CHEMOTHERAPEUTIC EFFICACY OF TRIDHAM AND 1,2,3,4,6-PENTA-O-GALLOYL-β-D-GLUCOSE ON ANTIOXIDANTS STATUS AND TUMOUR MARKERS IN EXPERIMENTAL MAMMARY CARCINOMA IN SPRAGUE-DAWLEY RATS

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ABSTRACT

Objective: To study the restorative effect of Tridham (TD) and 1,2,3,4,6-penta-o-galloyl-β-D-glucose (PGG) on 7,12-dimethyl benz(a)anthracene (DMBA) induced mammary carcinoma in female Sprague-Dawley rats.

Methods: Rats were divided into seven groups of six animals each. Group I rats served as control. Group II - mammary carcinoma was induced by DMBA. Group III and Group IV were induced with DMBA and subsequently treated with TD and PGG, respectively, for 48 days. Group V was treated with DMBA and subsequently with a standard drug, cyclophosphamide (CYC). Group VI and Group VII were given TD and PGG alone, respectively, for 48 days. After the experimental period, the levels of lipid peroxides (LPO), activities of enzymic and non-enzymic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, vitamin C and vitamin E were evaluated in the breast tissue of control and experimental rats. Levels of LPO, marker enzymes such as 5'-nucleotidase and lactate dehydrogenase, were also evaluated.

Results: The levels of enzymic and non-enzymic antioxidants were decreased in DMBA induced rats when compared to control rats. The levels of tumor markers were increased in DMBA induced rats when compared to control rats. These parameters were restored to near normal levels on treatment with TD and PGG.

Conclusions: The results suggest that TD and PGG have a cytoprotective role in DMBA induced breast cancer bearing rats. The effect of TD and PGG was found to be more pronounced than CYC, a standard drug.

Keywords: Breast cancer, Tridham, Penta galloyl glucose, Antioxidants, Tumor markers, Sprague-Dawley rats.

INTRODUCTION

Breast cancer is the second leading cancer of women worldwide. According to the International Agency for Research on Cancer, breast cancer is one of the most commonly diagnosed cancers worldwide and estimated at 1.38 million cases, i.e., 10.9% when compared to all cancers during 2008 [1]. Breast cancer is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Carcinogenesis is a multistep process, which involves deregulation of cell cycle, cell growth, cell proliferation, and genetic alterations, which results in aberrant cellular appearance and carcinoma [2]. Many factors such as age, early menarche, late menopause, family history, prolonged hormone replacement therapy, alcohol consumption, gene mutations, and environmental factors contribute to the development of breast cancer [3]. Among the environmental factors, chemical carcinogens, such as 7,12-dimethyl benz(a)anthracene (DMBA) and N-Nitroso-N-methylurea, play a major role in the development of breast cancer by alterations of cellular machinery [4].

DMBA is a type of polycyclic aromatic hydrocarbon, and it serves as a powerful organ-specific experimental carcinogen in mammary gland carcinogenesis in rats [5]. DMBA acts as a tumor initiator by making necessary mutations in DNA. The oxidation of DMBA by cytochrome P450 enzyme produces metabolites that initiate carcinogenesis. These metabolites form covalent adducts with DNA and the formation depurinated basic sites that lead to the formation of stable DMBA-DNA adducts [6]. Reactive oxygen species (ROS) are involved in the initiation and progression of carcinogenesis by ROS-induced oxidative

damage, which causes a decrease in the efficiency of antioxidant defense mechanism [7]. Proper balance between ROS and antioxidants should be maintained in the cell because of their potential importance in the pathogenesis of cancer. In the recent scenario, there has been a growing interest in modulating the role of phytochemicals in antioxidant status [8] and lipid peroxidation (LPO). Many medicinal plants and their isolated phytochemicals are interesting subjects for drug discovery because they are believed to protect cells from damage that could lead to cancer. The major groups of secondary metabolites in phytochemicals are polyphenols, flavonoids, tannins, alkaloids, etc.

Phytochemicals act in the following manner:
i.   Help to stop the formation of potential cancer-causing substances (carcinogens)
ii. Help to stop carcinogens from attacking cells
iii. Inhibit cell proliferation.

Based on the above facts, we have prepared a triherbal formulation called Tridham (TD) consisting of three major plant ingredients, viz., dried seed coats of *Terminalia chebula*, dried fruits of *Elaeocarpus ganitrus,* and dried leaves of *Prosopis cineraria*. These three plants are used for the treatment of various diseases in indigenous systems of traditional Indian medicine and also extensively used as folk medicine and remedy for various ailments. The anticancer effect of TD against hepatocellular carcinoma has already been studied and reported in our laboratory [9,10]. The combined phytochemical interactions of herbal drugs are effective in several ways, and hence, in the present study, the anticancer activity of herbal drug, TD has been investigated against DMBA-induced mammary carcinoma.

METHODS

Collection, authentication of plant material, and preparation of TD drug

TD is a triherbal formulation of seed coats of *T. chebula* (Family: Combretaceae), dry seeds of *E. ganitrus* (Family: Elaeocarpaceae), and leaves of *P. cineraria* (Family: Leguminosae). The botanical authentication was done in Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India. The ingredients were washed, shade dried then finely grounded and mixed in a definite proportion. The powder was extracted using various solvents (aqueous, ethanol, methanol, acetone, and hexane). Based on the phytochemical properties, the lyophilized dried pellets of the aqueous extract of TD were used.

Drugs and chemicals

DMBA (Catalogue No: D3254-100MG), 1,2,3,4,6-penta-o-galloyl-β-D-glucose (PGG) (Catalogue No: G7548-25MG), and cyclophosphamide (CYC) (Catalogue No: C3250000) were obtained from Sigma-Aldrich Chemical Company, St. Louis, USA. Pyrogallol, potassium dichromate, hydrogen peroxide, 5,5'-dithio-bis(2-nitrobenzoic acid), ethylene diamine tetra acetic acid, nicotinamide adenine dinucleotide phosphate, and all other chemicals and solvents used were of analytical grade with the highest purity.

Animals

The study was performed with the approval of the Institutional Animal Ethical Committee (IAEC NO: 01/21/12). Adult female albino rats of Sprague-Dawley strain weighing 100±10 g were purchased and maintained in Central Animal House Facility, DR. A.L.M. Post-Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai - 600 113, Tamil Nadu, India. The animals were maintained under standard conditions of humidity, temperature (25°C±2°C) and light (12 hrs light/12 hrs dark). They were fed with standard pelleted diet (M/S Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and had free access to tap water.

Experimental design

The nulliparous rats were divided into seven groups with six animals in each group as follows: Group I normal healthy rats maintained as a control group, which received a single dose of corn oil (0.5 ml) by gastric intubation at the age of 8th week (56th day). Group II mammary carcinoma was induced by DMBA with the dosage of 25 mg/kg/body weight dissolved in 0.5 ml of corn oil, at the age of 8th week (56th day) by gastric intubation. Mammary carcinoma was confirmed by regular palpation and histological examinations of breast tissue. Then, the tumor was allowed to grow to attain considerable mass and size. In Group III, Group IV, and Group V rats, the mammary carcinoma was induced by DMBA as in Group II, but after the tumor induction, treatment was started using aqueous extract of TD (400 mg/kg/body weight/day), PGG (30 mg/kg/body weight/day), and CYC Trade Name: Endoxan 50 (30 mg/kg/body weight/day), respectively, for 48 days daily and administered through gastric intubation. Group VI and Group VII rats served as drug controls, which received the aqueous extract of TD (400 mg/kg/body weight/day) and PGG (30 mg/kg/body weight/day), respectively, for 48 days daily and administered through gastric intubation.

At the end of experimental period, the overnight fasted rats were sacrificed by cervical decapitation. Six pairs of mammary gland were dissected from each group of animals and used for further analysis.

Experimental procedure of biochemical parameters in breast tissue

Estimation of ROS

ROS comprises superoxide radical, hydroxyl radical, and hydrogen peroxide. Superoxides were estimated by the method of Nishikimi *et al.* [11], in which superoxide dismutase (SOD) was inhibited with diethyl dithiocarbamate. Superoxide radical levels are expressed in terms of nmoles NBT reduced/10 minutes/10^{12} cells/hr. Hydroxyl radicals were estimated by the method of Gutteridge [12] by their reaction from 2-deoxyribose, resulting in the formation of thiobarbituric acid reacting species. The amount of hydroxyl radicals present in the tissues is expressed in terms of malondialdehyde (MDA) as nmoles/10^{12} cells/hr. Hydrogen peroxide (H_2O_2) was estimated by the method of Wolff [13]. The hydrogen peroxide levels are expressed as μmoles/10^{12} cells.

Estimation of enzymic antioxidants

The levels of superoxide dismutase were assayed by the method of Marklund and Marklund (1974) [14]. The assay mixture contained 1.0 ml of Pyrogallol-Tris-EDTA, 0.2 ml of tissue homogenate, and 0.8 ml of water. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogallol autooxidation. The rate of pyrogallol autoxidation is taken from the increase in absorbance at 420 nm. The enzyme activity is expressed as unit/mg protein. Catalase (CAT) activity was assayed by the method of Sinha (1972) [15]. The reaction was started by the addition of 0.4 ml of hydrogen peroxide to the reaction mixture containing 1.0 ml of phosphate buffer and 0.1 ml of enzyme solution. The utilization of H_2O_2 by the enzyme was read at 620 nm. CAT activity is expressed as μmoles of H_2O_2 consumed/min/mg protein. Glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al.* [16]. The reaction mixture in a total volume of 1.0 ml containing 0.2 ml of 0.4 mol/l phosphate buffer (pH 7.0), 0.2 ml of 0.4 mmol/l EDTA, 0.1 ml of 10 mmol/l sodium azide, and 0.2 ml of tissue homogenate was incubated with 0.1 ml of H_2O_2 and 0.2 ml of glutathione for 10 min. Oxidation of glutathione by the enzyme was read at 420 nm. GPx activity is expressed as μmol of glutathione oxidized/min/mg protein.

Estimation of non-enzymic antioxidants

Reduced glutathione (GSH) was determined by the method of Moron *et al.* [17]. 1 ml of homogenate was precipitated with 1.0 ml of TCA and the precipitate was removed by centrifugation. To 0.5 ml of supernatant, 2.0 ml of DTNB was added, and the total volume was made up to 3.0 ml with phosphate buffer. The absorbance was read at 412 nm. The amount of glutathione is expressed as mg/100 g wet tissue. Vitamin C was measured by the method of Omaye *et al.* [18], in which 1.0 ml of homogenate and 1.0 ml of ethanol were added and mixed thoroughly. Then, 3.0 ml of petroleum ether was added and shaken rapidly and centrifuged. 2 ml of supernatant was taken and evaporated to dryness. 0.2 ml each of batho phenanthroline, ferric chloride, and o-phosphoric acid were added, and the volume was made up to 3.0 ml with ethanol. The color developed was read at 530 nm. Vitamin C level is expressed as mg/g wet tissue. Vitamin E was measured by the method of Desai (1984) [19]. To 0.5 ml of tissue homogenate, 0.5 ml of H2O and 1.0 ml of TCA were added, mixed thoroughly, and centrifuged. To 1.0 ml of the supernatant, 0.2 ml of DTC reagent was added and incubated at 37°C for 3 hrs. Then, 1.5 ml of sulfuric acid was added and mixed well, and the solution was allowed to stand at room temperature for another 30 minutes. The color developed was read at 520 nm. Vitamin E level is expressed as mg/g wet tissue.

Estimation of tissue LPO

Tissue LPO was measured by the method of Devasagayam and Tarachand (1987) [20]. The values are expressed in terms of nmoles of MDA formed/mg protein.

Estimation of marker enzymes in breast tissue

5'-Nucleotidase (5'-NT) was assayed by the method of Walia *et al.* [21]. 5'-NT activity is expressed as μmoles of Pi liberated/min/mg protein. Lactate dehydrogenase (LDH) was assayed by the method of King [22]. LDH activity is expressed as μmoles of pyruvate liberated/min/mg protein.

Statistical analysis

The values are expressed as mean±standard deviation. The results were computed statistically (SPSS Software Package, Version 7.5) using

one-way analysis of variance. *Post-hoc* testing was performed for inter-group comparison using Student–Newman–Kuel and Duncan's multiple comparison test. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Estimation of ROS in control and experimental groups

Imbalance between production of ROS and antioxidant defense system has been defined as oxidative stress in various pathologic conditions. Fig. 1 depicts the effect of *TD* and PGG on superoxide radical (O^{2--}), hydroxyl radical (OH^{--}), and hydrogen peroxide (H_2O_2) of control and experimental groups. In the present study, the free radical generation was significantly elevated ($p < 0.05$) in mammary carcinoma-bearing (Group II) rats and treatment with TD (Group III) and PGG (Group IV) lowered the ROS levels. TD may act as antioxidant and inhibits ROS production possibly due to the free radical scavenging activity. No alterations of ROS levels in drug control rats such as TD control (Group VI) and PGG control (Group VII) showed no significant alterations when compared to control (Group I) rats.

ROS have been implicated in the pathology of many diseases [23]. The most common ROS include the superoxide anion (O^{2--}), the tremendously toxic hydroxyl radical (OH^{--}), peroxyl radical, and non-radical oxidants such as singlet oxygen (O_2) and hydrogen

Fig. 1: The values are expressed as mean±standard deviation for six rats in each group. Comparisons were made between, "a" - Group II versus Group I, "b" - Groups III, IV, and V versus Group II, "e" - Groups VI and VII versus Group I. The symbol, *represents the statistical significance at p<0.05, NS: Non-significant

peroxide (H_2O_2). ROS are produced during aerobic metabolism, and increased levels of these radicals are produced during various forms of oxidative stress. ROS are involved in cell growth, differentiation, progression, and cell death [24]. They play a major role in cancer initiation and promotion. Exposure of cells to free radicals gives rise to macromolecular damage such as DNA, protein, and lipid moieties. The main characteristic feature of an antioxidant is its ability to trap or scavenge free radicals. These free radicals may oxidize nucleic acids, proteins, lipids, or DNA and can initiate degenerative diseases [25]. Antioxidants such as phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, and lipid peroxyl radicals, thus inhibit the oxidative events that lead to a number of diseases [26].

To control the detrimental effects of ROS, organisms have developed a variety of antioxidant defense systems, especially the endogenous antioxidant system including SOD, CAT, GPx, glutathione-S-transferase (GST), GSH, vitamin C, and vitamin E. These naturally occurring enzymes safeguard the body against active oxygen free radicals by scavenging them in various ways. Hence, these antioxidant enzymes have been shown to be modulated in diseases caused by free radical attack. Thus, maintaining the balance between the rate of radical generation and the rate of radical scavenging is an essential part of biological homeostasis.

Estimation of enzymic antioxidants in control and experimental groups

Figs. 2 and 3 depict the effect of TD and PGG on SOD, CAT, GPx, and GST of control and experimental groups. The levels of enzymic antioxidants in breast tissues of DMBA administered cancer-bearing (Group II) rats were significantly decreased ($p < 0.05$) when compared to control (Group I) rats. Treatment with TD (Group III) and PGG (Group IV) resulted in significant increase in the level of these enzymic antioxidants when compared to DMBA administered (Group II) rats. TD-treated (Group III) rats showed more pronounced effect when compared to the rats treated with the standard drug, CYC (Group V). Drug control rats such as TD control (Group VI) and PGG control (Group VII) rats showed no significant alterations when compared to control (Group I) rats.

Superoxide dismutase is essential for cellular functions, but its total or partial inhibition leads to increased susceptibility to oxidative stress which leads to elevation of ROS [27]. It defends cells from oxidant stress by dismutating superoxide anion (O^{2--}) and hydrogen peroxide (H_2O_2), respectively. Intracellular SOD may play a central role in protecting
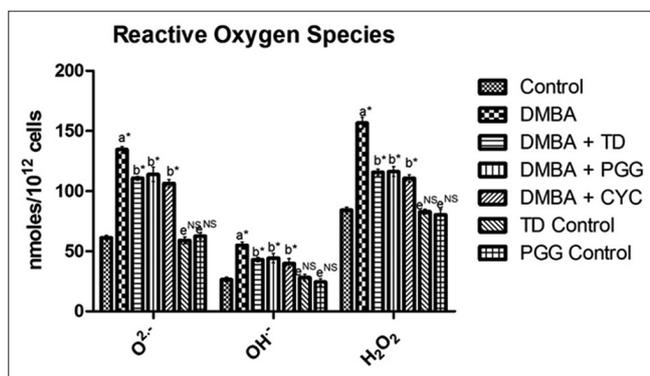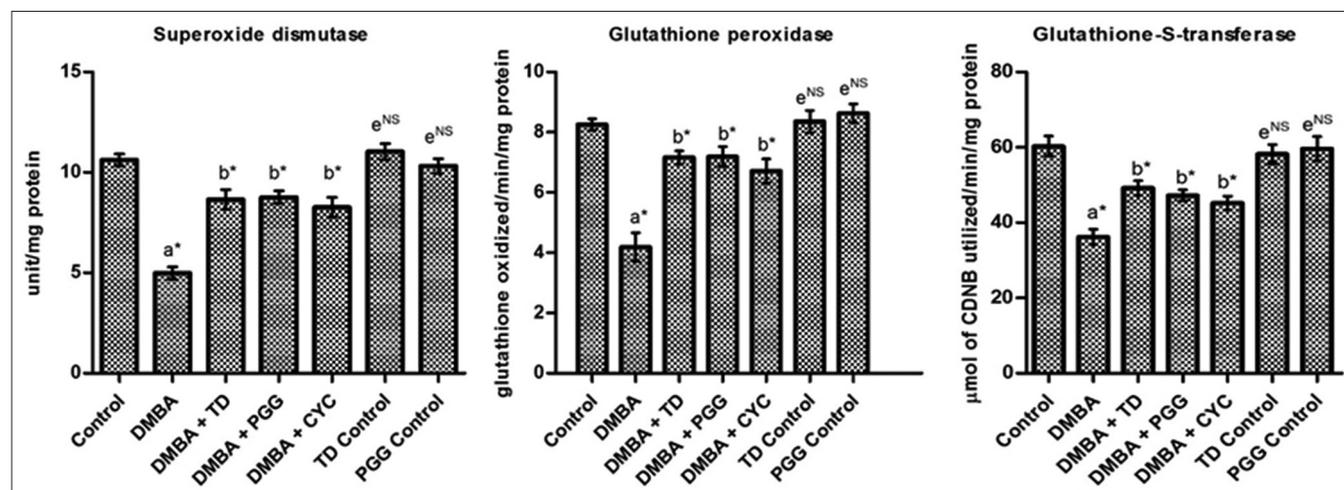
Fig. 2: The values are expressed as mean±standard deviation for six rats in each group. Comparisons were made between, "a" - Group II versus Group I, "b" - Groups III, IV, and V versus Group II, "e" - Groups VI and VII versus Group I. The symbol, *represents the statistical significance at p<0.05, NS: Non-significant

cancer cells against ROS generated by toxic chemicals and radiation. Thus, intracellular SOD level may be an important factor in determining the resistance of cancer cells against anticancer drugs or radiation [28]. Intracellular SOD levels have been reported to be much lower in cancer cells than in normal cells.

CATs are heme-containing proteins that play an important role as scavengers of free radicals of oxygen which are potentially dangerous and mutagenic [29]. It catalyzes the conversion of hydrogen peroxide to water and molecular oxygen, thereby protecting cells from the toxic effects of hydrogen peroxide. It has been reported that the CAT activity of the liver is greatly reduced by tumor growth in the host but restored to normal levels by removal of the tumor [30].

GPx is a selenium-containing antioxidant enzyme, located in both mitochondria and cytosol. Among the enzymic systems, GPx is the major defense against peroxides, superoxide anion, and hydrogen peroxide and assumes an important role in detoxifying lipid and hydrogen peroxides with the concomitant oxidation of glutathione [31]. GPx protects the cell and hypersensitive molecules from the attack of free radical [32]. In the absence of adequate GPx activity or glutathione levels, hydrogen peroxide and LPO are not detoxified and may be converted to hydroxyl radicals and lipid peroxyl radicals, respectively, by transition metals which lead to toxic insult for cells.

Estimation of non-enzymic antioxidants in control and experimental animals

Figs. 4 and 5 depict the effect of TD and PGG on GSH, vitamin C, and vitamin E of control and experimental groups. The levels of non-enzymic antioxidants were significantly decreased (p<0.05) in the DMBA administered cancer-bearing (Group II) rats when compared to control (Group I) rats. Treatment with TD (Group III) and PGG (Group IV) resulted in significant increase in the levels of these non-enzymic antioxidants when compared to DMBA administered (Group II) rats. TD-treated (Group III) rats showed a better effect when compared to the rats treated with the standard drug CYC (Group V). Drug control rats such as TD control (Group VI) and PGG control (Group VII) showed no significant alterations when compared to control (Group I) rats.

GSH is an important non-protein thiol which, in conjugation with GPx and GST, plays a significant role in protecting cells against cytotoxic and carcinogenic chemicals [33]. GSH acts directly as a free radical scavenger by donating a hydrogen atom and thereby neutralizing the hydroxyl radical. It also reduces peroxides and maintains protein thiols in the reduced state [34]. Changes in the rate of cancer cell proliferation are accompanied by changes in their intracellular GSH levels, and consequently, these could be reflected in their antioxidant machineries [35]. Polyphenols and flavonoids exhibit a wide range of biological effects including free radical scavenging and antioxidant activities [36].

Vitamin C or ascorbic acid is a water-soluble compound with a major antioxidant property in human plasma. Vitamin C is one of the most effective biological antioxidants, and it has been shown that vitamin C supplementation can reduce disease risk associated with oxidative stress such as cancer. Vitamin C protects cells mainly against ROS such as superoxide anion radical, hydroxyl radical, hydrogen peroxide, and singleton oxygen [37]. Vitamin C can protect cell membranes and lipoprotein particles from oxidative damage by regenerating the antioxidant form of vitamin E. Thus, vitamin C and E act synergistically in scavenging a wide variety of ROS.

Vitamin E or α-tocopherol is the major lipid soluble antioxidant protecting cellular membranes and lipoproteins against peroxidation. The reaction of α-tocopherol with free radicals generates tocopherol radicals, which can be reduced by vitamin C or GSH [38]. It is the most significant antioxidant that can protect against carcinogenesis and tumor growth. A decreased level of vitamin E content might be due to
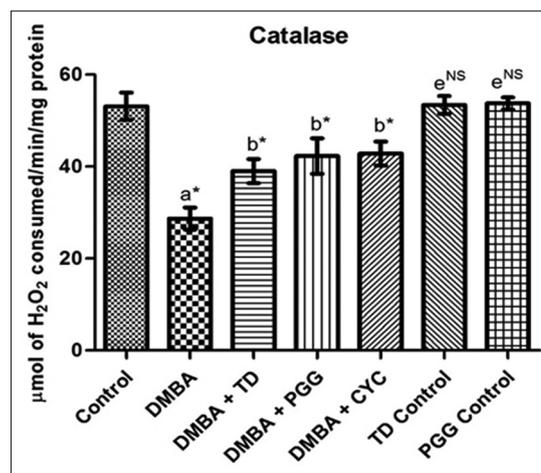

Fig. 3: The values are expressed as mean±standard deviation for six rats in each group. Comparisons were made between, "a" - Group II versus Group I, "b" - Groups III, IV, and V versus Group II, "e" - Groups VI and VII versus Group I. The symbol, *Represents the statistical significance at p<0.05, NS: Non-significant
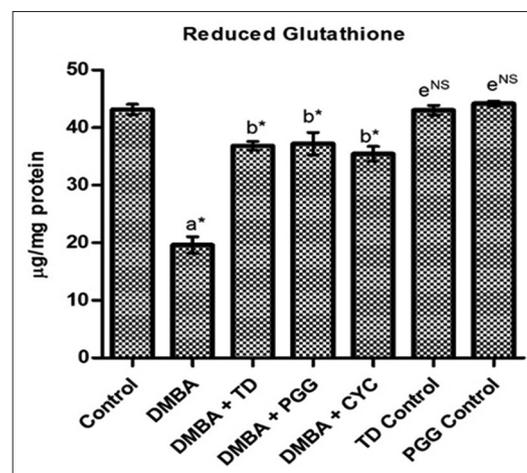

Fig. 4: The values are expressed as mean±standard deviation for six rats in each group. Comparisons were made between, "a" - Group II versus Group I, "b" - Groups III, IV, and V versus Group II, "e" - Groups VI and VII versus Group I. The symbol, *Represents the statistical significance at p<0.05, NS: Non-significant

Fig. 5: The values are expressed as mean±standard deviation for six rats in each group. Comparisons were made between, "a" - Group II versus Group I, "b"- Groups III, IV, and V versus Group II, "e" - Groups VI and VII versus Group I. The symbol, *Represents the statistical significance at p<0.05, NS: Non-significant
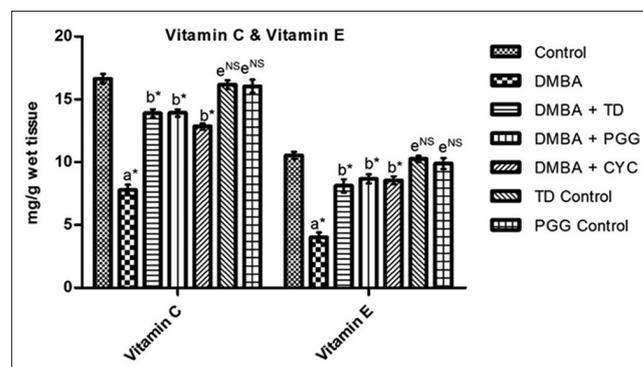
the excessive utilization of this antioxidant for quenching enormous free radicals produced in these conditions [39].

The levels of enzymic and non-enzymic antioxidants were significantly decreased in DMBA-induced (Group II) cancer-bearing rats when compared to control (Group I) rats. TD-treated (Group III) and PGG-treated (Group IV) rats showed significantly increased levels of enzymic and non-enzymic antioxidants which might be due to the presence of polyphenols, flavonoids, and tannins present in the herbal preparation. CYC-treated (Group V) rats showed decreased levels of enzymic and non-enzymic antioxidants when compared to Group III and Group IV rats. Group VI and Group VII rats showed no significant alterations in the levels of enzymic and non-enzymic antioxidants as compared to control (Group I) rats.

Estimation of LPO in control and experimental groups
Fig. 6 depicts the level of LPO in breast tissue of control and experimental groups. The levels were significantly increased (p<0.05) in the DMBA administered cancer-bearing (Group II) rats as compared to the control (Group I) rats. In TD-treated (Group III) and PGG-treated (Group IV) rats, the levels of LPO were restored to near normal levels when compared to DMBA administered (Group II) rats. Drug control rats such as TD control (Group VI) and PGG control (Group VII) rats showed no significant alterations when compared to control (Group I) rats.

LPO and decreased membrane fluidity in cancer conditions decrease the reactivity of thiol groups. Tissue protein oxidation results in charge modification, disulfide formation of thiols, and formation of covalent intermolecular association which cause loss of free thiols, decarboxylation, deamination, and generation of carbonyl moieties [40]. In the present study, the altered activities in the drug treated animals are probably due to the presence of therapeutic phytochemicals in TD. The levels of LPO were significantly increased in DMBA-induced (Group II) cancer-bearing rats when compared to control (Group I) rats. TD-treated (Group III) and PGG-treated (Group IV) rats showed significantly decreased levels of LPO when compared to control DMBA-induced (Group II) rats. Group VI and Group VII rats showed no significant alterations in LPO as compared to control (Group I) rats. These levels were restored to near normal levels in Group III and Group IV animals, probably due to the antioxidant and free radical quenching potential of TD and PGG, respectively.

**Estimation of marker enzymes in control and experimental groups**
The levels of marker enzymes such as 5'-NT and LDH were shown in Fig. 7. The level of 5'-NT and LDH in breast tissue was significantly increased (p<0.05) in the DMBA administered cancer-bearing (Group II) rats as compared to control (Group I) rats. In DMBA induced, TD-treated (Group III) and PGG-treated (Group IV) rats, the levels of 5'-NT and LDH were restored to near normal levels. TD-treated (Group III) and PGG-treated (Group IV) rats showed a better effect when compared to the standard drug, CYC (Group V)-treated group. Drug control rats such as TD control (Group VI) and PGG control (Group VII) rats showed no significant alterations when compared to control (Group I) rats.

5'-NT, also identified as 5'- ribonucleotide phosphohydrolase, is an intrinsic membrane-bound glycoprotein present in a variety of mammalian cells. It is widely found to be distributed in the tumor tissue. Nucleotidase is a hydrolytic enzyme which hydrolyzes the nucleotide into a nucleoside and phosphate. High 5'-NT activity results from the shedding of plasma membrane. These vesicles have a specific lipid and protein composition and high 5'-NT activity [41].

LDH is a perceptive diagnostic marker for solid neoplasia. LDH, a key glycolytic enzyme, is found in the cytoplasm of all cells and tissues in the body. LDH is a tetrameric enzyme which is involved in energy metabolism of cells. The increasing level of LDH in cancer-bearing animals might be due to its abnormal production by cancer cells [42].
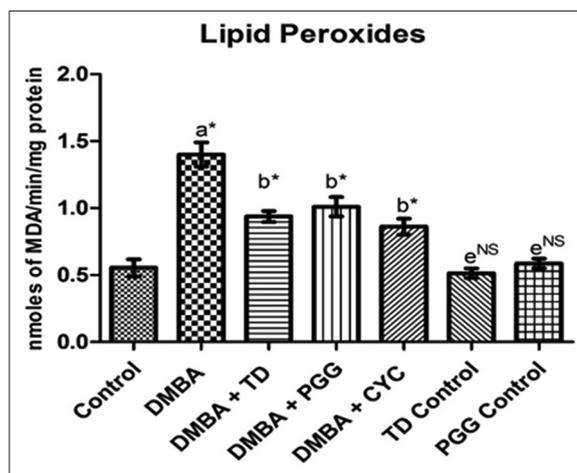
Fig. 6: The values are expressed as mean±standard deviation for six rats in each group. Comparisons were made between, "a" - Group II versus Group I, "b" - Groups III, IV, and V versus Group II, "e" - Groups VI and VII versus Group I. The symbol, *represents the statistical significance at p<0.05, NS: Non-significant
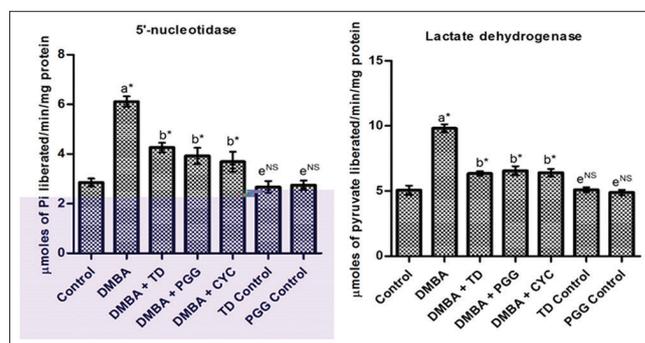
Fig. 7: The values are expressed as mean±standard deviation for six rats in each group. Comparisons were made between, "a" - Group II versus Group I, "b" - Groups III, IV, and V versus Group II, "e" - Groups VI and VII versus Group I. The symbol, *Represents the statistical significance at p<0.05, NS: Non-significant

The proliferating malignant cells show high levels of glycolysis which increases the LDH activity. In DMBA-induced (Group II) rats, the levels of 5'-NT and LDH were significantly elevated, but after treatment with TD (Group III) and PGG (Group IV), the levels were reverted to near normal. A significantly decreased level of 5'-NT and LDH in drug treated animals might be due to the synergistic action of aqueous extract in the herbal preparation of TD and polyphenolic nature of PGG. CYC-treated (Group V) rats showed increased levels of marker enzymes when compared to Group III and Group IV rats. TD control (Group VI) and PGG control (Group VII) rats showed no significant alterations in both 5'-NT and LDH expression.

From this study, it can be inferred that the anticarcinogenic effect of this triherbal preparation may be due to the presence of various classes of active ingredients in this drug which might be having synergistic activity. Further results, it can be speculated that the drug TD and PGG possesses a profound anticancer effect through its role in modulating the levels of enzymic and non-enzymic antioxidants, LPO and marker enzymes in animal model. A significant difference (p<0.05) was observed when Group V (CYC-treated) rats were compared with Group III (TD-treated) and Group IV (PGG-treated) rats, indicating that TD and PGG had a greater anticarcinogenic effect than the standard drug, CYC. Therapeutic activities of TD depend mainly on the presence of phytochemicals such as polyphenols and flavonoids.

The altered activities in the drug treated animals could be probably due to the presence of flavonoids, tannins, and other compounds in TD. Flavonoids have been proven to have antiproliferative effect on several cancer cells. The drug would have changed the permeability of the membrane affecting cellular growth [43]. It might also be due to the cytotoxic effects through the inhibition of several enzymes by flavonoids and other compounds present in TD. Plant polyphenols have been shown to exhibit anticarcinogenic effect on experimental tumors. Drug control animals did not show appreciable adverse side effects or any abnormal observable metabolic disturbance in the system. There were no toxic or deleterious effects, indicating that the drug acts as a safe positive pharmacological agent against mammary carcinoma in rats.

CONCLUSION

In conclusion, the altered biochemical parameters observed in the present study, in cancer-bearing rats may be due to reduction of antioxidant levels and the induction of LPO, marker enzymes because of DMBA administration. However, oral administration of 400 mg/kg/body weight/day of TD and 30 mg/kg/body weight/day of PGG significantly reversed the biochemical parameters to near normal level in DMBA-induced drug treated groups. The data of the present experiment suggest that oral administration of TD and PGG effectively modulate the antioxidant activity, LPO, marker enzymes and show a protective therapeutic role by quenching and detoxifying the free radicals in DMBA-induced mammary carcinoma in female Sprague-Dawley rats.

ACKNOWLEDGMENTS

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