ISBN: 978-93-90847-17-4

15. Evaluation of Biochemical Parameters and Antioxidant Activity of Alpha Terpineol on DMBA Induced Rats

Valsala P., Gunavathi V., Santhy, K. S.

Department of Zoology,
Avinashilingam Institute for Home Science and
Higher Education for Women, Coimbatore.

Abstract:

The present study aimed to assess the efficacy of Alpha terpineol, an important compound present in *M. piperita,* for its antitumor activity in DMBA induced breast tumour in rat model. Experimental animals are sorted into six groups, Group I: Normal Control, Group II: DMBA (25mg/Kg), Group III: DMBA + Tamoxifen (5mg/Kg), Group IV: DMBA + Alpha terpineol (20mg/Kg), Group V: DMBA + Alpha terpineol (40mg/Kg) and Group VI: Alpha terpineol alone (20mg/Kg). The DMBA is a powerful carcinogenic agent and is used to induce tumours in animals. Rats were treated twice per week for 4 weeks with DMBA (25 mg/Kg body weight dissolved in olive oil) orally. After the experimental period, the animals were fasted overnight and sacrificed by cervical decapitation, the blood was collected from the control and the experimental groups of rats; the serum was separated out for the analysis of the serum biochemical parameters and antioxidant parameters. The treatment with Alpha terpineol reduced the Cholesterol, uric acid, ALP, TP, TGL, BUN, SGOT and SGPT and brought to near normal levels. Alpha terpineol had significantly improved antioxidant enzymes such as SOD, catalase, GPX and GSH similar to the standard drug treated rat, whereas LPX levels were significantly reduced. The curative effect was found to be dose-dependent in animals treated with Alpha terpineol. Some of the damaged breast patterns were restored to near normal by the treatments.

Keywords: Biochemical parameters, antioxidant parameters, Alpha terpineol, DMBA, Tamoxifen

15.1 Introduction:

Breast cancer has been called the second most common cancer after lung cancer. It is one of the leading causes for death in women worldwide, surpassing the cervical cancer (Jemal *et al.,* 2011). In Malaysia, a current report shows breast cancer as the most often diagnosed cancer. The International Agency for analysis in Cancer (GLOBOCAN) estimate the ASR (age-standardized rate) of carcinoma in Malaysia as 38.7 per 100,000, reporting carcinoma as high among general population of Malaysia (Youlden *et al.,* 2014). Cancer-therapeutic medicine had associate impact of damage to the normal cells and tissues (Alakhova and Kabanov *et al.,* 2014). The relapse after chemotherapy, second primary neoplasm, and resistance to chemotherapeutic medicine have additionally been occurring in breast cancer patients (Beckwitt *et al.,* 2018). The best remedy to those unpreventable side effects is the use of natural products (Othman and Ahmed, 2013).

Alpha terpineol, a volatile monoterpenoid alcohol, is the major element of essential oils of many species of aromatic plants like *Origanium vulgare* L. and genus *Ocimum canum* Sims. It can be isolated from a variety of sources like cajeput oil, pine oil and petitgrain oil (Bauer *et al.,* 2001). A-Terpineol is usually present in a mixture of 3 isomers specifically β-γ-and terpinen-4-ol (Itani *et al.,* 2008).

Moreover, it exhibited an anti-proliferative activity, which may be used in the prevention or even treatment of tumour, because it was found that α-T had a potent antioxidant capability against completely different human neoplastic cell lines (breast, lung, prostate, female internal reproductive organs and leukaemia). This inhibits the progress and stimulation of the cell death in cancer cells by means of an inhibition of NF-κB activity (Hasan *et al.,* 2010).

Additionally, alpha terpineol possesses a huge range of biological applications as it shows an antihypertensive and antiproliferative result on human erythroleukemic cells (Sabino *et al.,* 2013), as well as anti-inflammatory properties (Held *et al.,* 2007). Therefore, the current study investigated the relationship between breast cancer and chemo preventive result of Alpha Terpineol to protect against DMBA (7,12-dimethylbenz(a)anthracene) induced mammary cancer in female Sprague Dawley rats.

15.2 Materials and Methods:

15.2.1 Chemicals:

DMBA was procured from Sigma Chemical Company St. Louis, MO, USA. All Other chemicals and materials used in the study are of highest purity and standards which are commercially available.

15.2.2 Experimental Animals:

Sprague–Dawley rats (Female, 130 -180g body weight) were used for the study. The experiments were planned and executed in compliance with ethical standards approved by the Institutional Animal Ethics Committee (IAEC) of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (AIW: IAEC: 2017: ZOO: 01 dated02/12/2017). The selected rats were kept in plastic cages at the institutional animal house and kept at room temperature of 22ºC under 12 h light/dark cycles. All the animals were fed with standard food and water adlibitum.

15.2.3 Tumour Induction and Drug Treatment:

The DMBA is a powerful carcinogenic agent and is used to induce tumours in animals. Rats were treated twice per week for 4 weeks with DMBA (25 mg/kg body weight dissolved in olive oil) orally. After the experimental period, the animals were fasted overnight and sacrificed by cervical decapitation, the blood was collected from the control and the experimental groups of rats; the serum was separated out for the biochemical analysis. All the vital organs were washed with ice cold saline, removed, trimmed and stored.

15.2.4 Experimental Design:

Animals are grouped and treatment was given as follows,

- **Group I:** Normal Control {rats were given normal olive oil orally for 16 weeks}.
- **Group II:** DMBA (25mg/Kg) {rats were treated twice per week for 4 weeks with DMBA (25 mg/kg body weight dissolved in olive oil) orally and then continued with or without the vehicle for additional 12 weeks}
- **Group III:** DMBA + Tamoxifen (5mg/Kg) {rats were treated twice per week for 4 weeks with DMBA (as in Group II). Subsequently, they were treated with tamoxifen (5 mg/kg body weight dissolved in olive oil orally) daily for 12 weeks}
- **Group IV:** DMBA + Alpha terpineol (20mg/Kg) {rats were treated with DMBA (as in Group II) for 4 weeks and subsequently, they were treated with Alpha terpineol 20mg/kg and continued for 12 weeks}
- **Group V:** DMBA + Alpha terpineol (40mg/Kg) {rats were treated with DMBA (as in Group II) for 4 weeks and subsequently, they were treated with Alpha terpineol 40mg/kg and continued for 12 weeks}
- **Group VI:** Alpha terpineol alone (20mg/Kg) {rats were treated with Alpha terpineol alone 20mg/kg for 16 weeks to find out cytotoxicity, if any, induced by Alpha terpineol}.

15.2.5 Serum Biochemical Parameters:

Appropriate auto analyser kits (Roche Diagnostics, Indianapolis, USA) were employed for the assessment of various biochemical parameters like alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminases (SGPT), total protein (TP), creatine, urea, uric acid, total cholesterol and Triglycerides.

15.3 Antioxidant Evaluation in Breast Tissue:

Breast tissues were removed and blood was wiped, blotted and gauged with super cold PBS. In potassium chloride solution (1.15 % w/v) tissue homogenate has been prepared (10 % w/v). To get a clear supernatant for estimating anti-oxidant and biochemical parameters, the homogenate obtained was centrifuged for 10 min at 8000 rpm (4°C).

15.4 Statistical Analysis:

The values are expressed as mean values of six rats in each group ± standard deviation. One-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons with least significance difference test were done by statistical software package SPSS 17. A value of $p < 0.01$ was considered statistically significant.

15.5 Result and Discussion

The cholesterol level was analysed in all the experimental groups of animals. In the control group, cholesterol level was found to be 83.63 ± 2.09mg/dl. In the case of DMBA treated

rats, cholesterol level was found to be increased to 94.48 ± 3.04 mg/dl, which was significantly decreased in Alpha terpineol treated rat, 91.54 ± 1.40 and 87.16 ± 1.98 mg/dl respectively. The level of uric acid was found to be significantly decreased in both the Alpha terpineol treated groups, as compared with the DMBA treated animals. Alpha terpineol alone treated group (20mg/kg) did not show any marked change when compared to normal control with regard to the uric acid. In all the control and experimental groups of animals the serum alkaline phosphate level was studied. The ALP level was found to be 76.20 ± 1.83IU/L in the control group. In the case of DMBA treated rats ALP level was found to be enhanced to 95.00 ± 1.56 IU/L, which was markedly decreased in Alpha terpineol treated rats 20mg/kg and 40mg/kg (78.60 ± 1.23 and 78.80 ± 1.55 IU/L) respectively.

The total protein content was determined in all the treated and control groups of rats. In the control group, total protein level was found to be 4.50 ± 0.21 IU/L. In the case of DMBA treated rats total protein level was found to increase to 6.87 ± 0.23IU/L, which was considerably ($P < 0.05$) decreased in Alpha terpineol treated groups 20mg/kg and 40mg/kg (4.85 ± 0.15 and 4.68 ± 0.18IU/L) respectively. The treatment of Alpha terpineol considerably decreased the triglyceride level in 20 and 40 mg/kg treated groups (132.2 ± 5.40 and 133.2 ± 5.89mg/dl) when compared with DMBA treated animals (145.80 ± 7.12mg/dl). The effect of lower dose was found to be significantly higher than the higher dose of Alpha terpineol. The BUN level was analysed in all the treated and control groups of rats. In the untreated group, BUN level was determined to be 16.89 ± 0.44mg/dl. In the case of DMBA treated rats, BUN was found to be increased to 19.57 ± 0.22 mg/dl, which was significantly decreased in Alpha terpineol treated rat,17.88 ± 0.46 and 17.96 ± 0.43 mg/dl respectively.

SGOT level was discovered to be reduced considerably in all the other treated groups when compared with DMBA treated group (39.13 ±1.13 IU/L). The lower dose of Alpha terpineol (20 mg/kg) was found to be more significant (36.31 ± 0.88 IU/L) in comparison with the higher dose, Alpha terpineol 40 mg/kg (36.90 ± 1.40 IU/L). SGPT level shown in Table I indicated an elevated level of enzyme in DMBA treated rat (40.04 ± 0.82 IU/L) when compared to the normal animals (35.28 ± 0.98 IU/L). Alpha terpineol treated groups differ significantly from DMBA treated rat (35.01 ± 1.52 and 34.45 ± 0.88 IU/L) (Table.15.a).

Table 15.a: Effect of Alpha terpineol on serum biochemical parameters

Parameters	**Cholesterol (mg/dl)**	**Uric acid (mg/dl)**	**ALP (IU/L)**	**Total protein (gm/dl)**	**Triglyceride (mg/dl)**	**Blood Urea Nitrogen (mg/dl)**	**SGOT (IU/L)**	**SGPT (IU/L)**
Normal control	83.63 ± 2.09b	4.01 ± 0.44b	76.2 ± 1.83b	4.50 ± 0.21b	116.60 ± .92b	16.89 ± 0.44b	33.62 ± 1.34	35.28 ± 0.98
DMBA (25mg/kg)	94.48 ± 3.04a	6.28 ± 0.32a	95.0 ± 1.56a	6.87 ± 0.23a	145.80 ± 7.12a	19.57 ± 0.22a	39.13 ± 1.13	40.04 ± 0.82
DMBA (25mg/kg) + Tamoxifen(20mg/kg)	86.01 ± 2.81b	4.19 ± 0.4b	77.60 ± 1.98b	4.74 ± 0.2b	120.20 ± 6.01b	17.68 ± 0.35b	36.32 ± 0.81	37.88 ± 0.78

Parameters	**Cholesterol (mg/dl)**	**Uric acid (mg/dl)**	**ALP (IU/L)**	**Total protein (gm/dl)**	**Triglyceride (mg/dl)**	**Blood Urea Nitrogen (mg/dl)**	**SGOT (IU/L)**	**SGPT (IU/L)**
DMBA (25mg/kg) + Alpha terpinol (20mg/kg)	91.54 ± 1.40a	5.61 ± 0.38a	78.60 ± 1.23b	4.85 ± 0.15b	132.20 ± 5.40b	17.88 ± 0.46b	36.31 ± 0.88	35.01 ± 1.52
DMBA (25mg/kg) + Alpha terpenol (40mg/kg)	87.16 ± 1.98b	4.24 ± 0.26b	78.80 ± 1.55b	4.68 ± 0.18b	133.20 ± 5.89b	17.96 ± 0.43b	36.90 ± 1.40	34.45 ± 0.88
Alpha terpenol (20mg/kg)	84.39 ±1.99b	4.02 ± 0.34b	76.40 ± 1.87b	4.60 ± 0.13b	119.0 ± 5.74b	17.41 ± 0.60b	36.01 ± 1.45	34.40 ± 0.79
F- test	*	**	**	**	*	*	*	*
SEd CD (p<0.05)	1.448 3.795	0.752 1.971	5.491 14.386	0.749 1.963	3.724 9.758	0.586 1.535	0.758 1.986	0.632 1.657

Values are expressed as the mean ± S.E.M (n=6); Statistical significance (p) calculated by one way ANOVA followed by Duncan's multiple range test. Significant levels were ** $p < 0.01$; * $p < 0.05$; NS –Non significant. *In vivo* antioxidant studies were carried out on DMBA induced mammary carcinoma in female Sprague Dawley rats because oxidative stress is associated with cancer. The following antioxidant enzymes were determined in the liver tissues of all tested rats. A deep decrease of SOD level was observed in DMBA induced rats (1.06 ± 0.26 U/mg) when compared to normal rats (1.90 ± 0.61U/mg). In the case of DMBA induced rats treated with Alpha terpineol extract (20 mg/kg and 40mg/kg U/mg), the SOD levels found to be increased to (1.84±0.56 and 2.07 ± 0.30 U/mg) as seen in Table II. However, the study was found to be non-significant. A deep decrease of CAT level was observed in DMBA induced rats (15.65 ± 1.69 U/mg) when compared to normal rats (26.44±3.60U/mg). The decrease seems to be statistically significant. When DMBA induced rats were treated with Alpha terpineol extract (20 mg/kg and 40mg/kg), the CAT levels significantly increased to (23.88±1.36 and 24.17 ± 1.60 U/mg). The present study showed a significant decrease in GPx in the DMBA treated group (21.36 ± 1.34 U/mg) when compared with normal control rat (31.27 ± 1.46 U/mg). Both Alpha terpineol treatment groups (20 and 40 mg/kg) showed a significant increase in GPx (29.73 ± 0.80 and 29.41 ± 1.10 U/mg) when compared with DMBA treated group. A deep decrease of GSH level was observed in DMBA induced rats (0.41 ± 0.11 µg/mg) when compared to normal rats (1.33 ± 0.65µg/mg). In the case of DMBA induced rats, GSH level of both the Alpha terpineol treated groups (20 and 40 mg/kg) was found to increase to 1.28 ± 0.54 and 1.27 ± 0.51µg/mg respectively. With administration of Alpha terpineol to the DMBA induced rat, the level of LPx was found to be significantly decreased compared to that of DMBA treated rat. LPx in both the treatment groups Alpha terpineol 20 mg/kg (33.03 ± 1.56 µg of MDA/mg) and 40 mg/kg (33.06± 1.64 µg of MDA/mg) were found to be decreased (Table II). Alpha terpineol alone (20mg/kg) did not show any marked difference when compared to normal control in the LPX (Table.15.b).

Table 15.b: Effect of Alpha terpineol on antioxidant parameters

Parameters	SOD (U/mg protein)	Catalase (U/mg protein)	GPX (U/mg protein)	GSH (µg/mg tissue)	LPX (µg of MDA/mg protein)
Normal control	1.90±0.61a	26.44±3.60	31.27±1.46	1.33±0.65	30.71±0.63
DMBA (25mg/kg)	1.06±0.26b	15.65±1.69b	21.36±1.34b	0.41±0.11b	35.66±0.80a
DMBA (25mg/kg) +Tamoxifen(20mg/kg)	1.82±0.63a	24.59±1.35a	30.01±1.38a	1.30±0.66a	33.36±1.36b
DMBA (25mg/kg) + Alpha terpineol(20mg/kg)	1.84±0.56a	23.88±1.36a	29.73±0.80a	1.28±0.54a	33.03±0.1.56b
DMBA (25mg/kg) + Alpha terpineol(40mg/kg)	2.07±0.30a	24.17±0.86a	29.41±1.10a	1.27±0.51a	33.06±1.64b
Alpha terpineol (20mg/kg)	1.83±0.58a	23.70±1.60a	29.32±1.00a	1.26±0.49a	32.86±1.15b
F- test	NS	*	*	NS	*
Sed CD ($p < 0.05$)	0.3280.859	1.2353.236	0.7642.003	0.3350.878	0.7922.076

Values are expressed as the mean ± S.E.M (n=6); Statistical significance (p) calculated by one way ANOVA followed by Duncan's multiple range test. Significant levels were ** $p < 0.01$; * $p < 0.05$; NS –Non significant.

Biochemical examination of DMBA induced rats in the present study showed marked elevation in ALP, SGOT, and SGPT indicating the hepatotoxic effect of the tumor (Sreejaya *et al.,* 2017). Similarly, triglycerides, lipid profile, and protein profile were also normalized by treatment with Alpha terpineol. Hence, the present study shows that Alpha terpineol extract can mediate suppression of elevated levels of ALP in DMBA-induced rats which suggests the possibility of this test compound to stabilize the plasma membrane (Kalaiselvi *et al.,* 2014). This is also in accordance with the work of Dharmalingam *et al.,* 2016, who reported that a tri herbal formulation of seed coats of Terminalia chebula, dry seeds of E. ganitrus, and leaves of P. cineraria had potential in treating DMBA-induced mammary carcinoma in female Sprague Dawley rats. Uric acid is regarded as a marker of oxidative stress and as an end product of purine metabolism. At its elevated level, it can act as a pro-oxidant. The rapid destruction of tumor cells leads to a release of their intracellular content into the circulation with a marked rise in potassium and phosphate. The increased nucleotide release and turnover results in increased synthesis of uric acid. In the present study, a decrease in uric acid levels in Alpha terpineol treated groups indicate restoration of a normal renal function (Zahan *et al.,* 2011).

Intracellular alterations in cholesterol were accompanied by specific changes of cholesterol in plasma (Sreejaya *et al.,* 2017). The present study reports that Alpha terpineol significantly restores cholesterol level to near normal value. The liver produces triglycerides that may change to cholesterol. In the present study, Alpha terpineol treatment in both doses (20 and 40mg/kg) could restore the total protein content and lipid to a near-normal level suggesting

the stabilization of endoplasmic reticulum leading to protein synthesis. Decreased SOD activity was observed in various cancerous conditions (Selvendiran *et al.,* 2003).

The enzymic antioxidant catalase is widely distributed in all tissues and catalyzes the breakdown of hydrogen peroxide produced by tumor cells. The source of hydrogen peroxide is mainly SOD-mediated dismutation of superoxide radicals, which is generated by various enzyme systems as well as by non-enzymic pathways. Several reports have cited decreased activities of SOD and catalase in various carcinogenic conditions (Kamaraj *et al.,* 2009). CAT is an intracellular antioxidant enzyme that promotes the removal of hydrogen peroxide (H_2O_2) and its conversion to molecular oxygen (O_2) and water. CAT activity is directly regulated by the build-up of H_2O_2 in the tissues (Ogueji *et al.,* 2017a; Nwani *et al.,* 2017). The reduced activities of catalase found in the cancerous condition may be due to the exhaustion of these enzymes in catalyzing the overproduction of hydrogen peroxide by the malignant cells. Several studies had reported the decreased activities of GPx in many cancerous conditions. It is an important intracellular enzyme that breaks down hydrogen peroxide (H_2O_2) to water; and lipid peroxides to their corresponding alcohols mainly in the mitochondria and sometimes in the cytosol (Ighodaro *et al.,* 2018).

The protection of GSH is by scavenging the free radicals, acting as a cofactor for antioxidant enzymes, and accelerating xenobiotic detoxification (Morsy *et al.,* 2020). The decrease in GSH levels in the present study may also be due to a reduction in the substrate obtainable for GSH synthesis. Periyasamy *et al.,* 2015 reported that breast cancer-bearing rats showed an increased level of mammary lipid peroxidation, which involves the process of oxidative degradation of polyunsaturated fatty acids (PUFA), that occur in biological membranes causing the impaired structural integrity, decreased membrane fluidity, inactivation of several membrane-bound enzymes and functions (Massaccesi *et al.,* 2020). Thus, it is reasonable to speculate that carcinogen exposure may result in the peroxidation of PUFA and finally leads to cellular deterioration in the breast tissue (Periyasamy *et al.,* 2015). Bhat *et al.,* 2008 also suggested a strong correlation between carcinogen-induced breast cancer and the initiation of LPO. This is in line with the present study which shows increased lipid peroxidase in DMBA induced rats.

15.6 Conclusion:

The present study evaluated the Biochemical parameters and Antioxidant activity of Methanol extract of Alpha terpineol against DMBA induced Female Sprague Dawley Rats. The Methanol extract treatment of 20mg/kg and 40mg/kg inhibited the tumour activity by restoring the serum biochemical parameters and antioxidant activity. The restoration of these parameters to near normal levels indicate the anti-cancer effect of Alpha terpineol against DMBA induced Female Sprague Dawley Rats.

15.7 References:

1. Alakhova, D.Y. and Kabanov, A.V., 2014. Pluronics and MDR reversal: an update. *Molecular pharmaceutics*, *11*(8), pp.2566-2578.
2. Bauer, K., Garbe, D. and Surburg, H., 2008. *Common fragrance and flavor materials: preparation, properties and uses*. John Wiley & Sons.

3. Beckwitt, C.H., Brufsky, A., Oltvai, Z.N. and Wells, A., 2018. Statin drugs to reduce breast cancer recurrence and mortality. *Breast Cancer Research*, *20*(1):1-11.
4. Bhat, S., Rao, G., Murthy, K.D. and Bhat, P.G., 2008. Seasonal variations in markers of stress and oxidative stress in rats. *Indian Journal of Clinical Biochemistry*, 23(2):191-194.
5. Dharmalingam, Karthick., Ramakrishnan, Stalin., Panchanatham, Sachidanadam.and Palanivelu, Shanthi., 2016. Chemotherapeutic efficacy of tridham and 1, 2, 3, 4, 6-penta-o-galloyl-β-dglucose on antioxidants status and tumour markers in experimental mammary carcinoma in Sprague-Dawley rats. *Asian J Pharm Clin Res.*, 9(5):202-208.
6. Hassan, S.B., Gali-Muhtasib, H., Göransson, H. and Larsson, R., 2010. Alpha terpineol: a potential anticancer agent which acts through suppressing NF-κB signalling. *Anticancer Research*, 30(6):1911-1919.
7. Held, S., Schieberle, P. and Somoza, V., 2007. Characterization of α-terpineol as an anti-inflammatory component of orange juice by in vitro studies using oral buccal cells. *Journal of agricultural and food chemistry*, 55(20):8040-8046.
8. Ighodaro, O.M. and Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, *54*(4):287-293.
9. Itani, W.S., El-Banna, S.H., Hassan, S.B., Larsson, R.L., Bazarbachi, A. and Gali-Muhtasib, H.U., 2008. Anti-colon cancer components from Lebanese sage (*Salvialibanotica*) essential oil: mechanistic basis. *Cancer biology & therapy*, 7(11):1765-1773.
10. Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. and Forman, D., 2011. Global cancer statistics. *CA: a cancer journal for clinicians*, *61*(2), pp.69-90.
11. Kalaiselvi, M., Gomathi, D., Ravikumar, G., Devaki, K. and Uma, C., 2014. Therapeutic effect of *Ananuscomosus* peel on breast cancer induced by 7, 12-dimethylbenz (alpha) anthracene. *Plant Science Today*, 1(1):13-21.
12. Kamaraj, S., Ramakrishnan, G., Anandakumar, P., Jagan, S. and Devaki, T., 2009. Antioxidant and anticancer efficacy of hesperidin in benzo (a) pyrene induced lung carcinogenesis in mice. *Investigational New Drugs*, *27*(3):214-222.
13. Massaccesi, L., Galliera, E. and Romanelli, M.M.C., 2020. Erythrocytes as markers of oxidative stress related pathologies. *Mechanisms of Ageing and Development*, 191:111333.
14. Morsy, M.A., Abdel-Aziz, A.M., Abdel-Hafez, S., Venugopala, K.N., Nair, A.B. and Abdel-Gaber, S.A., 2020. The Possible Contribution of P-Glycoprotein in the Protective Effect of Paeonol against Methotrexate-Induced Testicular Injury in Rats. *Pharmaceuticals*, 13(9):223.
15. Nwani CD, Somdare PO, Ogueji EO, Nwani JC, Ukonze JA, Nwadinigwe AO., 2017. Genotoxicity assessment and oxidative stress responses in freshwater African catfish Clarias gariepinus exposed to fenthion formulations. *Drug Chem Toxicol*., 40(3):273–280.
16. Ogueji EO, Nwani CD, Iheanacho SC, Mbah CE, Okeke OC, Ibrahim BU., 2017. Acute toxicity of ibuprofen on selected biochemical and oxidative stress parameters of liver in Clarias gariepinus juveniles (Burchell, 1822). *J Entomol Zool Stud*., 5(4):1060–1068.
17. Othman, E. and Ahmed, A., 2013. Challenges of mega construction projects in developing countries. *Organization, technology & management in construction: an international journal*, *5*(1), pp.730-746.

18. Periyasamy, K., Baskaran, K., Ilakkia, A., Vanitha, K., Selvaraj, S. and Sakthisekaran, D., 2015. Antitumor efficacy of tangeretin by targeting the oxidative stress mediated on 7, 12-dimethylbenz (a) anthracene-induced proliferative breast cancer in Sprague–Dawley rats. *Cancer chemotherapy and pharmacology*, 75(2):263-272.
19. Rao, K.R. and Yip, P., 2014. *Discrete cosine transform: algorithms, advantages, applications*. Academic press.
20. Sabino, E.C., Ribeiro, A.L., Salemi, V.M., Di Lorenzo Oliveira, C., Antunes, A.P., Menezes, M.M., Ianni, B.M., Nastari, L., Fernandes, F., Patavino, G.M. and Sachdev, V., 2013. Ten-year incidence of Chagas cardiomyopathy among asymptomatic Trypanosoma cruzi–seropositive former blood donors. *Circulation*, *127*(10), pp.1105-1115.
21. Selvendiran, K., Prince Vijeya Singh, J., Baba Krishnan, K., Sakthisekaran, D., 2003. Cytoprotective effect of piperine against benzo(a)pyrene induced lung cancer with reference to lipid peroxidation and antioxidant system in Swiss albino mice. *Fitoterapia*, 74:109–115.
22. Sreejaya, S.B., Archana, D. and Santhy, K.S., 2017. Biochemical changes in the serum of experimental animals treated with acorus calamus rhizome. *Studies on Ethno-Medicine*, 11(3):216-220.
23. Youlden, D.R., Cramb, S.M., Yip, C.H. and Baade, P.D., 2014. Incidence and mortality of female breast cancer in the Asia-Pacific region. *Cancer biology & medicine*, 11(2):101.
24. Zahan, R., Alam, M., Islam, S., S Chowdhury, N., B Hosain, S., Mosaddik, A., Jesmin, M. and Haq, M.E., 2011. Anticancer activity of *Alangium salvifolium* flower in Ehrlich Ascites carcinoma bearing mice, 7(3):254-262.