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ANTIOXIDANT ACTIVITY OF STEM BARK EXTRACT OF *ZANTHOXYLUM TETRASPERMUM* W.A. AGAINST MNU INDUCED BREAST CARCINOMA IN MICE

NARAYANASAMY, K.[1*] AND B. RAGAVAN[2]
[1]Department of Biochemistry,
Sree Narayana Guru College,
Coimbatore – 641 105, Tamil Nadu, India.
[2] Department of Biochemistry,
PSG College of Arts and Science,
Coimbatore – 641 014, Tamil Nadu, India.

ABSTRACT

Breast carcinoma is a heterogeneous disease that appears to progress from an *in situ* tumor to invasive cancer, thought to occur via a multistep process. Even then, despite improvements in surgery and use of adjuvant therapy, it continues to be fatal in many patients. Metastatic disease is the most common cause of breast cancer death. The genus *Zanthoxylum* is known as "Timoor" which 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 *in vivo* antioxidant activity of stem bark extracts of *Z. tetraspermum* W.A. has been evaluated against N-methyl-N-nitrosourea (MNU) induced breast carcinoma in mice. Biochemical estimations of enzymic antioxidants such as Glutathione-S-transferase (GST), Glutathione reductase (GR) and non-enzymic antioxidants like Vitamin-A and Vitamin-E were done in liver and kidney tissue homogenate. These parameters were declined in cancer induced mice and restored back to near normal after treatment with 50% 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 showed no significant variations in these enzymic and non-enzymic parameters. From these findings, it is revealed that 50% hydroethanolic stem bark extract of *Z. tetraspermum* W.A. has shown good effect on *in vivo* antioxidant activity against MNU induced breast carcinoma in mice.

Key words: *Zanthoxylum tetraspermum*, Breast carcinoma, MNU, antioxidants.

INTRODUCTION

Breast cancer is associated with high morbidity and mortality. Around 1.6 million new cases of breast cancer are diagnosed and over 500,000 women die from this disease. The annual incidence of breast cancer is increasing in both industrialized and developing countries [1]. This is the most

common cancer in women and second leading cause of death in India [2]. It is likely to emerge as a major malignancy among females due to recent changes in lifestyle, food habits and industrialization. As reported in the annual report of 2005 of National Cancer Registry Programme, Indian Council of Medical Research, the incidence of breast cancer in India varied from 23 to 32 per 100,000 women [3]. The uncontrolled growth of breast cells develops into a malignant tumor. Metastatic disease is the most common cause of breast cancer death [4] 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 loss of cellular adhesion and gain of motility [5].

The animal model of carcinogen induced breast carcinoma is widely used for breast cancer [6-9]. There is extensive evidence demonstrating similarities between these chemically induced mammary carcinomas and human breast cancers, including their origination from mammary ductal epithelial cells [10-13]. It has previously been reported that N-methyl-N-nitrosourea (NMU) induced primary animal tumors are similar to estrogen receptor (ER)-positive [14]. Though NMU-induced primary mammary tumors are typically low grade *in situ* carcinomas, following serial transplantation they develop the capacity for invasion [15]. 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 efficient antioxidant systems in the body. When the generation of these radicals exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases [16, 19]. 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 [20].

Traditional medicines are considered to be effective and safe alternative treatment for cancers. 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. As per the WHO assessment, almost 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 [19, 20, 21]. 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 [22, 23.].

Zanthoxylum has been studied for several types of biological activities such as larvicidal, anti-inflammatory, analgesic, antinociceptive, antioxidant, antibiotic, hepatoprotective, antiplasmodial, cytotoxic, antiproliferative, anthelminthic, antiviral, anticonvulsant and antifungal [24-35]. Toothpaste containing *Z. nitidum* extract decreased the incidence of dental plaque and enhanced gingival health [36].An alkaloid extract of the stem barks of *Z.chiloperone* exhibited antifungal activity against *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* [37]. Bafi-Yeboa *et.al,* [38] investigated *Z. americanum* leaf, fruit, stem, bark and root for antifungal activity with 11 strains of fungi. All extracts exhibited a broad spectrum of antifungal activity. Alkamides isolated from the leaves of *Z. syncarpum* showed moderate antiplasmodial activity against p*lasmodium falciparum* [39]. Ethanolic extracts of the trunk bark of *Z. fagara*, *Z.elephantiasis* and *Z. martinicense* showed antifungal activity [40]. The petroleum ether, chloroform and methanol extracts of the leaves and barks of *Z. budrunga* have been evaluated for their antibacterial, antifungal and cytotoxic properties [41]. 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 [42].

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 [43] 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 Srilanka with stimulant, astringent and digestive properties and is prescribed in dyspepsia and diarrheas [44- 46]. 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[47] and decrine from *Z. tetraspermum, Z. caudatum* and *Zanthoxylum limonella*[48].The presence of the alkaloids such as Liriodenine, sesamin, lichexanthone and piperitol gamma-gamma-diethyl ether from the *Z. tetraspermum* has been reported and they have shown significant anti-bacterial and anti-fungal activity[49]. The presence of an alkaloid Norsanguinarine, a polyhydroxy and a phenolic compound cyclohexanetetrol, methoxyphenol, Gallopamil in the aqueous extract and a phenolic compound 2-methoxy-4-vinylphenol in the ethanolic extract of *Z. tetraspermum* has also been reported[50].

Since the scientific evaluation of the stem bark extract of Z. *tetraspermum* on *in vivo* antioxidant activity is not yet carried out, the objective of this study was focused to assess enzymic and non-enzymic antioxidants activity to evaluate the effect of *Z.tetraspermum* against MNU induced breast carcinoma in experimental mice.

MATERIALS AND METHODS

Plant Collection and Extraction:

The plant stem bark of *Zanthoxylum tetraspermum* Wight & Arn. [Syn.*Fagara tetrasperma*][51] 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 50% hydroethanolic stem bark extract of *Zanthoxylum 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 [52].

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 50% hydroethanolic extract according to the acute toxic classic methods as per OECD - 423 guidelines [53] 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 b.w; ip).

Group –III = Bark extract treated mice (MNU + 300mg extract/kg b.w, oral; daily) for 4 weeks.

Group –IV = Bark extract treated mice (MNU + 600mg extract/kg b.w, oral; daily) for 4 weeks.

Group –V = 5FU treated mice (MNU + 5-Fluoro Uracil 300mg/kg b.w, oral; daily) for 4 weeks.

Group –VI = 5FU treated mice (MNU + 5-Fluoro Uracil 600mg/ kg b.w, oral; daily) for 4 weeks.

Group –VII = Plant extract only (Plant extract 300mg/ kg b.w, oral; daily) for 4 weeks.

Group –VIII= Plant extract only (Plant extract 600mg/ kg b.w, 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 activity of enzymic antioxidants such as Glutathione-S-transferase (GST) was determined by the method of Habig and Jakoby [54] and Glutathione reductase (GR) was assayed using the method of Goldberg and Spooner [55]. GR catalyzes the reduction of oxidized glutathione in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in absorbance is measured at 340 nm. Non enzymic antioxidants such as Vitamin-A [56] and Vitamin-E [57] were estimated in liver and kidney homogenates of the experimental mice.

Statistical analysis:

The values were expressed as mean ± SD. The statistical analysis was carried out by one way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, version 16.0) statistical analysis program. Individual differences between treatments were examined using Tukey's HSD test. Statistical significance was considered at $P< 0.05$.

REULTS 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,tannins,sterols,thiols saponins, fats and oils [49, 50].

Acute Toxicity

Acute toxicity studies were performed for the stem bark extract of *Z.tetraspermum* 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.

Enzymic Antioxidants

The effects of *Z. tetraspermum* stem bark extract on enzymic antioxidants Glutathione-S-transferase (GST) and Glutathione reductase (GR) in liver and kidney of control and experimental mice are depicted in Table No.1, 2 and Figures 1-4.

Table.1. Effect of liver Glutathione-S-transferase and Glutathione reductase in control and experimental mice

Treatment Groups	Liver Glutathione-S-transferase (GST) (µmoles of CDNB conjugated/min/mg protein)	Liver Glutathione reductase (GR) (Units/min/mg protein)
Group – I (Normal)	26.7700 ± 0.43964	12.0367 ± 0.06976
Group – II (Control-MNU Induced)	16.1017 ± 0.11514^{a*}	7.1900 ± 0.08462^{a*}
Group – III (MNU + 300mg extract)	20.3300 ± 0.17239^{b,f*}	9.4950 ± 0.05683^{b,f*}
Group – IV (MNU + 600mg extract)	23.2500 ± 0.08786^{b,f*}	10.8433 ± 0.03670^{b,f*}
Group – V (MNU + 300 mg 5FU)	20.4867 ± 0.12565^{c}NS	9.7817 ± 0.11890^{c*}
Group – VI (MNU + 600 mg 5FU)	23.4200 ± 0.26616^{d}NS	11.2117 ± 0.11652^{d*}

Group – VII (300 mg plant extract)	26.5583 ± 0.10852[e]NS	11.9533 ± 0.08165[e]NS
Group – VIII (600 mg plant extract)	26.7583 ± 0.12352[e]NS	12.0133 ± 0.02582[e]NS

Values are expressed as Mean ± S.D (n = 6). NS – Non-significant *P < 0.05

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 V is compared with Group III; d–Group VI is compared with Group IV; e–Group VII, VIII is compared with Group I; f–Group III is compared with Group IV.

Figure.1. Liver Glutathione-S-transferase in control and experimental mice

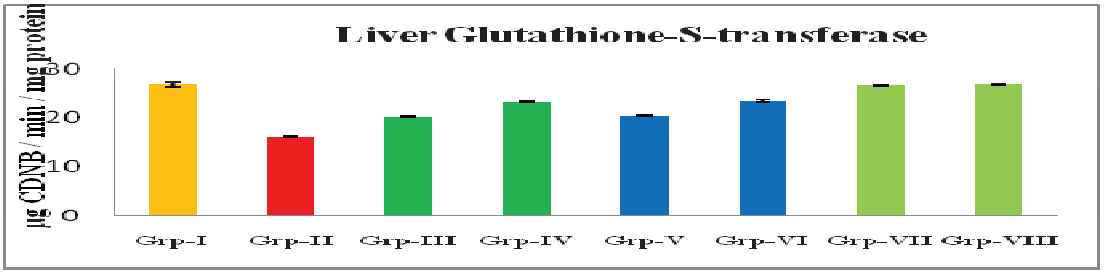


Figure.2. Liver Glutathione reductase in control and experimental mice

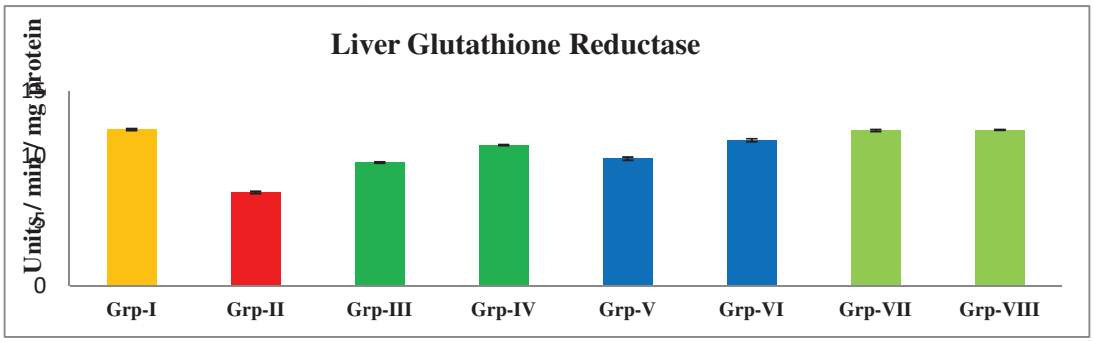


Table.2. Effect of kidney Glutathione-S-transferase and Glutathione reductase in control and experimental mice

Treatment Groups	Kidney Glutathione-S-transferase (GST) (µmoles of CDNB conjugated/min/mg protein)	Kidney Glutathione reductase (GR) (Units/min/mg protein)
Group – I (Normal)	22.9083 ± 4.13653	10.5600 ± 0.08741
Group – II (Control-MNU Induced)	10.1583 ± 0.03764[a*]	5.5667 ± 0.02875[a*]

Group – III (MNU + 300mg extract)	$15.9750 \pm 0.05010^{b,f*}$	$7.8350 \pm 0.06189^{b,f*}$
Group – IV (MNU + 600mg extract)	$18.1517 \pm 0.04070^{b,f*}$	$9.0333 \pm 0.13677^{b,f*}$
Group – V (MNU + 300 mg 5FU)	16.4417 ± 0.14784^{c}NS	$8.8883 \pm 0.04834^{c*}$
Group – VI (MNU + 600 mg 5FU)	18.4500 ± 0.07537^{d}NS	$10.0050 \pm 0.05992^{d*}$
Group – VII (300 mg plant extract)	20.7983 ± 0.07250^{e}NS	10.4350 ± 0.03271^{e}NS
Group – VIII (600 mg plant extract)	21.1817 ± 0.17406^{e}NS	10.5150 ± 0.02739^{e}NS

Values are expressed as Mean ± S.D (n = 6). NS – Non-significant *P < 0.05 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 V is compared with Group III; d–Group VI is compared with Group IV; e–Group VII, VIII is compared with Group I; f–Group III is compared with Group IV.

Figure.3. Kidney Glutathione-S-transferase in control and experimental mice

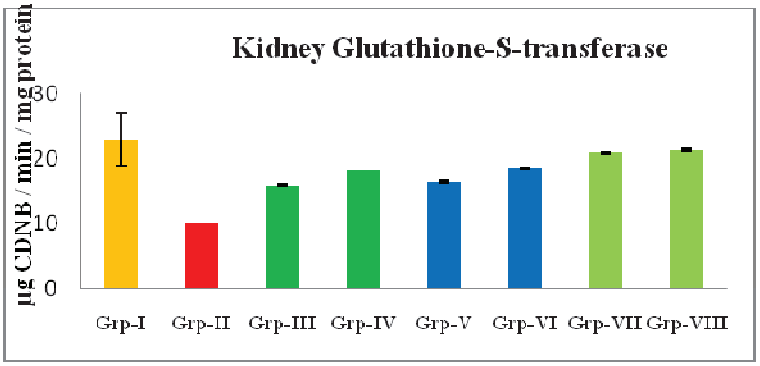
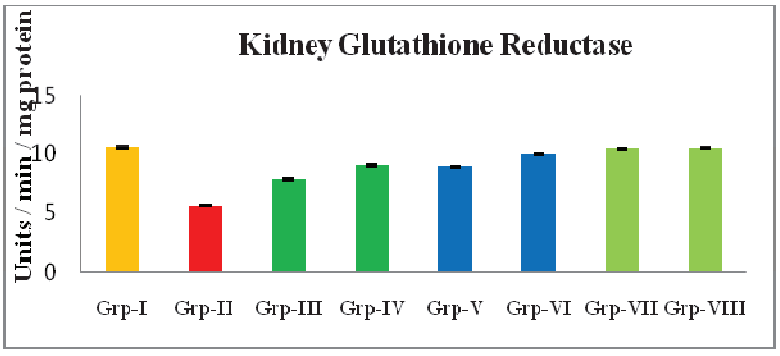


Figure.4. Kidney Glutathione reductase in control and experimental mice



In nature, there is a dynamic balance between the amount of free radicals generated in the body and antioxidant defense system that quench or scavenge them and protect the body against their deleterious effects. This antioxidant status is suggested as a useful tool in estimating risk of oxidative damage induced carcinogenesis [58]. Antioxidant enzymes GST and GR represent protection against oxidative tissue damage. GST work together with GSH in the decomposition of hydrogen

peroxide or other organic hydroperoxides to non-toxic products. GSH and functionally related GR enzyme are responsible for the regeneration of GSH [59, 60, 61]

The activities of Glutathione-S-transferase and Glutathione reductase were significantly (P<0.05) declined in liver and kidney of MNU induced Group II animals when compared to normal control. Treatment with 50% hydroethanolic stem bark extract of *Z. tetraspermum* (300mg, 600mg / kg body weight) and standard drug 5-FU (300mg, 600mg / kg body weight) dose-dependently for 30 days, significantly (P<0.05) elevated the activities of these antioxidant enzymes in Group III, IV, V and VI animals when compared to Group II. GST did not show significant (P<0.05) variation in Group III and IV animals when compared with Group V and VI respectively. On the other hand, GR showed a significant (P<0.05) variation in Group III and IV animals when compared with Group V and VI respectively. The stem bark extract at 600mg / kg body weight of dose (Group IV) showed significantly (P<0.05) effective response on these enzymatic antioxidants than Group III mice. There was no significant (P<0.05) variation in the levels of these enzymes in crude stem bark extract alone (300mg, 600mg / kg body weight) treated Group VII and VIII animals when compared to Group I.

MNU intoxication induced significant (P<0.05) reduction in antioxidant enzymes Glutathione-S-transferase and Glutathione reductase in liver and kidney of cancerous animals when compared to normal control. The reduction in the activities of these enzymes obtained from the present investigation is almost due to the altered antioxidant defense system caused by enormous production of free radicals, weak free radical defence system against oxidative stress and decreased synthesis of enzymes or oxidative inactivation of enzyme proteins in chemically induced mammary carcinogenesis [62, 63., 64] The present results are consistent with many previous studies done by Heikal *et al.,* 2011 [65], Mansour and Mossa 2010 [66], Heikal and Soliman 2010 [67], Verma *et al.,* 2007 [68] and Hayes *et al.,* 2005[69] on enzymatic antioxidants in experimental animal models.

Oral administration of 50% hydroethanolic stem bark extract of *Z. tetraspermum* (300mg, 600mg / kg body weight) for 30 days after MNU injection, caused significant (P<0.05) alleviation in the activity of antioxidant enzymes GST and GR when compared to cancerous mice. This could be attributed to the antioxidant capacity of stem bark extract that attenuates LPO and antioxidant enzymes capacity which in turn restore the integrity of the cell membrane and improve the disturbance in membrane permeability [62]. The presence of phenolics, fatty acid, flavonoids and various other components in the stem bark extract of *Z. tetraspermum* may have possibly contributed to this effect. These findings were corresponded with the observations of Shen-Kang *et al.,* 2012 [70], who reported enhanced activities of GSH-dependent enzymes in experimental gastric carcinogenesis

by protecting against carcinogen induced oxidative damage. Pracheta *et al.,* 2011[71], in a previous finding have reported a decline in the levels of glutathione related antioxidants in cancer induced mice which were brought back to near normal level on administration with *Euphorbia neriifolia*. GR, at the dose of 300mg and 600mg / kg body weight of stem bark extract treatment and GPx at the dose of 300mg / kg body weight of stem bark extract treatment showed a significant (P<0.05) variation with the corresponding dose of 5-FU drug treated Group V and VI animals. This could be due to the moderate activity and crude nature of the stem bark extract of *Z.tetraspermum.*

Non- enzymic Antioxidants

The levels of non-enzymatic antioxidants such as Vitamin-A (β-Carotene) and Vitamin-E (α-Tocopherol) in liver and kidney of control and experimental mice are summarized in Table No.3, 4 and Figures 5-8. Vitamin-A and E comprise the well known non-enzymatic antioxidant defense system that protects the cells against free radicals and ROS [72].

Table.3. Effect of Liver Vitamin-A and Vitamin-E in control and experimental mice

Values as Mean ± S.D (n = 6). NS – Non-significant *P < 0.05	Liver Vitamin-A (µg / mg protein)	Liver Vitamin-E (µg / mg protein)
Group – I (Normal)	15.8967 ± 0.38250	2.7217 ± 0.01472
Group – II (Control-MNU Induced)	9.9700 ± 0.19183[a*]	2.0050 ± 0.02588[a*]
Group – III (MNU + 300mg extract)	12.2417 ± 0.10439[b,f *]	2.2567 ± 0.02160[b,f *]
Group – IV (MNU + 600mg extract)	13.5183 ± 0.19343[b,f *]	2.5367 ± 0.02422[b,f *]
Group – V (MNU + 300 mg 5FU)	12.2733 ± 0.08287[c]NS	2.2933 ± 0.01862[c]NS
Group – VI (MNU + 600 mg 5FU)	13.7717 ± 0.16376[d]NS	2.5917 ± 0.01722[d *]
Group – VII (300 mg plant extract)	15.5783 ± 0.02137[e]NS	2.7200 ± 0.02449[e]NS
Group – VIII (600 mg plant extract)	15.6217 ± 0.08035[e]NS	2.7400 ± 0.02366[e]NS

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 V is compared with Group III; d–Group VI is compared with Group IV; e–Group VII, VIII is compared with Group I; f–Group III is compared with Group IV.

Figure.5. Effect of Liver Vitamin-A in control and experimental mice

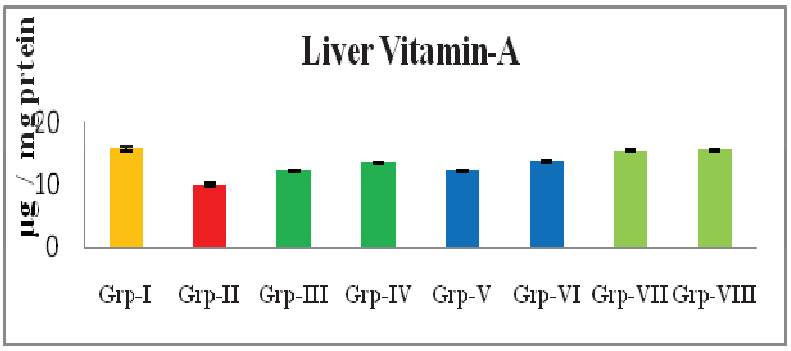


Figure.6. Effect of Liver Vitamin-E in control and experimental mice

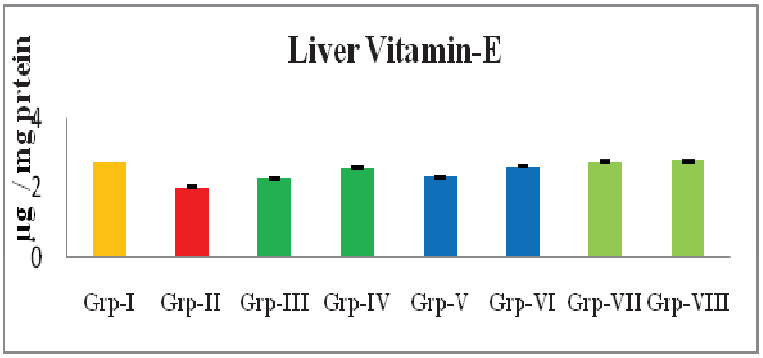


Table.4. Effect of Kidney Vitamin-A and Vitamin-E in control and experimental mice

Treatment Groups	Kidney Vitamin-A (µg / mg protein)	Kidney Vitamin-E (µg / mg protein)
Group – I (Normal)	3.6100 ± 0.05514	1.7400 ± 0.02280
Group – II (Control-MNU Induced)	1.3583 ± 0.10870[a*]	0.7383 ± 0.02229[a*]
Group – III (MNU + 300mg extract)	2.1950 ± 0.04506[b,f *]	1.1567 ± 0.01506[b,f *]
Group – IV (MNU + 600mg extract)	2.6367 ± 0.05203[b,f *]	1.5550 ± 0.01871[b,f *]
Group – V (MNU + 300 mg 5FU)	2.2750 ± 0.07176[c]NS	1.1700 ± 0.02898[c]NS
Group – VI (MNU + 600 mg 5FU)	2.6400 ± 0.05292[d]NS	1.6233 ± 0.01506[d*]
Group – VII (300 mg plant extract)	3.5383 ± 0.04792[e]NS	1.7333 ± 0.01211[e]NS
Group – VIII (600 mg plant extract)	3.6117 ± 0.03189[e]NS	1.7417 ± 0.03764[e]NS

Values are expressed as Mean ± S.D (n = 6). NS – Non-significant *P < 0.05

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 V is compared with Group III; d–Group VI is compared

with Group IV; e–Group VII, VIII is compared with Group I; f–Group III is compared with Group IV.

Figure.7. Effect of kidney Vitamin-A in control and experimental mice

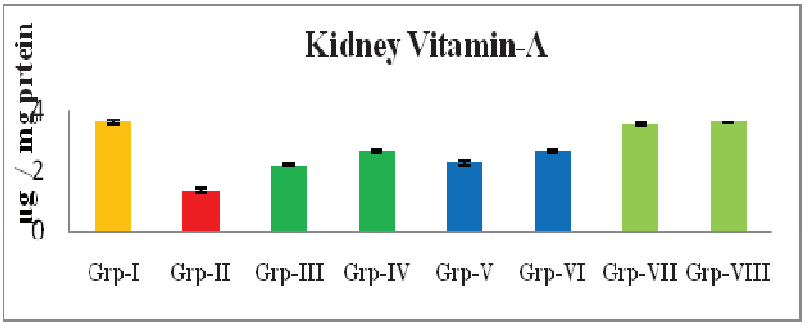
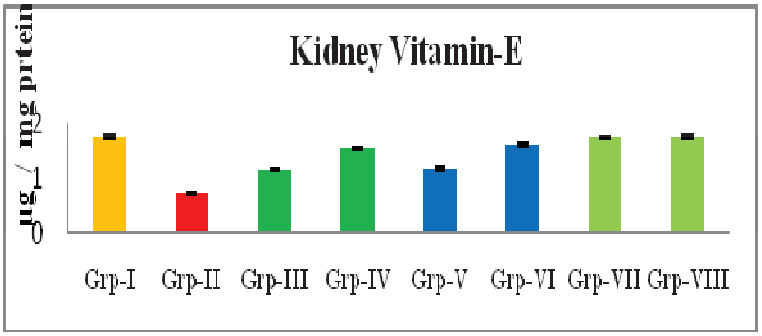


Figure.8. Effect of kidney Vitamin-E in control and experimental mice



The levels of non-enzymatic antioxidants such as Vitamin-A and E were significantly (P<0.05) lowered in liver and kidney of cancerous mice when compared to normal control mice. Treatment with 300mg, 600mg / kg body weight of plant stem bark extract and standard drug 5-FU for four weeks significantly (P<0.05) improved the levels of Vitamins A and E in Group III, IV, V and VI treated mice when compared to Group II. No significant (P<0.05) change was noticed on Vitamin-A level in Group III and IV animals when compared with Group V and VI respectively. However, There was a significant (P<0.05) variation on the level of Vitamin-E when Group IV mice compared with Group VI mice only. The stem bark extract (600mg / kg body weight) treated Group IV mice significantly (P<0.05) improved the levels of these vitamins more than Group III mice. No significant (P<0.05) difference noticed on these vitamins in plant stem bark extract alone (300mg, 600mg / kg body weight) treated Group VII and VIII mice when compared to Group I.

MNU induced oxidative DNA damage mediated free radicals stimulation showed a significant (P<0.05) drop on the levels of Vitamin-A and E. The low levels of non-enzymic antioxidants in Group II mice might be due to excessive production of free radicals in cancer cells and utilization of vitamin-A and E to scavenge the free radicals to keep up the cellular levels for cell proliferation.

When there is reduction in the levels of GSH, cellular levels of β-carotene and α-tocopherol are also depleted in cancerous condition [73] .These findings were in agreement with that of Fouzia Banu *et al.,* 2014 [74] who observed the reduced levels of non-enzymic antioxidants activity of Geraniol on N-Nitrosodiethylamine-induced hepatocarcinogenesis in *wistar albino* rats.

The 50% hydroethanolic stem bark extract of *Z. tetraspermum* (300mg, 600mg / kg body weight) significantly (P<0.05) improved the levels of vitamins A and E in dose a dependent manner with 600 mg/kg body weight showing best improvement on these vitamin levels. Possible protective, synergistic effect and antioxidant activities of *Z. tetraspermum* plant stem bark extract derived phytochemicals are by scavenging excess of free radicals and improving the antioxidant vitamins level with redox regulation of some important physiological processes [59]. The present observation is favorable with the findings of Padmavathi *et al.,* 2006[75] who reported improved concentrations of vitamins A and E on the effect of paclitaxel and propolis on antioxidant system in DMBA induced breast cancer in female rats. Surya Surendren *et al.,* 2012[76], found a significant elevation of Vitamin-A and E levels in breast cancer tissues after treatment with methanolic extract of *Alstonia scholaris* leaves. A significant (P<0.05) difference was noticed on the level of vitamin-E in Group IV mice when compared with Group VI mice may probably due to slower activity of the crude stem bark extract (600mg / kg body weight) when compared with standard drug 5-FU (600mg / kg body weight) activity in treated mice.

CONCLUSION

In these findings, *Z.tetraspermum* stem bark extract has effectively enhanced the activities of antioxidant enzymes such as Glutathione-S-transferase, Glutathione reductase and the levels of Vitamins A and E in dose dependent manner against MNU induced breast carcinogenesis in animal models. This extract has improved the activities of antioxidant enzymes and also efficiently reduced the intracellular ROS levels of liver and kidney. Thus, preventing the oxidative stress induced cellular damage. This clearly indicates that stem bark extract of *Z. tetraspermum* has an immense *in vivo* antioxidant property over MNU induced mammary carcinoma in experimental animals. The 600mg / kg body weight dose was significantly (P<0.05) more effective than the dose of 300mg / kg body weight.

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