Antiproliferative activity of Zanthoxylum tetraspermum W.A. stem bark extract against Breast carcinoma in mice

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ABSTRACT

Breast carcinoma is a heterogeneous complex disease thought to occur via a multistep process, which despite improvements in surgery and the use of adjuvant therapy, continues to be fatal in many patients. Metastatic disease is the most common cause of breast cancer death. The genus Zanthoxylum is used as mouth fresh, tooth care, spice and possesses several types of biological activities. Zanthoxylum tetraspermum (Wight & Arn.) belongs to the family “Rutaceae” possesses some biological activities. In the present study antiproliferative activity of Z. tetraspermum W.A. stem bark extract has been evaluated against N-methyl-N-nitrosourea (MNU) induced breast carcinoma in mice. Biochemical estimations like Lipid peroxidation, enzymic antioxidants and non enzymic antioxidants activity done in the liver and kidney tissue homogenate were restored after treatment with hydroethanolic stem bark extract of Z. tetraspermum. Comparison of normal mice, mice administered only with stem bark extract and mice administered with 5-Fluoro Uracil (5-FU) as positive drug control group showed no significant variations in these enzyme activities. The antiproliferative activity of Z. tetraspermum stem bark extract was studied using breast cancer cell lines and measured by MTT assay. From these findings it is reported that the hydroethanolic stem bark extract of the plant has shown good inhibition on lipid peroxidation and proliferation which indicated the antitumor activity of the plant extract in in vivo model against MNU induced breast carcinoma in mice.

KEY WORDS: Zanthoxylum tetraspermum, Breast carcinoma, MNU, antioxidants, MTT assay.

INTRODUCTION

Breast carcinoma is the most common cancer in women in India. It is likely to emerge as a major malignancy among females due to recent changes in lifestyle, food habits and industrialization. The incidence of breast cancer in India, as reported in the National Cancer Registry Programme, Indian Council of Medical Research (Annual Report 2005), varied from 23 to 32 per 100,000 women. It is the uncontrolled growth of breast cells developing to a malignant tumor. Usually breast cancer either begins in the cells of the lobules, which are the milk-producing glands, or the ducts, the passages that drain milk from the lobules to the nipple. Less commonly, breast cancer can begin in the stromal tissues, which include the fatty and fibrous connective tissues of the breast. Metastatic disease is the most common cause of breast cancer death and is preceded by a sequence of events leading to the transformation of normal breast epithelium. Progression may proceed through stages and the first critical step in this process is invasion, which requires the loss of cellular adhesion and gain of motility.

The animal model of carcinogen induced mammary tumors is widely used for breast cancer. There is extensive evidence demonstrating similarities between these chemically induced mammary carcinomas and human breast cancers, including their origination from mammary ductal epithelial cells. It has previously been reported that N-methyl-N-nitrosourea (NMU) induced primary animal tumors are similar to estrogen receptor (ER)-positive. Though NMU-induced primary mammary tumors are typically low grade in situ carcinomas, following serial transplantation they develop the capacity for invasion. This model replicates the events observed in patients with breast cancer and therefore serves as a useful and relevant model for studying the disease.

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions.
pecially degenerative diseases [15]. Many natural complex related derivatives have recently drawn much attention due to its broad pharmacological activities including anti-bacterial, anti-thrombotic, antimutagenic, scavenging of reactive oxygen species, and anti-tumourigenic effect [16]. Many plant derivatives have special ability to scavenge reactive oxygen species (ROS) - free radicals, such as hydroxyl radicals, superoxide radicals and to influence processes involving free radical-injury. They have also been found to inhibit lipid peroxidation and to possess vasorelaxant, anti-inflammatory and antiproliferative effect [17].

Traditional medicines are considered to be effective and safe alternative treatment for tumor. The use of medicinal plants in modern medicine for the prevention of cancer is an important aspect. This has led to chemical and pharmacological investigations and general biological screening of medicinal plants all over the world. According to the assessment of WHO approximately 80% of the world’s population relies on the use of traditional medicine and more than 30% of the pharmaceutical preparations are based on plant materials. In recent years there has been considerable emphasis on the identification of plant products as possible anticarcinogens with antioxidant properties [18, 19]. Ellagic acid and a whole range of flavonoids, carotenoids and terpenoids present in Fragaria vesca and Rubus idaeus have been reported to be responsible for antioxidant activity. These chemicals block various hormone actions and metabolic pathways that are associated with the development of cancer [20]. Antioxidants are compounds that protect cellular systems from the potentially harmful effects of processes that can cause excessive oxidations. By implication, they may inhibit the pathogenesis of the many diseases which involve oxidative reactions [21, 22].

An extract of the seeds and leaves of Athrixia elata has exhibited moderate growth inhibitory, cytostatic and cytotoxic effects against the cancer cell lines, such as TK10 (Renal cancer), MCF7 (Breast cancer) and UACC62 (Melanoma) [23]. Rosmarinus officinalis contains substantial amounts of carnosol and ursolic acid, the potent antioxidants that possess antitumor activity [24]. Cytotoxic activity of essential oil of Z. rhoifolium was evaluated against HeLa (human cervical carcinoma), A-549 (human lung carcinoma), HT-29 (human colon adenocarcinoma), Vero (monkey kidney) cell lines and mice macrophages by Da Silva et al. [25]. The fruit essential oils of Z. leprieurii and Z. xanthoxyloides could be used as food supplements to protect against emergent diseases such as cardiovascular problems, cancer and diabetes [26].

Zanthoxylum tetraspermum (Rutaceae) is a potent unidentified medicinal plant. It is vernacularly called “Tooth ache tree”. It is an aromatic, spiny, thorny, stout, deciduous climbing shrub or small tree, with brown bark and alternate branches are armed with strong brown prickles. The wood is yellowish and soft and found in the Western Ghats in the Nilgiris, Aanaimalai hills, Kollis hills at attitudes of 1,200 to 1,800m and in Kerala and Karnataka. The plant is credited in Sri Lanka with stimulant, astringent and digestive properties and is prescribed in dyspepsia and diarrheas [46-48]. This is used for treating microbial infections, antifungal activities, tumors and tooth ache. The phytochemical investigations of Z.tetraspermum stem bark have revealed the presence of two benzophenanthrene alkaloids such as 8-acetonyl dihydronitidine, 8-acetonyl dihydro avicine [49] and decrine from Z. tetraspermum, Z. caudatum and Zanthoxylum limonella [50]. The presence of the alkaloids such as Liriodenine, sesamin, lichexanthone and piperitol gamma-gamma-diethyl ether from the leaves of Z. tetraspermum, Z. caudatum and Z. budrunga have been evaluated for their antibacterial, antifungal and cytotoxic properties [44].

Since the scientific evaluation of the stem bark extract of Z. tetraspermum on antiproliferative and antioxidant effect is not yet carried out, the objective of this study was focused to assess the antiproliferative potential and in vivo antioxidant activity to evaluate the effect of Z.tetraspermum against MNU induced breast carcinoma in experimental mice.
MATERIALS AND METHODS

Plant Material and Extraction:
The whole plant material of Zanthoxylum tetraspermum, Wight & Arn. [Syn. Fagara tetrasperma] was collected from the silent valley, the evergreen forest of Western Ghats, Palakkad district, Kerala, South India and its identity was confirmed by the Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, South India. The shade dried stem bark of the plant (500 g) was subjected to size reduction to coarse powder. The powder was then subjected to extraction. It was extracted with a mixture of ethanol and water (1:1 ratio) for 72 hrs. Later it was concentrated under vacuum to get the residue. The yield of the extract was found to be 12.6 grams.

Preliminary Phytochemical Screening:
Ten grams of the hydroethanolic extract of stem bark of Z. tetraspermum was dissolved in 100ml of its own mother solvent to prepare the plant extract. The extract thus obtained with a concentration of 10% (w/v) was used for preliminary phytochemical screening.

Animals used:
Female Sprague-Dawley albino mice between 40-50 days old were used for the experiments. The animals were maintained at standard housing conditions (room temperature 23-25°C, relative humidity 55%). A controlled 12 h light / 12 h dark cycle was maintained. The animals were housed in spacious cages and they were fed with standard pellet diet and water ad libitum. The study was approved by Institutional Animal Ethical Committee constituted for the purpose of CPCSEA, by the approval No. 158 / 99 / 10.

Acute Toxicity Study
The acute toxicity study was performed for hydroethanolic extract according to the acute toxic classic methods as per OECD - 423 guidelines on female albino mice. The animals were kept fasting overnight providing only water after which the extract was administered orally in increasing dosage and found safe up to the dose of 2000mg/kg.

Induction of Breast carcinoma:
Breast carcinoma was induced in female mice by a single intraperitoneal dose of N-methyl-N-nitrosourea (MNU) injected into each of 30 female Sprague-Dawley mice (aged 50 days). At day 50, all mice received a single dose of MNU 50 mg/kg intraperitoneally (MNU, reagent grade, was obtained from Sigma, USA, dissolved in 0.9% saline). Two weeks after MNU treatment, a time by which the animals had recovered from MNU-induced toxicity, the mice were divided into groups. The tumor was allowed to grow for three months and the mice were palpated regularly to determine the appearance of mammary tumor. After three months, breast carcinoma was confirmed by histological examination.

Experimental design:
The animals were divided into eight groups of six animals each. The groups were formed as follows:
Group – I = Normal healthy control mice.
Group – II = Breast cancer control mice (MNU induced, 50mg MNU/kg; ip)
Group – III = Bark extract treated mice (MNU + 300mg extract/kg; oral; daily) for 4 weeks.
Group – IV = Bark extract treated mice (MNU + 600mg extract/kg; oral; daily) for 4 weeks.
Group – V = 5FU treated mice (MNU + 5-Fluoro Uracil 300mg/kg; oral; daily) for 4 weeks.
Group – VI = 5FU treated mice (MNU + 5-Fluoro Uracil 600mg/kg; oral; daily) for 4 weeks.
Group – VII = Plant extract only (Plant extract 300mg/kg; oral; daily) for 4 weeks.
Group – VIII = Plant extract only (Plant extract 600mg/kg; oral; daily) for 4 weeks.

Groups III to VI were induced with breast carcinoma and after three months, treatment began with plant extract, 5-FU administered orally for four weeks as indicated above. Groups VII and VIII animals were administered with Z.tetraspermum only on the same dosage as Groups III and IV animals and by a similar route.

Biochemical analysis:
At the end of the experimental period, animals were fasted overnight and then killed by cervical decapitation. The liver and kidneys from all the animals were removed, washed in ice-cold isotonic saline and blotted individually on ash-free filter paper. The tissues were homogenized in 0.1M Tris HCl buffer (pH 7.4) and used for biochemical estimations. The levels of lipid peroxidation, enzymic antioxidants such as Superoxide dismutase (SOD), Catalase, Glutathione peroxidase (GPx) and reduced glutathione were estimated in liver and kidney homogenates of the experimental mice.

Maintenance of cell line:
The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air and...
100% relative humidity. Maintenance of cultures was passaged weekly, and the culture medium was changed twice a week.

**MTT – cell proliferation assay:**

The cell growth inhibition was determined by MTT assay\[^{63}\]. 3-[(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. This assay is a simple non-radioactive colorimetric assay to measure cell cytotoxicity, proliferation or viability.

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x10^5 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO\(_2\), 95% air and 100% relative humidity. After 24hrs the cells were treated with serial concentrations of hydroethanolic stem bark extract of *Z. tetraspernum*. They were initially dissolved in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, two fold serial dilutions were made to provide a total of five different concentrations. Aliquots of 100 µl of these different dilutions of stem bark extract were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following the addition of the plant extract, the plates were incubated for an additional 48hrs at 37°C with same conditions. The medium containing without the plant extracts were served as control, a blank with cell free control and triplicate was maintained for all concentrations. After 48hrs of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4hrs. The medium with MTT was then flicked off and the formed formasan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

\[
\text{% cell Inhibition} = \frac{100-\text{Abs (sample)}}{\text{Abs (control)}} \times 100
\]

Non linear regression graph was plotted between % Cell inhibition and Log\(_{10}\) concentration and IC\(_{50}\) was determined using Graph Pad Prism software.

**Statistical analysis:**

The values were expressed as mean ± SD. The statistical analysis was carried out by one way analysis of variance using SPSS (version 16) statistical analysis program. Individual differences between treatments were examined using Tukey’s HSD test. Statistical significance was considered at P< 0.05.

**RESULTS AND DISCUSSION**

**Phytochemical Screening:**

The results of preliminary phytochemical screening was found that the major chemical constituents of the stem bark extract were alkaloids, flavonoids, glycosides,lignins,phenols,sterols,thiols saponins, fats and oils \[^{51, 52}\].

Acute toxicity studies were performed for the stem bark extract of *Z. tetraspernum* according to the toxic classic methods as per guidelines-423 prescribed by OECD \[^{53}\]. The hydroethanolic extract did not cause any mortality up to 2000mg/kg body weight and hence considered as safe. The extract was found to be safe up to 2000 mg/kg body weight. Hence, the biological dose of the extract was fixed as 300 mg/kg body weight and 600 mg/kg body weight of the safe dose.

**Lipid peroxidation:**

Lipid peroxidation in higher animals was known to cause destabilization and disintegration of the cell membrane leading to cell injury and kidney damage \[^{65}\]. It is one of the main causes of oxidative damage initiated by ROS and linked with altered membrane structure and enzyme inactivation \[^{66}\]. In the present study, the increased level of lipid peroxidation in the liver and kidney of normal and experimental animals are shown in Table-1and 2.

The lipid peroxidation activity (LPO) in the liver and kidney of cancerous group II animals (P<0.05) were significantly increased when compared with the normal (group I) animals. Administration of two different doses (300mg, 600mg) of plant extract for 4 weeks in breast carcinoma-bearing mice (group III and IV) showed significantly reduced activity of LPO (P<0.05). Similarly administration of same doses of 5-FU for 4 weeks in breast carcinoma-bearing mice (group V and VI) also showed the significantly reduced activity of LPO. However, the plant extract alone treated animals (groups VII and VIII) did not show any significant changes when compared with normal control mice (group I). No significant variation is shown when the group III and IV compared with Group V and VI respectively. The increased lipid peroxidation observed in the present study is in accordance with the work done by Gurukumar *et al.*, \[^{67}\].

**Enzymic Antioxidants:**

SOD, CAT and GPx enzymes are important scavengers of free radi-
The present data reveals that a significant decrease in the antioxidant enzymes were found in group II mice compared to the normal group animals in the liver and the kidney homogenates as represented in the Table-1 and 2. But after administration of hydroethanolic stem bark extract of *Z. tetraspermum* there was a significant increase in the enzymic antioxidants as observed in groups III to VI. The increased level of lipid peroxidation in group II significantly (*P* < 0.05) decreased in groups III to VI after treatment with hydroethanolic stem bark extract of *Z. tetraspermum*. There was no significant changes shown in these enzymes activities in the plant extract alone treated animals (groups VII and VIII) when compared with normal control animals. No significant variation is shown between the groups III and IV compared with the groups V and VI respectively. In the demonstration of oxidative stress, the changes determined either in the form of the inhibition or stimulation of a high level of free radicals in tissues proves that antioxidant enzymes play an active role in the conversion of these harmful and very effective compounds into less harmful or harmless metabolites. SOD, CAT and GPx constitute a mutually supportive team of defense against reactive oxygen species (ROS). SOD is the first enzyme involved in the antioxidant defense by lowering the steady state of $O_2^-$. CAT is a hemoprotein, localized in the peroxisomes and catalyses the decomposition of $H_2O_2$ to water and oxygen. GPx, a selenoenzyme, present predominantly in the liver and catalyses the reaction of hydro peroxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. SOD, Catalystase and glutathione peroxidase activity in control and experimental mice

Table-1: Liver lipid peroxidation, SOD, Catalase and glutathione peroxidase activity in control and experimental mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxidation (LPO) (Units / mg protein)</th>
<th>Super oxide dismutase (SOD) (Units / mg protein)</th>
<th>Catalase (CAT) (Units / min / mg protein)</th>
<th>Glutathione peroxidase (GPx) (µg GSH / min / mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Normal control)</td>
<td>12.7910 ± 2.36263</td>
<td>0.0583 ± 0.05283</td>
<td>1.4066 ± 1.17541</td>
<td>8.5867 ± 0.24582</td>
</tr>
<tr>
<td>Group – II (Tumor control)</td>
<td>20.8616 ± 2.31318</td>
<td>0.0107 ± 0.00103</td>
<td>0.9931 ± 0.04932</td>
<td>0.9397 ± 0.51210</td>
</tr>
<tr>
<td>Group – III (Bark extract-300mg)</td>
<td>15.0310 ± 0.77535</td>
<td>0.0375 ± 0.00164</td>
<td>1.0950 ± 0.11502</td>
<td>7.4283 ± 0.33552</td>
</tr>
<tr>
<td>Group – IV(Bark extract-600mg)</td>
<td>11.2457 ± 1.63404</td>
<td>0.0443 ± 0.00543</td>
<td>1.6039 ± 0.57101</td>
<td>8.2133 ± 0.70834</td>
</tr>
<tr>
<td>Group – V (5FU-300 mg)</td>
<td>15.4883 ± 3.03820</td>
<td>0.0452 ± 0.00916</td>
<td>1.2986 ± 0.16810</td>
<td>7.8950 ± 0.51290</td>
</tr>
<tr>
<td>Group – VI (5FU-600 mg)</td>
<td>12.9567 ± 0.50899</td>
<td>0.0499 ± 0.02875</td>
<td>1.3817 ± 0.03545</td>
<td>8.5950 ± 0.16245</td>
</tr>
<tr>
<td>Group – VII (Plant control-300 mg)</td>
<td>12.9367 ± 0.57778</td>
<td>0.0622 ± 0.01094</td>
<td>1.4050 ± 0.11077</td>
<td>8.5000 ± 0.36436</td>
</tr>
<tr>
<td>Group – VIII (Plant control-600 mg)</td>
<td>12.7950 ± 0.29433</td>
<td>0.0469 ± 0.00810</td>
<td>1.3332 ± 0.10905</td>
<td>8.5683 ± 0.25996</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D (n = 6). Statistical comparison by Tukey’s HSD: a – Group II is compared with Group I; b – Group III, IV is compared with Group II; c – Group III is compared with Group V; d – Group IV is compared with Group VI; e – Group VII, VIII is compared with Group I; f – Group VIII is compared with Group IV. NS – Non-significant.*P < 0.05.

Table-2: Kidney lipid peroxidation, SOD, Catalase and glutathione peroxidase activity in control and experimental mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxidation (LPO) (Units / mg protein)</th>
<th>Super oxide dismutase (SOD) (Units / mg protein)</th>
<th>Catalase (CAT) (Units / min / mg protein)</th>
<th>Glutathione peroxidase (GPx) (µg GSH / min / mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Normal control)</td>
<td>6.1911 ± 1.64939</td>
<td>0.0462 ± 0.00366</td>
<td>6.1027 ± 1.25184</td>
<td>4.5333 ± 0.41462</td>
</tr>
<tr>
<td>Group – II (Tumor control)</td>
<td>14.6900 ± 2.83662</td>
<td>0.0115 ± 0.00187</td>
<td>1.6880 ± 0.17166</td>
<td>1.5932 ± 0.40009</td>
</tr>
<tr>
<td>Group – III (Bark extract-300mg)</td>
<td>8.9119 ± 0.84119</td>
<td>0.0312 ± 0.00960</td>
<td>4.7465 ± 0.79175</td>
<td>3.3967 ± 0.27522</td>
</tr>
<tr>
<td>Group – IV(Bark extract-600mg)</td>
<td>5.4250 ± 2.39961</td>
<td>0.0440 ± 0.00723</td>
<td>5.2598 ± 1.14805</td>
<td>3.7633 ± 0.13049</td>
</tr>
<tr>
<td>Group – V (5FU-300 mg)</td>
<td>8.2417 ± 1.07007</td>
<td>0.0387 ± 0.03475</td>
<td>4.8333 ± 0.55788</td>
<td>3.7185 ± 0.22393</td>
</tr>
<tr>
<td>Group – VI (5FU-600 mg)</td>
<td>6.0917 ± 0.72550</td>
<td>0.0419 ± 0.01502</td>
<td>5.3967 ± 0.23526</td>
<td>4.4507 ± 0.22029</td>
</tr>
<tr>
<td>Group – VII (Plant control-300 mg)</td>
<td>5.8583 ± 0.70839</td>
<td>0.0496 ± 0.02592</td>
<td>5.5617 ± 0.20173</td>
<td>4.8050 ± 0.11095</td>
</tr>
<tr>
<td>Group – VIII (Plant control-600 mg)</td>
<td>6.0303 ± 0.68533</td>
<td>0.0479 ± 0.02623</td>
<td>5.6817 ± 0.15118</td>
<td>4.7050 ± 0.27165</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D (n = 6). Statistical comparison by Tukey’s HSD: a – Group II is compared with Group I; b – Group III, IV is compared with Group II; c – Group III is compared with Group V; d – Group IV is compared with Group VI; e – Group VII, VIII is compared with Group I; f – Group VIII is compared with Group IV. NS – Non-significant.*P < 0.05.
Antioxidant systems such as antioxidant vitamins like A, C and E, superoxide dismutase (SOD), Catalase (CAT), glutathione (GSH), ceruloplasmin and glutathione peroxidase (GSH-Px) protect the cells against lipid peroxidation, which is the base for many pathological processes [69, 70].

MTT Assay:
Cell viability was measured using MTT assay. The cells were incubated with the hydroethanolic stem bark extract of *Z. tetraspermum* at different concentrations. The results of the cell viability test are shown in Fig.Nos.1-7. The plant extract has shown a concentration dependent growth inhibition of the cancer cells. IC\(_{50}\) value for the plant extract treated cells was 245\(\mu\)g/ml. The cell DNA content was estimated by diphenyl amine method using MCF-7 cells.

**Table-3: Liver Vitamin-C and Reduced glutathione activity in control and experimental mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin- C (µg / mg protein)</th>
<th>Reduced glutathione (GSH) (µg / mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Normal control)</td>
<td>4.0183 ± 0.60383</td>
<td>7.2407 ± 1.46827</td>
</tr>
<tr>
<td>Group – II (Tumor control)</td>
<td>1.0433 ± 0.04457 * *</td>
<td>3.4350 ± 0.33183 * *</td>
</tr>
<tr>
<td>Group – III (Bark extract- 300mg)</td>
<td>2.5250 ± 0.14124 * *</td>
<td>6.4667 ± 0.14949 * *</td>
</tr>
<tr>
<td>Group – IV (Bark extract- 600mg)</td>
<td>3.3350 ± 0.14639 * *</td>
<td>6.9367 ± 0.22740 * *</td>
</tr>
<tr>
<td>Group – V (5FU-300 mg)</td>
<td>3.6217 ± 0.11427 * NS</td>
<td>6.5041 ± 0.10789 * NS</td>
</tr>
<tr>
<td>Group – VI (5FU-600 mg)</td>
<td>4.0433 ± 0.12517 * NS</td>
<td>7.1633 ± 0.03266 * NS</td>
</tr>
<tr>
<td>Group – VII (Plant control-300 mg)</td>
<td>3.8145 ± 0.16154 * NS</td>
<td>7.1567 ± 0.05854 * NS</td>
</tr>
<tr>
<td>Group – VIII (Plant control-600 mg)</td>
<td>3.9864 ± 0.17423 * NS</td>
<td>7.1550 ± 0.03082 * NS</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D (n = 6). Statistical comparison by Tukey’s HSD: a – Group II is compared with Group I; b – Group III, IV is compared with Group II; c – Group III is compared with Group V; d – Group IV is compared with Group VI; e – Group VII, VIII is compared with Group I; f – Group III is compared with Group IV. NS – Non-significant.*P < 0.05.

**Table-4: Kidney Vitamin-C and Reduced glutathione activity in control and experimental mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin- C (µg / mg protein)</th>
<th>Reduced glutathione (GSH) (µg / mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Normal control)</td>
<td>2.2840 ± 0.20978</td>
<td>3.4106 ± 0.43496</td>
</tr>
<tr>
<td>Group – II (Tumor control)</td>
<td>0.8250 ± 0.05891 * *</td>
<td>1.2600 ± 0.15773 * *</td>
</tr>
<tr>
<td>Group – III (Bark extract- 300mg)</td>
<td>1.7650 ± 0.02811 * *</td>
<td>2.6583 ± 0.08424 * *</td>
</tr>
<tr>
<td>Group – IV (Bark extract- 600mg)</td>
<td>2.0109 ± 0.14994 * *</td>
<td>3.0733 ± 0.08618 * *</td>
</tr>
<tr>
<td>Group – V (5FU-300 mg)</td>
<td>2.0970 ± 0.05835 * NS</td>
<td>3.1542 ± 0.16775 * NS</td>
</tr>
<tr>
<td>Group – VI (5FU-600 mg)</td>
<td>2.1083 ± 0.08060 * NS</td>
<td>3.1976 ± 0.14213 * NS</td>
</tr>
<tr>
<td>Group – VII (Plant control-300 mg)</td>
<td>2.0917 ± 0.06014 * NS</td>
<td>3.1187 ± 0.09902 * NS</td>
</tr>
<tr>
<td>Group – VIII (Plant control-600 mg)</td>
<td>2.1414 ± 0.09263 * NS</td>
<td>3.2350 ± 0.21455 * NS</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D (n = 6). Statistical comparison by Tukey’s HSD: a – Group II is compared with Group I; b – Group III, IV is compared with Group II; c – Group III is compared with Group V; d – Group IV is compared with Group VI; e – Group VII, VIII is compared with Group I; f – Group III is compared with Group IV. NS – Non-significant.*P < 0.05.

(groups VII and VIII) when compared with normal control animals and compared between the groups III and IV with the groups V and VI respectively as represented in the Table-3 & 4. The antioxidant systems such as antioxidant vitamins like A, C and E, superoxide dismutase (SOD), Catalase (CAT), glutathione (GSH), ceruloplasmin and glutathione peroxidase (GSH-Px) protect the cells against lipid peroxidation, which is the base for many pathological processes [69, 70].
The percentage cell inhibition against the various concentrations of stem bark extract are shown in the Table-5. Oxidative stress leads to the damage of membrane lipids, DNA, protein and cellular organelles, contributing to the development of cancer, early-aging, cardiovascular diseases, degenerative and neurological diseases and others [71]. Our result revealed that MCF-7 human breast cancer cells treated with stem bark extract of *Z. tetraspermum* exhibited reduced cell DNA content.

**Table-5: MTT cell proliferative assay**

<table>
<thead>
<tr>
<th>Stem Bark extract of <em>Z. tetraspermum</em> (%)</th>
<th>% Cell Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.75</td>
<td>1.085271</td>
</tr>
<tr>
<td>37.5</td>
<td>8.217054</td>
</tr>
<tr>
<td>75</td>
<td>15.11628</td>
</tr>
<tr>
<td>150</td>
<td>28.60465</td>
</tr>
<tr>
<td>300</td>
<td>59.53488</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>244.8 µg/ml</td>
</tr>
<tr>
<td>R²</td>
<td>0.9881</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The results from the present study revealed the antiproliferative activity of the hydroethanolic stem bark extract of *Z. tetraspermum*.
against N-methyl-N-nitrosourea (MNU) induced breast carcinoma in in-vivo model.

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