Antioxidant and Cytotoxic Activity of Different Extracts of *Zanthoxylum Alatum*

Indrajit Karmakar*, Sagnik Haldar, Mainak Chakraborty, Saikat Dewanjee, Pallab Kanti Haldar

Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India.

ABSTRACT

Objectives: The present study evaluates the antioxidant and cytotoxic activity of different extract (methanol, chloroform, petroleum ether) of *Zanthoxylum alatum*. **Methodology:** The antioxidant activities of all the three above mentioned extracts of *Zanthoxylum alatum* were measured by different *in vitro* standard methods like 1,1-diphenyl-2-picrylhydrazil radical (DPPH), superoxide anions, nitric oxide, hydroxyl radicals and reductive assay. On the basis of antioxidant properties total phenolic, total flavonoid, CCl_4-induced *in vivo* antioxidant and *in vitro* cytotoxicity were measured for methanol extract of *Zanthoxylum alatum* (MEZA). Cytotoxicity was determined in Ehrlich Ascites Cancer (EAC) cells by trypan blue exclusion method. **Results:** The extracts exhibited antioxidant activities in a dose dependent manner. The IC_{50} values of Methanol, Chloroform, Petroleum ether extracts and standard for DPPH are 3.63 ± 0.14 µg/mL, 9.33 ± 0.42 µg/mL, 7.70 ± 0.31 µg/mL and 7.87 ± 0.39 µg/mL; for Superoxide are 19.80 ± 0.96 µg/mL, 129.70 ± 2.57 µg/mL, 168.10 ± 2.26 µg/mL and 11.76 ± 0.72 µg/mL; for Nitric oxide are 105.0 ± 1.64 µg/mL, 157.60 ± 1.99 µg/mL, 185.30 ± 2.48 µg/mL and 28.48 ± 1.06 µg/mL; for Hydroxyl radical are 28.10 ± 0.75 µg/mL, 81.50 ± 1.88 µg/mL, 72.47 ± 1.76 µg/mL and 12.24 ± 0.82 µg/mL. Reductive ability of the extracts was also tested where dose dependent reducing capability was observed. In case of *in vivo* antioxidant lipid peroxidation, reduced glutathione, superoxide dismutase and catalase parameters significantly change towards the normal levels. MEZA showed satisfactory phenolic and flavonoid content which support the cytotoxicity with IC_{50} value 102.30 ± 0.81 µg/mL. **Conclusion:** The study showed that among all the three extract MEZA has better dose dependant antioxidant and cytotoxic activity. Further research is going on to find out the active principle(s) of MEZA for its cytotoxic activity.

Key words: Antioxidant, Cytotoxicity, Ehrlich Ascites Cancer, *Flavonoid, Zanthoxylum alatum.*

INTRODUCTION

Free radicals are ubiquitous in our body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms.[1] Free radicals are formed naturally in the body and play an important role in many normal cellular processes. At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health disorders like diabetes, liver damage, nephrotoxicity, inflammation, cancer, neurological, cardiovascular disorders and aging.[2,3] Research in humans has not demonstrated convincingly that taking antioxidant supplements can help reduce the risk of developing or dying from cancer, and some studies have even shown an increased risk of some cancers.[4] Plants are the primary sources of naturally occurring antioxidants for humans.[5] Indeed, molecules derived from natural sources including plants, marine organisms and micro-organisms, have played, and continue to play, a dominant role in the discovery of leads for the development of conventional drugs for the treatment of most human diseases.[6]

***Corresponding address:**
Dr. Indrajit Karmakar
Department of Pharmaceutical Technology, Jadavpur University, Kolkota-700032, India.
E mail: indrajit.k83@gmail.com

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Zanthoxylum alatum Roxb. [Syn. *Zanthoxylum armatum* DC] (family: Rutaceae) is an evergreen or sub-deciduous common shrub of the Himalayan regions in India.[7] Local names of this plant are: Tejphal (Hindi), Tejowati (Sanskrit), Mukthrubi (Manipuri) and Timur (Nepal).[8] The ethno medicinal importance of its seeds has been well known for a long time in Indian medical system as a stomachic, carminative, disinfectant, antiseptic, and for the treatment of fever, dyspepsia, cholera, anthelmintic, indigestion and general debility.[9-11]

The essential oil of fruits of *Zanthoxylum armatum* DC exhibited good antibacterial, antifungal and anthelmintic activities.[12] It has been reported that the petroleum extract of *Zanthoxylum armatum* DC, shows significant insecticidal activity against Culex spp. Recently larvicidal activities of essential oil from the seeds of *Zanthoxylum armatum* DC against mosquito vectors has been reported.[7] It has been reported that the ethanolic extract of bark and leaves of this plant has significant hepatoprotective activity.[13,14] It has also been reported that the stem and root has antinociceptive and anti-inflammatory and antipyretic activities.[15] Whereas aqueous extracts of dried fruits has the capacity to induce cellular and nuclear damage.[16] The aim of the present study was to evaluate the antioxidant and cytotoxic activity of *Zanthoxylum alatum* leaves extract.

MATERIALS AND METHODS

Chemicals

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Chemicals, USA. Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), sodium nitroprusside, napthyl ethylene diamine dihydrochloride, ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), deoxyribose, potassium ferricyanide [$K_3Fe(CN)_6$], were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Trypan blue was purchased from Loba Chemie Pvt. Ltd., India. All other chemicals were used in high analytical grade.

Plant collection, identification and extraction

Zanthoxylum alatum leaves were collected from the hilly region of Gangtok, Sikkim, India. The plant material was identified by the Botanical Survey of India, Howrah, West Bengal, India. A voucher specimen (CNH/38/2014/Tech. II/78) has been preserved in our laboratory for future reference. The leaves were cleaned and air dried for a week at 35–40°C and pulverized in electric grinder. Powdered leaves (1.1 kg) were consecutively extracted by petroleum ether (7.4% w/w, yield), chloroform (9.1% w/w, yield) and methanol (10.6% w/w, yield) by using soxhlet apparatus. The solvents were completely removed under reduced pressure in a rotary vacuum evaporator (Buchi R-210). The concentrated extracts were stored in vacuum desiccators for further use.

In vitro antioxidant activity

in vitro antioxidant activity of above mentioned all extracts (methanol, chloroform and petroleum ether) of *Zanthoxylum alatum* (ZA) were determined by following scavenging assays.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of different extracts of ZA leaves (1, 5, 10, 25, 50, 100 μg/ml) was measured according to the method.[4] The 50% inhibitory concentrations (IC_{50}) of the extracts were calculated from graph as concentration versus percentage inhibition. The experiments were performed in triplicate.

Determination of superoxide radical scavenging activity

Superoxide radical scavenging activity of different extracts of ZA leaves (5, 10, 25, 50, 100, 200 μg/ml) was measured (triplicate) by the reduction of NBT according to the previously used methods in our laboratory.[4] The IC_{50} values of the extracts were calculated from graph as concentration versus percentage inhibition.

Determination of nitric oxide (NO) radical scavenging activity

The nitric oxide (NO) radical scavenging activity of different extracts of ZA leaves (5, 10, 25, 50, 100, 200 μg/mL) was measured according to the previously used method in our laboratory.[4] The IC_{50} values of the extracts were calculated from graph as concentration versus percentage inhibition. The experiments were performed in triplicate.

Determination of hydroxyl radical scavenging activity

The scavenging activity of different extracts of ZA leaves (5, 10, 25, 50, 100, 200 μg/mL) on hydroxyl radical activity was measured (triplicate) according to the previously described method.[4] The hydroxyl radical scavenging activity of the sample extracts was evaluated as % of antioxidant activity and IC_{50} values.

Determination of reductive activity

Reducing power of different extracts of ZA leaves (5, 10, 25, 50, 100 μg/mL) was determined by the previously described method.[4] A higher absorbance of the reaction mixture indicated greater reducing power ability. All tests

were run in triplicate.

In vivo antioxidant activity

Test animals

Healthy Wistar Albino male rats (180 ± 20 g) were divided into five groups (n=6) for the present experiment. They were kept in a controlled environment at 27 ± 2°C and 55 ± 5% relative humidity with a 12 h light and dark cycle. The animals were fed a standard pellet diet and water ad libitum (Hindustan Lever, Kolkata, India). All the procedures described were reviewed and approved by Jadavpur University Animal Ethics Committee (367001/C/CPCSEA).

Carbon tetrachloride (CCl_4)-induced oxidative toxicity

After seven days of acclimatization, treatment was done for 10 days as follows. Group I (normal control) was given distilled water with 0.3% sodium carboxymethyl cellulose (CMC-Na) (1 mL/kg bodyweight, p.o.) daily for 5 days and olive oil on days 2 and 3. Group II (CCl_4 control) was given 0.3% CMC-Na (1 mL/kg body weight, p.o.) solution for 5 days and a 1:1 mixture of CCl_4 and olive oil (2 mL/kg body weight, s.c.) on days 2 and 3. Groups III and IV (test group) were orally given 100 and 200 mg/kg body weight of MEZA, respectively, for 5 days and a 1:1 mixture of CCl_4 and olive oil (2 mL/kg bodyweight, p.o.) on days 2 and 3. Group V (positive control) was given the standard Vitamin E (100 mg/kg body weight, p.o.) daily for 5 days and a 1:1 mixture of CCl4 and olive oil (2 mL/kg bodyweight, s.c.) on days 2 and 3. At the end of the experiment (on day 6), the animals were sacrificed and liver samples were dissected, cleaned of blood with ice-cold saline, and immediately stored in a refrigerator for further experiment.

In vivo antioxidant parameters

The tissue antioxidant assay was performed with liver homogenate and the evaluation was carried out by measuring the level of lipid peroxidation, the amount of enzymatic Catalase, nonenzymatic reduced glutathione[17] and superoxide dismutase.[18]

Determination of total phenolic and flavonoid content

Total phenolic content of MEZA (best free radical scavenger among the three extract) was determined using Folin-Ciocalteu (FC) reagent according to the reference method.4 The total flavonoid content of MEZA was determined by the previously described method with some modification and total flavonoid content was expressed in milligram of quercetin equivalents/g extract.[19]

Determination of in vitro cytotoxic activity

Ehrlich ascites carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India prior to the experiment in Swiss albino mice. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7–8 of tumor bearing) of the tumor cells for the present experiment.[20] On the basis of antioxidant activity *in vitro* cytotoxicity was checked for methanol extract of *Zanthoxylum alatum* (MEZA) by previously used trypan blue exclusion method.[6] The experiment was performed in triplicate.

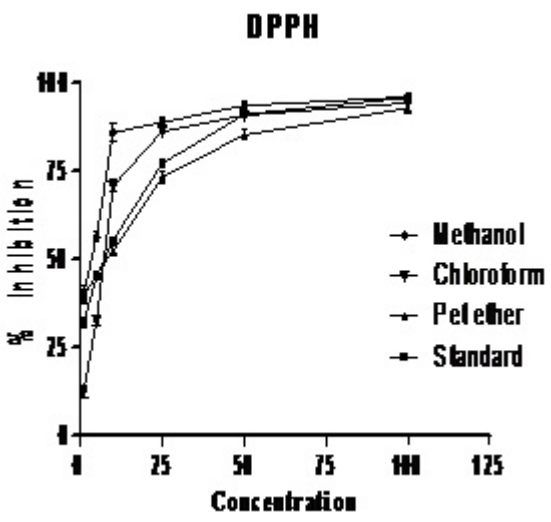

Figure 1: 1, 1-diphenyl-2-picrylhydrazil (DPPH) scavenging activity of different extracts and standard. The data represent the percentage of DPPH inhibition. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM).

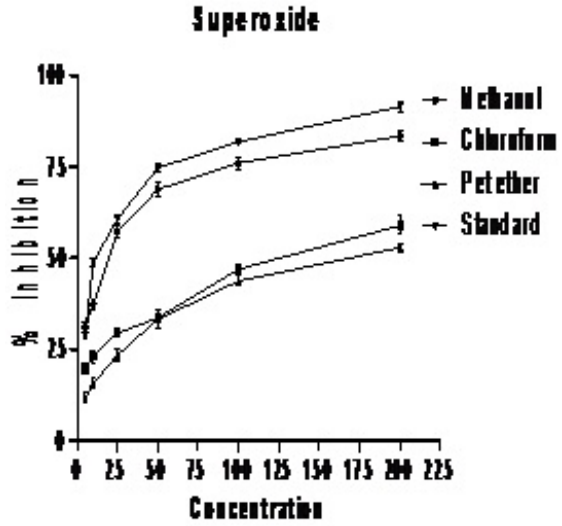

Figure 2: Superoxide radical scavenging assay of different extracts and standard. The data represent the percentage of superoxide inhibition. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM).

Statistical analysis

All the data are given as the mean ± SEM of three individual measurements. 50% inhibitory concentrations (IC_{50}) were calculated by plotting the data in the graph as concentration versus percentage inhibition using Graph Pad Prism software, version 5.0, San Diego, California.

RESULTS

In vitro antioxidant activity

The methanol, chloroform and petroleum ether extracts from the leaves of *Zanthoxylum alatum* show the capacity to scavenge all the tested reactive species and all the IC_{50} values (mean ± SEM) for three individual experiments being found at the µg/ml level. Among the all above mentioned extract MEZA shows better antioxidant properties compared to chloroform and petroleum ether extract.

DPPH radical scavenging activity

The assay showed the inhibitory ability of three extract and standard ascorbic acid on DPPH in a concentration dependent manner (Figure 1). But methanol extract demonstrated comparatively stronger antioxidant activity as compared to the petroleum ether and chloroform extract. The IC_{50} values for DPPH scavenging of methanol, chloroform, petroleum ether and standard ascorbic acid were found to be 3.63 ± 0.14 µg/ml, 9.33 ± 0.42 µg/ml, 7.70 ± 0.31 µg/ml and 7.87 ± 0.39 µg/ml (Figure 5) which indicates the efficient DPPH scavenging activity.

Superoxide radical scavenging activity

The increase in inhibition capability indicates the three extracts and standard has superoxide radicals scavenging activity (Figure 2). The IC_{50} values of methanol, chloroform, petroleum ether and ascorbic acid were found to be 19.80 ± 0.96 µg/ml, 129.70 ± 2.57 µg/ml, 168.10 ± 2.26 µg/ml and 11.76 ± 0.72 µg/ml (Figure 5) which favour methanol extract for its further study.

Nitric oxide (NO) radical scavenging activity

Methanol, chloroform, petroleum ether extract and ascorbic acid showed concentration dependent inhibition on nitric oxide (Figure 3). The IC_{50} values of methanol, chloroform, petroleum ether extract and ascorbic acid were found to be 105.0 ±1.64 µg/ml, 157.60 ± 1.99 µg/ml, 185.30 ± 2.48 µg/ml and 28.48 ± 1.06 µg/ml (Figure 5) respectively.

Hydroxyl radical scavenging activity

The methanol extract showed better concentration dependent hydroxyl radical scavenging activity compared to chloroform and petroleum ether extract (Figure 4). The IC_{50} values of methanol, chloroform, petroleum ether extracts and standard for hydroxyl radical are 28.10 ± 0.75 µg/ml, 81.50 ± 1.88 µg/ml, 72.47 ± 1.76 µg/ml and 12.24 ± 0.82 µg/ml (Figure 5).

Reductive activity

Figure 6 showed the reductive ability of the extract in a concentration dependent manner, which indicates the good reducing power of methanol extract as compared to chloroform, petroleum ether extract and standard ascorbic acid.

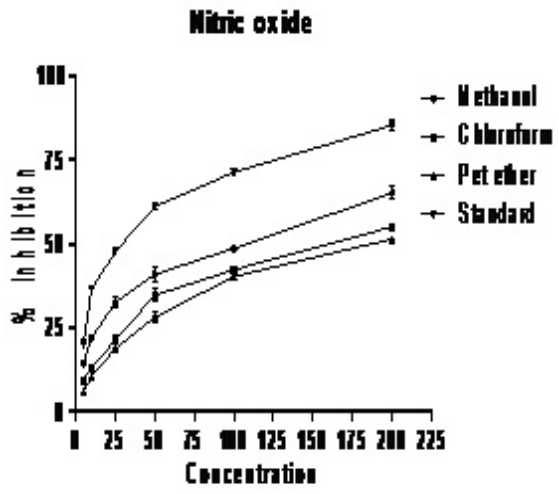

Figure 3: The nitric oxide radical scavenging activity of different extracts and standard. The data represent the percentage of nitric oxide inhibition. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM).

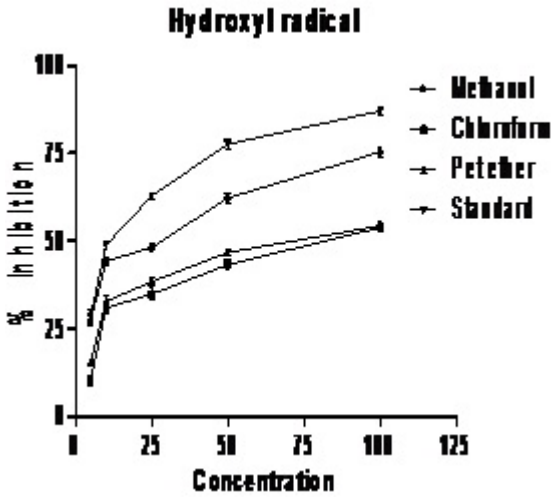

Figure 4: Hydroxyl radical scavenging activities of different extracts and standard. The data represent the percentage of hydroxyl radical inhibition. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM).

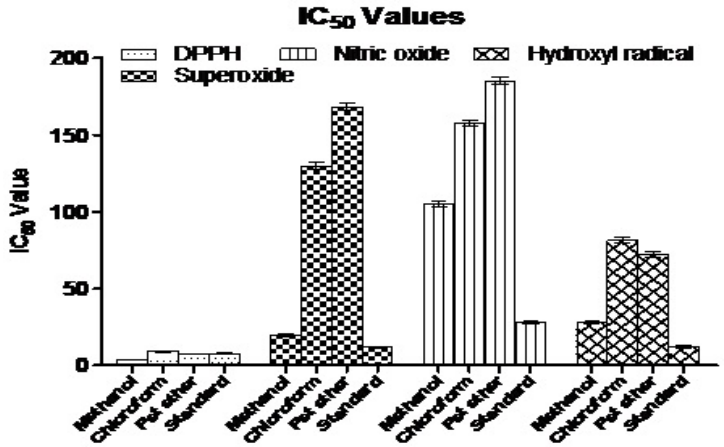

Figure 5: IC_{50} values. The IC_{50} values of Methanol, Chloroform, Pet ether extracts and standard for DPPH are 3.63 ± 0.14 µg/mL, 9.33 ± 0.42 µg/mL, 7.70 ± 0.31 µg/mL and 7.87 ± 0.39 µg/mL; Superoxide are 19.80 ± 0.96 µg/mL, 129.70 ± 2.57 µg/mL, 168.10 ± 2.26 µg/mL and 11.76 ± 0.72 µg/mL; Nitric oxide are 105.0 ± 1.64 µg/mL, 157.60 ± 1.99 µg/mL, 185.30 ± 2.48 µg/mL and 28.48 ± 1.06 µg/mL; Hydroxyl radical are 28.10 ± 0.75 µg/mL, 81.50 ± 1.88 µg/mL, 72.47 ± 1.76 µg/mL and 12.24 ± 0.82 µg/mL. The results are mean ± SEM of three experiments.

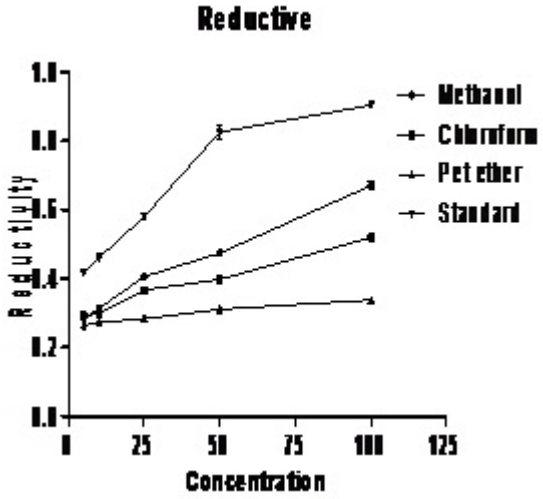

Figure 6: Reductive effect of different extracts and standard. The data represent the percentage of reductive ability. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM).

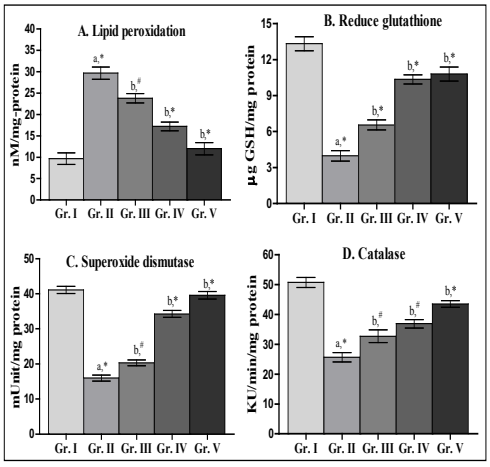

Figure 7: *In vivo* antioxidant activity of MEZA. Lipid peroxidation (A), Reduce glutathione (B), Superoxide dismutase (C) and Catalase (D). Values are represented as mean±SEM, where n=6. [a]CCl_4 control vs normal control group; [b]treated groups vs CCl_4 control group, #P<0.05; *P<0.01.

The result of *in vitro* antioxidant activity showed that among the three extract methanol extract is better free radical scavenger which supports its further study.

In Vivo antioxidant parameters

There were significant (P<0.01) decrease in the activities of SOD, CAT and GSH whereas lipid peroxidation level was found to increase in EAT bearing control group as compared to normal animals (Figure 7). Treatment with MEZA 100 and 200 mg/kg significantly (P<0.05 and P <0.01) increased the levels of SOD and CAT whereas the GSH level was increased. The lipid peroxidation level was also decreased with the treatment of MEZA at the doses of 100 mg/kg (P<0.05) and 200 mg/kg (P<0.01) respectively.

Total phenolic and flavonoid content

The total phenolic and flavonoid content was determined for MEZA and it contains 89.2 ± 0.59 mg/g and 63.8 ± 0.34 mg/g of extract of phenolic compound and flavonoid compound respectively which is mainly accounted for its free radical as well as antioxidant activity.

Cytotoxic activity

In vitro cytotoxicity of MEZA was evaluated by the trypan blue exclusion method. The assay showed direct cytotoxic effect of MEZA on the EAC cell line in a concentration dependent manner. The IC_{50} value was found to be 102.3 ± 0.81 µg/ml by plotting the graph of concentration versus percentage inhibition (Figure 8).

DISCUSSION

The term 'oxyradical overload' encompasses a wide range of diseases that are associated with an increased cancer risk. Key reasons for this increased risk include DNA damage, protein modification and changes in the transcriptional activation and/or repression of genes that are responsible for cellular homeostasis. Any chronic shift in the maintenance of this cellular homeostasis can lead to permanent changes associated with carcinogenesis. Because of the complexity of free radicals, it has been difficult to delineate the specific role of each radical in carcinogenesis.[1]

Recently, several dietary supplements containing vitamins, polyphenols, or flavones also play a significant role in this matter. Phenolic compounds are very important plant constituents because they exhibit an antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals.[21]

The antioxidant potential of the extracts were determined by five methods namely DPPH assay, super oxide radical scavenging assay, nitric oxide radical scavenging assay, hydroxyl radical scavenging assay and reductive assay; cytotoxic activity was studied by using trypan blue exclusion method.

Different therapeutic approaches can be used to decrease the oxidative stress and include scavenging of free radicals, inhibition of free radical producing enzymes, enhancing the antioxidant system or by targeting the signalling routes and expression of molecules involved in the inflammatory cascade.[4] Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplement as traditional medicines.

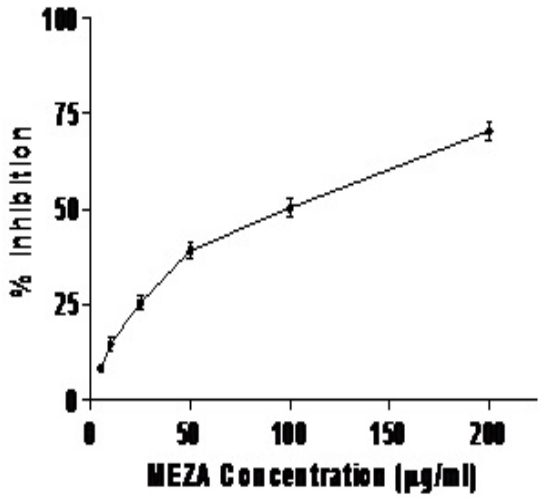

Figure 8: Cytotoxic effect of MEZA on *in vitro* EAC cell line. Values are Mean ± SEM; where n=3.

DPPH is a stable free radical, which can accept an electron or hydrogen radical to become a stable diamagnetic molecule hydrazine. DPPH radical reacts with suitable reducing agents and then electrons become paired off and the solution loses color from violet to yellow stoichiometrically with the number of electrons taken up which is demonstrated by the decrease in absorbance at 517 nm.[22,23]

Generation of free radicals through endogenous cellular processes creates an oxidative environment which, if not removed by the delicately balanced antioxidant enzyme machinery, causes oxidative damage in DNA and proteins. Polymorphism of the gene encoding the antioxidant enzyme manganese superoxide dismutase, which converts O_2•– to H_2O_2, alters protein trafficking and is associated with increased breast cancer risk.1 Flavonoids are effective antioxidant mainly because they scavenge superoxide anions. In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The results suggest that concentration-dependent increasing of superoxide radical scavenging activity.[24]

Nitric oxide plays a vital role in various inflammatory processes. The toxicity of nitric oxide increases when it reacts with superoxide radical forming highly reactive peroxy nitrate anion (ONOO−).[5] In nitric oxide scavenging assay, the radicals generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions which is estimated with Griess reagent. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extracts inhibit nitrite formation by directly competing with oxygen in the reaction with nitric oxide.[25] The present study proved that the extracts have good nitric oxide scavenging activity.

Hydroxyl radicals are extremely reactive free radicals formed in biological systems and have been implicated as a highly damaging species in free radical pathology. These radicals can be formed from a superoxide anion and hydrogen peroxide in the presence of iron ions. There is no specific enzyme to defend against them in human. Therefore, it is important to discover natural compounds with good antioxidant properties.[23] Phenolic compound comprising with hydroxyl group and aromatic ring serves as potent antioxidant.[26] The concentration dependant antioxidant properties with its phenolic and flavonoid contents confirmed it.

The reducing property of extracts indicate they can be used as electron donors which reduce the oxidized intermediates of lipid peroxidation processes, therefore, they can act as primary and secondary antioxidants. However, the activities of antioxidant have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging.[27] The result showed that the extract has good reductive ability.

The above study shows free radical scavenging property of three extract of ZA. Among the three extract MEZA has potent antioxidant property as compared to the chloroform and petroleum ether extract indicating its food supplement as a rich source of phenolic and flavonoid compounds which is responsible for its potency. These data inspire to carry forward the further work of MEZA for its total phenolic and flavonoid content determination as well as cytotoxic activity.

In CCl_4-induced toxicity, CCl_4 is metabolized through the cytochrome P450 monooxygenase system to produce the trichloromethyl radical, which then reacts with oxygen to form the trichloromethyl peroxyl radical (CCl_3O_2•). These radicals further attack cellular macromolecules such as proteins or lipids, which lead to the lipid peroxidation. They also affect antioxidant defence mechanisms, which in turn decrease the activity of antioxidant enzymes.[28]

Excessive production of free radicals results in oxidative stress, which leads to damage of macromolecule cells such as lipids, and can induce lipid peroxidation *in vivo*. Increased lipid peroxidation causes degeneration of tissues. The endogenous antioxidant system of the body comprising of Glutathione (GSH), SOD and Catalase exert a protective role by scavenging the notorious free radicals.[29] Glutathione is found in high concentrations in liver. SOD and catalase are present in all oxygen-metabolizing cells. Decrease in SOD activity in EAT bearing mice might be due to loss of mitochondrial SOD in the EAT cells.[30] Inhibition of SOD and CAT activities as a result of tumor growth was also reported. Similar findings were obtained in the present investigation with EAT bearing mice. Treatment with MEZA at 100 mg/kg and 200 mg/kg significantly increased the SOD and CAT levels in a dose dependant manner.

The evaluation of the phenolic compounds uses the Folin-Ciocalteau reagent, which forms blue complexes in the presence of reducing agents. The intensity of blue-colored complex is related to the presence of hydrogen donating groups in the phenolic compounds. Flavonoids are effective antioxidant mainly because they scavenge superoxide anions. A major function of this group of compounds is to protect plants against ultraviolet, and as the leaves are more exposed than the seeds, the higher amounts of flavonoids in the leaves are justified.[31]

The EAC cells were initially described as a spontaneous murine mammary rapidly growing adenocarcinoma with a very aggressive behavior and can proliferate in almost all strains of mice.[5] The Ehrlich ascites tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascites fluid accumulation.[32] Trypan blue is a vital stain used in the identification of dead tissue or cells. Living cells or tissues with intact cell membrane are not colored, because the dye is not absorbed through the intact cell membrane. However it traverses the membrane of dead cells. Hence dead cells are shown as a distinctive blue color under the microscope.[33] The assay confirmed that the reduction on viable cell number was due to the cytotoxic action of MEZA to EAC cells. The IC_{50} value and this cytotoxicity probably reflect the cell response to particular kinds of damage, in this case, mitochondria insult and/or oxidative stress.

CONCLUSION

The results of the present study indicate that the different extract of *Zanthoxylum alatum* possess significant antioxidant activities when tested against different in vitro and *in vivo* models. It also gives preliminary positive results in cytotoxic study. Future investigations will focus on in vivo studies assessing the effects of MEZA in different cancer cell lines.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

ACKNOWLEDGEMENT

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