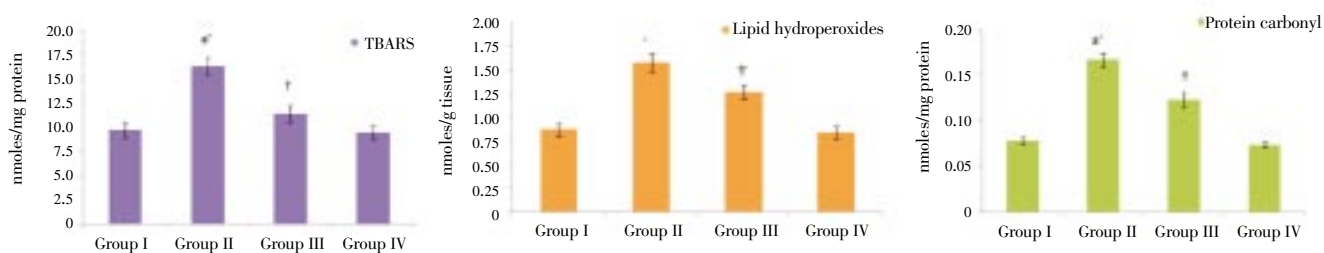

Figure 4. Figures 4a–4c depict the levels of lipid peroxidation estimated as thiobarbituric acid reactive substances (4a), lipid hydroperoxides (4b) and protein oxidation (4c) in the mammary gland of control and experimental animals (mean $\pm$ SD; $n=6$). (Group I, control; group II, tumor−induced; group III, tumor induced + SM treated; group IV, control+ SM). a*Comparisons are made between groups I and II. †Comparisons are made between groups II and III (*, †$P<0.05$).
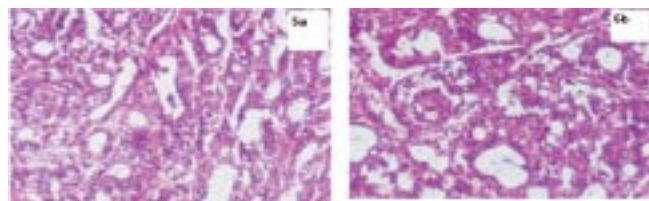

Figure 5. DMBA−induced rats (group II) show parts of a tumour composed of hyperchromatic, pleomorphic, vescicular nuclei and moderate cytoplasm arranged in nests, sheets and acinar structures with numerous mitotic Figures (5a). Tumour induced + SM treated rats (group III) show fatty tissue with small lobules and tumour regression is seen (5b).

4. Discussion

Expanding knowledge in the role of physiologically active food components from plant sources has changed the role of diet in health. Phenolic compounds known to possess high antioxidant activity are commonly found in fruits, vegetables, and herbs. Thus, antioxidants that have been shown able to neutralize free radicals, may be of central importance in the prevention of carcinogenicity, cardiovascular, and neurodegenerative changes associated with aging[32]. In the present study, qualitative analysis carried out on SM drug showed the presence of phytochemical constituents such as tannins, saponins, flavonoids, terpenoids and alkaloids. The antioxidant activity of the SM extract was tested as its

capacity to scavenge free radicals of DPPH, which has been widely used to evaluate the antioxidant activity of natural products from plant sources[33]. The antioxidant effects of the extract may be due to its phenolic content.

The body weight of the rats was recorded from the day of tumour induction until the completion of the experimental period. In tumour−bearing rats, there was a notable reduction in body weight. This may be due to tumour cachexia, characterized by weakness, lethargy, anorexia, depletion of host components, tissue wasting and a progressive fading of vital functions[34]. The drug treated animals showed a gradual increase in their body weights indicates the counteractive property of the drug. The drug effectively decreased the tumour volume in cancer−induced animals. This might be due to the inhibitory action of the drug on tumour growth in rats. The flavonoids, phenolic acids, quercetin, catechins, niacin, ascorbic acid, β −carotene and other bioactive components present in the SM might influence the growth due to their antioxidant, immunopotentiating and anti−inflammatory properties.

LPO refers to the reaction of oxidative deterioration of polyunsaturated lipids. Peroxidation involves the direct reaction of oxygen and lipid to form radical intermediates and to produce semi stable peroxidases, which in turn damage the enzymes, nucleic acids, membranes and proteins. The increased levels of LPO in group II cancer− bearing animals of the present investigation may be due to free radicals produced by DMBA administration and

is consistent with the earlier findings of Bhuvaneswari *et al*[35]. However, the administration of SM drug decreased the LPO levels in induced animals (group III) which may be due to the free radical scavenging activity of the flavonoids in the drug. In this connection, several studies have shown that flavonoids act as a powerful consumer of superoxide, singlet oxygen and hydroxyl radicals thereby contributing significantly to the intracellular antioxidant defence system[36].

Antioxidants act as the primary line of defence against ROS and suggest their usefulness in estimating the risk of oxidative damage induced during carcinogenesis. During cell membrane damage, various enzymes leak down to the circulatory fluid and their assessment in serum serves as markers in clinical studies. SOD is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide (O_2^-) to hydrogen peroxide (H_2O_2). CAT is a peroxisomal haem protein that catalyses the removal of H_2O_2 formed during the reaction catalysed by SOD. Thus, SOD and CAT acts mutually supportive antioxidative enzymes, which provide protective defence against reactive oxygen species. The present study reveals that SOD and CAT levels are decreased in the cancer–bearing animal, which may be due to altered antioxidant status caused by carcinogenesis which was consistent with published studies[37]. GPx, a selenium–containing enzyme, works together with glutathione in the decomposition of hydrogen peroxide or other organic hydroperoxides to non–toxic products at the expense of GSH. The decreased activity of GPx in mammary carcinoma rats obtained from the present investigation indicates the weak free radical defence system against oxidative stress.

The non–enzymic antioxidant systems are the second line of defence against free radical damage. GSH is an important non–protein cellular thiol that in conjunction with GPx plays a regulatory role in cell proliferation. We also observed decreased activity of GSH in cancer–bearing animals. GSH serves as a marker for evaluation of oxidative stress and it acts as an antioxidant at both extra cellular and intracellular levels. Ascorbic acid is a good scavenger of free radicals and it protects cellular membranes thereby preventing degenerative disease like cancer[38]. Decreased levels of water soluble antioxidants found in cancer–bearing animal may be due to the utilization of antioxidants to scavenge the free radicals. Vitamin E is a potent oxygen radical scavenger that protects cell membranes from oxidative damage initiated by carcinogens[39]. The present study found decreased levels of Vitamin E in the cancerous condition which may be due to excessive production of ROS by cancerous cells.

The administration of SM drug significantly reversed the status of antioxidants to near normal level in cancer–bearing animals, which indicate the additive and synergistic effect of combination of *H. rosasinensis* and *P. emblica* in drug. The presence of flavonoids, phenolics and various aromatic compounds may have contributed to this effect. These results put forward the protective role of the SM flavonoids against the toxicity of the chemotherapeutic agents in rats giving us hope that they may have similar protective action in humans.

The successful therapy of breast cancer will have to identify natural products that have significant therapeutic and preventive potential without toxic side effects of the common anticancer drugs. The drug SM, a potent antioxidant agent may prove to be one of the useful candidates in the search for effective, non–toxic substances with free radical scavenging activity. Considering the antioxidant property of SM, the bioactive compounds derived from these plants can be supplemented with anticancer medicines. Further investigation on the anticancer activity mechanisms of SM remains to be studied in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

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