ANTI-CANCER, ANTI-OXIDANT ACTIVITIES OF ALBENDAZOLE DERIVATIVES AND ITS COMPUTATIONAL STUDIES

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ABSTRACT: Albendazole is anti-helminitic agent and used mostly in the treatment of echinococcosis.It is a derivative of benzimidazole and its anti cancer and antitumor effect has been of ABZ has been reported in both animals and human in clinical trails.According to the cytotoxic effects of ABZ on breast cancer and melanoma cells,it can be used as a promising adjuvant along with other chemotherapy drugs. The main aim/objective of our study was to evaluate the antioxidant activity of the newly synthesized albendazole compounds ,compared with that of standard antioxidant such as Ascorbic acid and Ethylenediaminetetra acetate (EDTA),by using different antioxidant activities that includes the DPPH radical scavenging assay,Ferrous ion-chelating ,Ferric ion-reducing assay,Total antioxidant capacity assay and hydroxyl radical scavenging assay.

I. INTRODUCTION

Antioxidants are the compounds that inhibit the oxidation or are the potent substances that strongly removes damaging oxidizing agents in biological system. These substances can prevent or slow down the damage to cells caused by free radical or unstable molecules that are produced in body as a consequence of environmental or some other pressures. They are also sometimes referred as "free-radical scavengers". (Halliwell , 2017) reported an antioxidant as "any substance that delays, prevents or removes oxidative damage to the targeted molecule" .They are strong inhibitor in the process of oxidation and acts as a warrior against it. Some of the antioxidants includes Thiols, glutathione, vitamin A, vitamin E, carotenes etc. Some enzymes such as catalase , superoxide dismutase and various peroxides. Chemically, antioxidants can be described as radical scavenger, hydrogen donor, electron donor, singlet oxygen quencher, enzyme inhibitor, synergist and metal- chelating agents .Early the term antioxidant originally was used to refer a chemical that prevents the consumption of oxygen .In the late 19^{th} and 20^{th} century ,the extensive study was devoted towards the useage of antioxidants in industrial process such as for the prevention of metal corrosion etc. Its history includes its importance too as one of the early research was based upon the role of antioxidants in biology ,which were use for the prevention of oxidation of unsaturated fats which was considered to be one of the source of rancidity. Then vitamin E as a lipid antioxidant was one of the major contribution in the history of science.The identification of Vitamin A,E and C was the bombastic revolution in the field of biochemistry as it led to the realization of their importance .

II. ENDOGENOUS ANTIOXIDANTS

These are the antioxidants that can be prepared or manufactured inside the living body .Common types of endogenous antioxidants are;

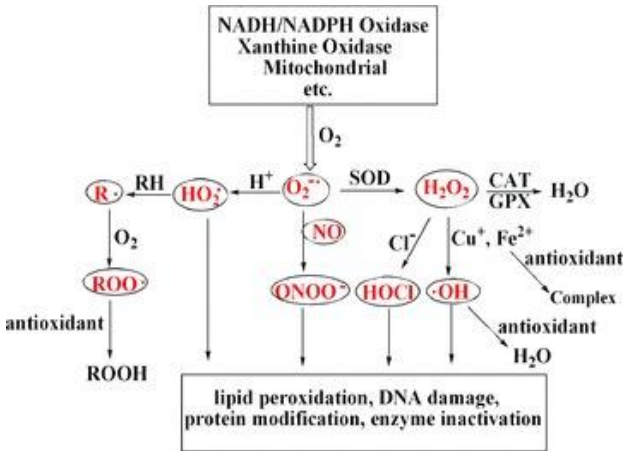
- Glutathione
- Alpha – lipoic Acid (ALA)
- CoQ10 (Ubquinone)
- Cholestrol

EXOGENOUS ANTIOXIDANTS: These type of antioxidants cannot be prepared by the body so can be obtained by the intake of antioxidant rich fruits and vegetables or potent antioxidant supplements .These types of antioxidants includes ;

- Polyphenols
- Carotenes
- Vitamin A
- Vitamin C
- Vitamin E

BACKGROUND:

These are the atoms or ions or molecules that has the unpaired electrons .They are very reactive and autocatalytic species .Organic radicals are having short life span while inorganic has more life span than organic radicals. Due to their reactivity and unstability,they pull electron from the other molecules and causes the affected molecule to behave as a free radical .e.g, superoxide anion, hydroxide radical, peroxynitrite , hydrogen radical ,nitric oxide, lipid peroxyl.Free radicals may also cause a depletion of the immune system antioxidants, a change in the gene expression and may induce the synthesis of abnormal proteins. About 5% or more of the inhaled oxygen (O_2) is converted to the reactive oxygen species (ROS) such as O_2^-, H_2O_2, and OH^- radicals. ROS represents the major type of free radicals in any biological system. They are produced through the mitochondrial electrons transport chain.The imbalance between the naturally produced free radical and body's antioxidant defense mechanism is said to be oxidative stress.Higher levels of oxidative stress are potentially damaging to the entire living body.The main reason of the oxidative damage is the higher oxidative stress baseline and this is implicated in a number of diseases i.e., from heart failure to the that of cancer, autism and even Alzheimer's disease.It also represents the decrease in the production of antioxidants status in our entire body. Decrease or disturbance in the normal redox state of the tissues can cause toxic effects and production of the free radicals damaging the DNA.



Summary of ROS types and sources, and action point of antioxidants. $O_2^{-\bullet}$, superoxide anion; $HO_2^{\bullet}$, perhydroxyl radical; ▪OH, hydroxyl radical; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; $ONOO^-$, peroxynitrite; R▪, lipid alkyl radical; RH, lipid; ROO▪, lipid peroxyl radical; ROOH, lipid hydroperoxide; SOD, superoxide dismutase; CAT, catalase and GPX, glutathione peroxidase.

III. METHOD

CHEMICALS:

Albendazole compounds were prepared in the organic chemistry laboratory of Abdul Wali Khan University Mardan and its antioxidant anticancer activities were done in the inorganic chemistry laboratoty of the same university and prepared concentrations with DMSO and Ethanol.

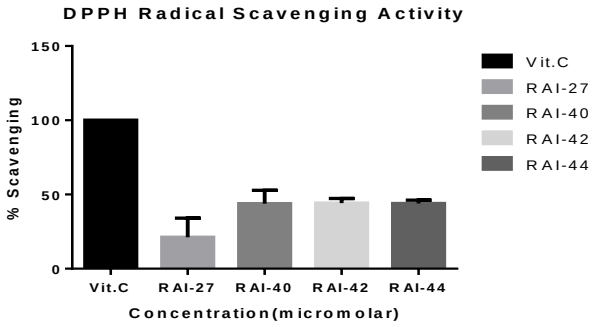
DETERMINATION OF ANTIOXIDANT ACTIVITIES:

DPPH Free Radical-Scavenging Assay

The antioxidant capacity of the synthesized complexes wAS measured as previously described (Choi *et al*., 2002). A stock solution of 85 µm DPPH in ethanol and stock solution of 3000 µM of test compound will be prepared and different concentration of samples will be mixed with fixed amount of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution. Absorbance at 517 nm of all tests would be measured immediately up to 2 hrs. Vitamin C will be used as the positive control.The following formula was used to calculate the percentage of inhibition;

DPPH Inhibition (%) =

$$\frac{Absorbance\ \ of\ control - Absorbance\ \ of\ sample}{Absorbance\ \ of\ control} \times 100$$

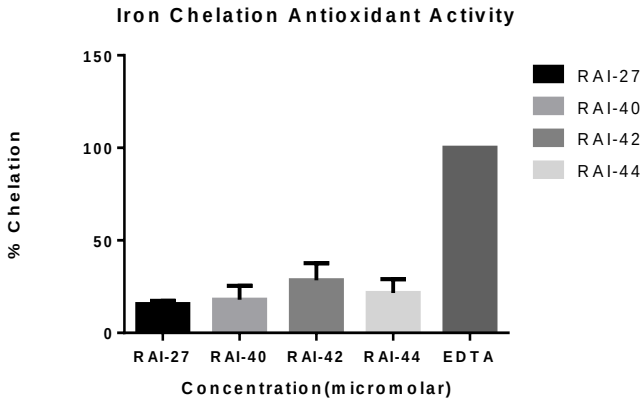


Ferrous Ion- Chelating Assay

The ferrous ion chelating ability of the synthesized complexe was measured as previously described (Puntel*et al*., 2005). Different concentrations of samples will be mixed with *O*-phenanthroline and Iron(II) sulphate solutions. All mixtures will be incubated for 10 minutes at room temperature. Ethylenediaminetetraacetic acid (EDTA) will be used as a positive control. The absorbance at 490 nm will be measured. A decrease in absorbance of ferrous ion and *O*-phenanthroline complex by the drug will show chelation of ferrous ion of the test compounds.The Fe^{+2} chelating capacity can be calculated by using the following formula;

Chelation(%) =

$$\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

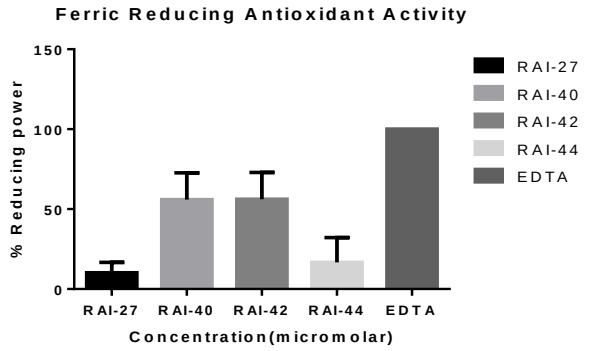


Ferric Ion- Reducing Power Assay(FRAP)

The reducing power of sample drugs was measured by using the reported method (Zhao *et al*., 2006).The complexes in different concentration will be mixed with Iron(III) chloride, phosphate buffer (pH 6.6) and O-phenanthroline. The absorbance will be measured at 490 nm. The increase in absorbance will show reducing power of the compounds. Ethylenediaminetetraacetic acid (EDTA) will be used as positive control and mixture without sample drug will be used as blank.The reducing power can be calculated as;

Reducing power (%)

$$= \frac{Absorbance\ \ of\ sample - Absorbance\ \ of\ the\ control}{Absorbance\ \ of\ sample} \times 100$$

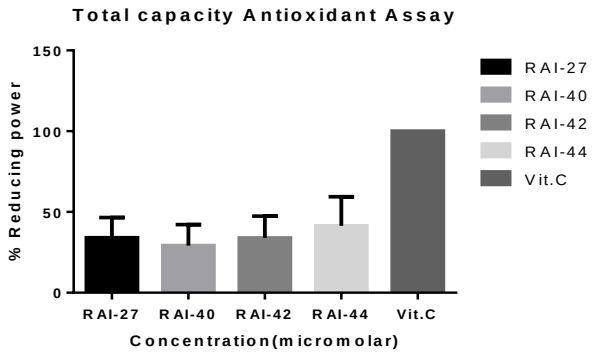


Total Antioxidant Capacity Assay

Total antioxidant capacity of the tested compounds was determined by the reported method (Kumaran&karunakaran 2007).All Compounds in different concentration will be mixed with sodium phosphate, ammonium molybdate and

sulfuric acid in Eppendorf tube. All tubes will be heated in a thermal block at 95°C for 1.5 hrs. After cooling the mixtures to room temperature, the increase in absorbance will be measured at 695 nm using Vitamin C as a standard.The reducing power can be calculated as;

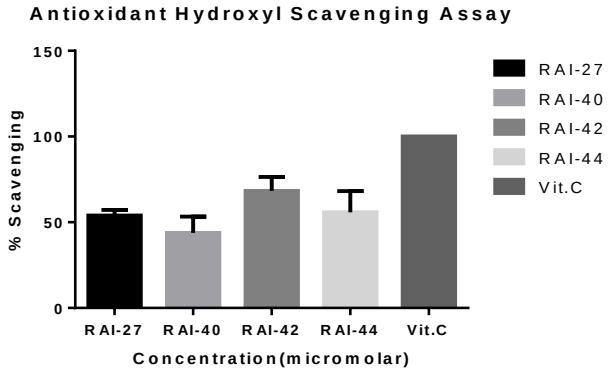
$$\text{Reducing Power (\%)} = \frac{Absorbance\ \ of\ sample - Absorbance\ \ of\ control}{Absorbance\ \ of\ sample} \times 100$$



Hydroxyl radical scavenging activity

The scavenging activity of the Albendazole co2pounds for hydroxyl radicals was measured with fenton reaction as describe by (Huo *et al.*, 2011). Reaction mixture of various concentrations of Oxadiazole derivatives,7.5 mM O-phenanthroline, 0.2 M phosphate buffer (pH 6.6), of 7.5 mM ferrous sulfate and 0.1 ml of H_2O_2 (0.1%) and diluted with distilled water. The reaction mixture will be incubate at room temperature for 30 min and the absorbance will be measure at 510 nm using UV spectrophotometer. The reaction mixture without albendazole complexes will be used as control and without albendazole complexes and H_2O_2 as a blank. The percent scavenging can be calculated as;

$$\text{Scavenging(\%)} = \frac{Absorbance\ \ of\ sample - Absorbance\ \ of\ control}{Absorbance\ \ of\ blank - Absorbance\ \ of\ sample} \times 100$$



Molecular Docking

Target Retrieval…. From pdb database

PDB… 1UK5

Preparation of Ligand

The molecular structures of 1,2,3 and 4 were made by using the Chem-Draw ultra-version 12.0.2.1076 (2010) and then saved in mol format aiming to open these files in MOE and were protonate 3D and energy minimized through MOE using default parameters.

Preparation of Protein

The target protein was 3D protonated and then energy minimization was performed by using the MOE software with default parameters. Protein-ligand docking score, 2D and 3D structures were saved.

Active Site Prediction

The active site was predicted by using site finder option of MOE software. Site Finder option were used to calculate possible active sites in 1UK5 from the 3D atomic coordinates of the receptor. Calculations were used to determine potential sites for ligand binding docking calculations or for calculating restriction sets for rendering partial molecular surfaces [1].

(GLY1 SER2 SER3 GLY4 SER5 SER6 GLY7 ALA8 PRO9 ALA10 GLU11 PRO12 ALA13 LYS25 VAL29 GLU33 ALA34 LEU36 GLU37 GLN40 GLU43 GLN44 ASP47 ASP82 GLN85 ALA86 ARG88 ASP89 GLY90 ARG92 LYS93 THR96 ILE97 GLU99 LYS100 GLN103 LYS104 SER106 GLY107 PRO108 SER109 SER110)

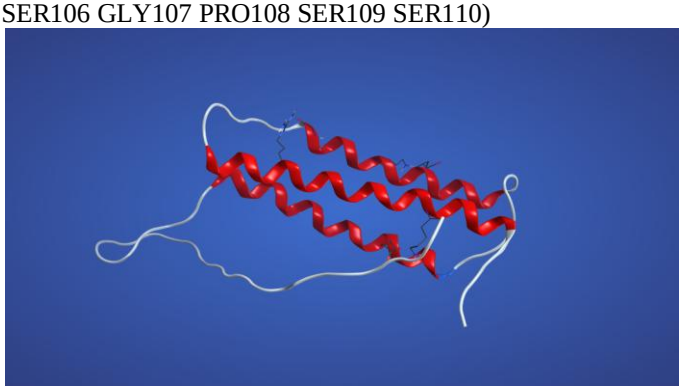


Fig 1UK5 structure

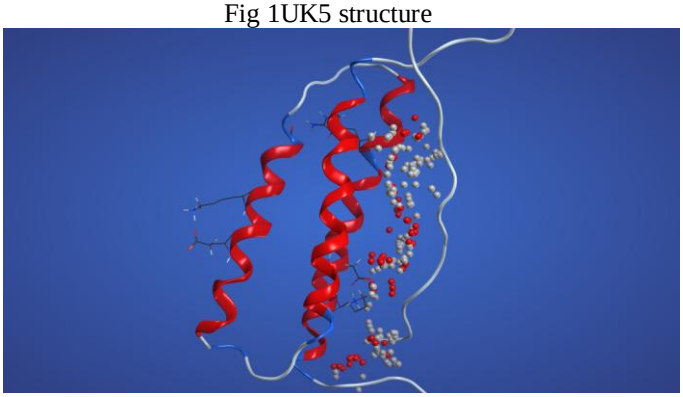
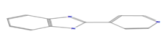
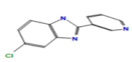
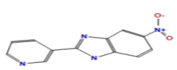


Figure: Active site residues

Molecular Docking

Table: Docking Results

Target (Receptor)	Name (M.wt)	Compound Structure	Docking score
1UK5	195_1		-9.05474186
1UK5	229_2		-9.95534229 (Best docking score)
1UK5	240_3		-9.04665375
1UK5	273_4		-9.32280254

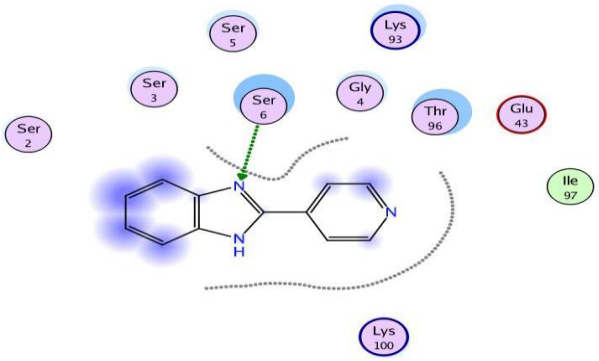
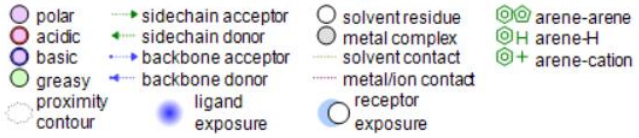


Fig: 2D interaction of comp_1 with 1UK5

Description of the interaction



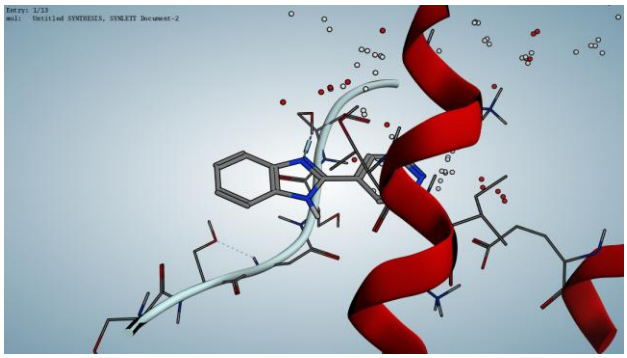


Fig: 3D interaction of comp_1 with 1UK5

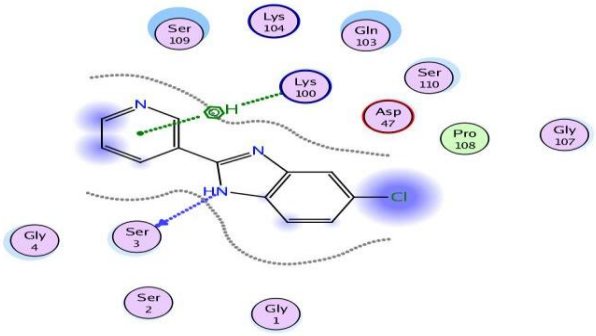


Fig: 2D interaction of comp_2 with 1UK5

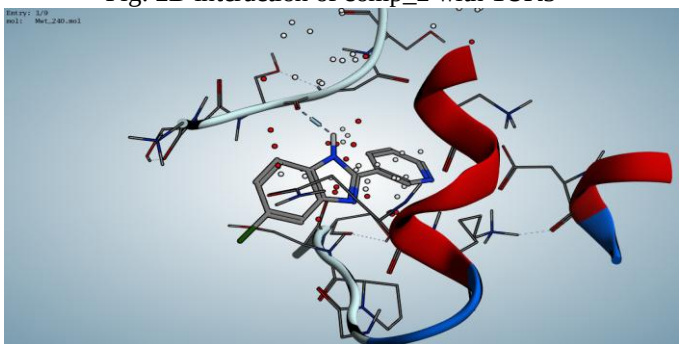


Fig: 3D interaction of comp_2 with 1UK5

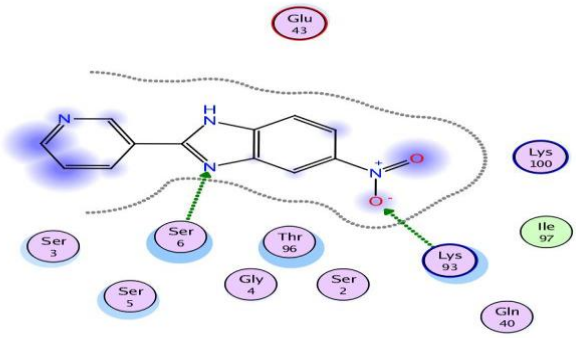


Fig: 2D interaction of comp_3 with 1UK5

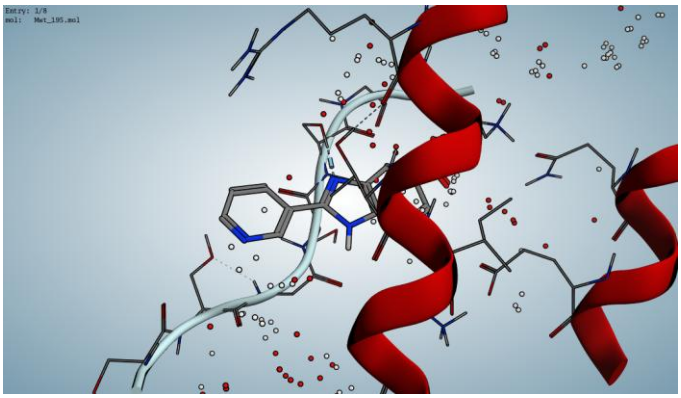


Fig: 3D interaction of comp_3 with 1UK5

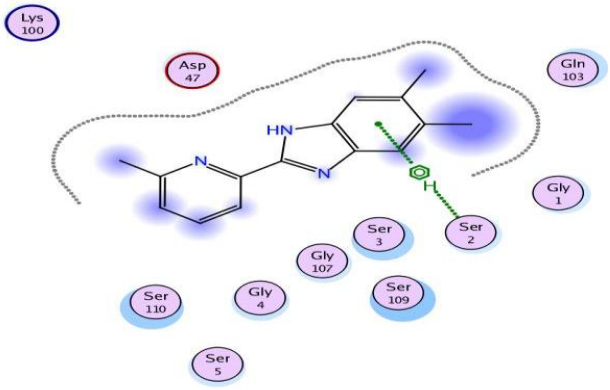


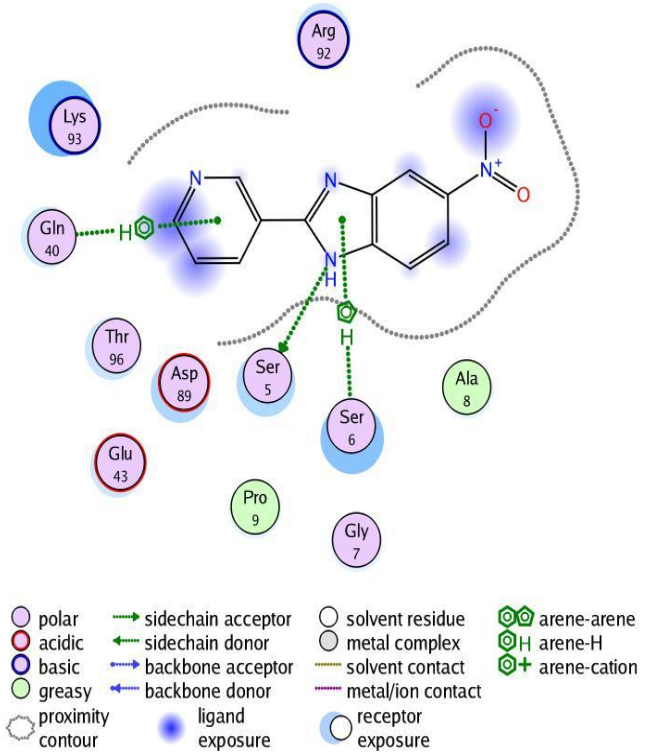
Fig: 2D interaction of comp_4 with 1UK5

Fig: 3D interaction of comp_4 with 1UK5

Result

1	-4.3228731	1.847275
2	-4.6328201	1.847275
3	-4.5015459	0.93984967
4	-4.2746115	2.6612713

Four compounds were docked with receptor protein. Among the docked compounds, compound2 showed good interaction with receptor protein having a docking score of -4.6328201.The interactions of compound2 with receptor protein are shown in Fig…..



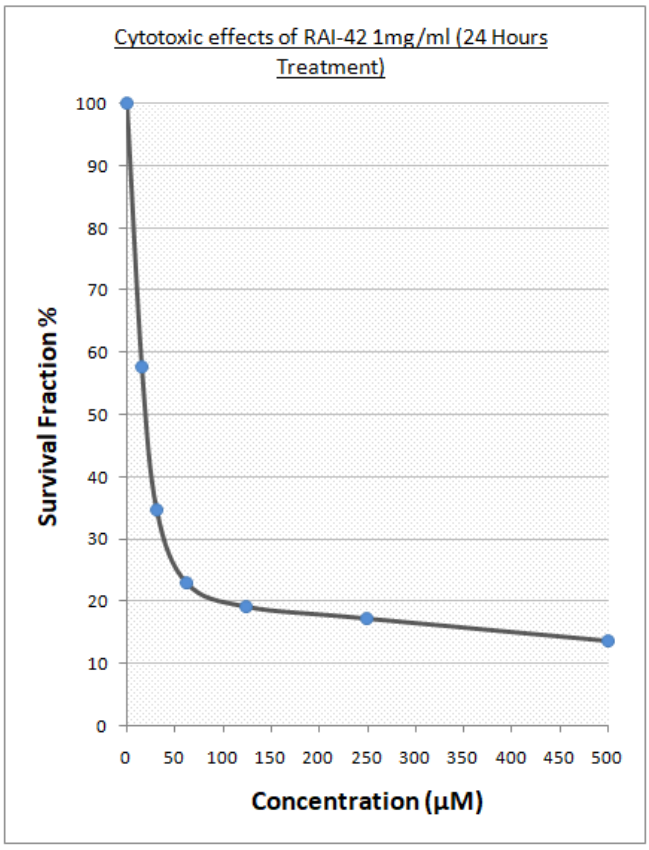
Growth inhibition assays:

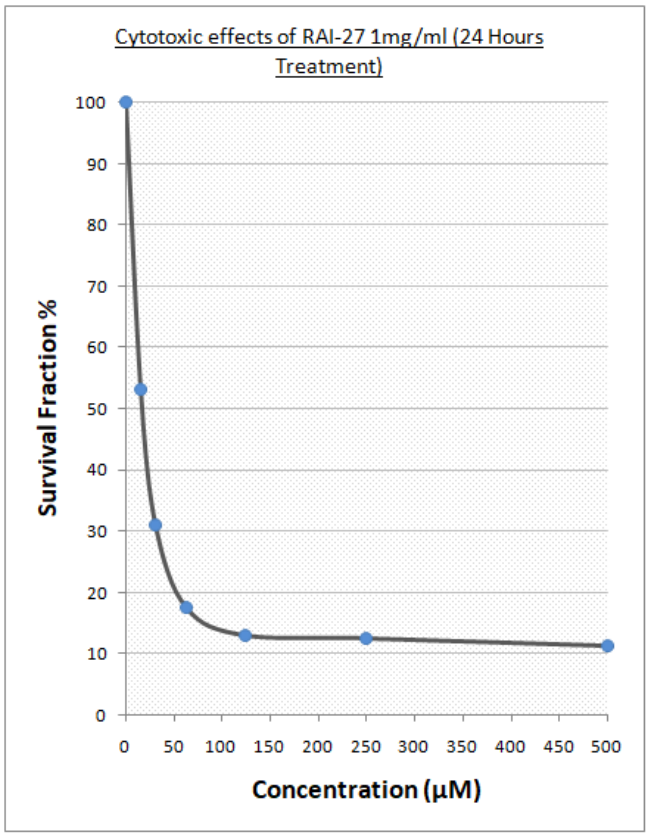
For drug assay/growth inhibition assays, cells were plated in 96 well plates. We plated 5000
cells per well. For this purpose cell counting technique was used. 5000 cells were seeded in
200 microliters of media per well. The plate was placed in CO2 incubator for 24hr. After that
desired concentration of drugs were applied.

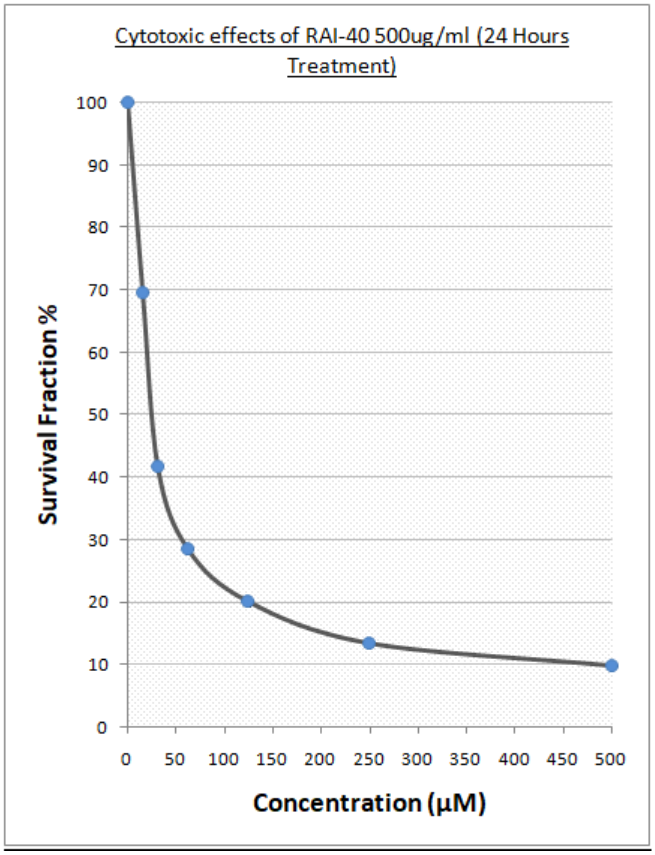
Drug assay

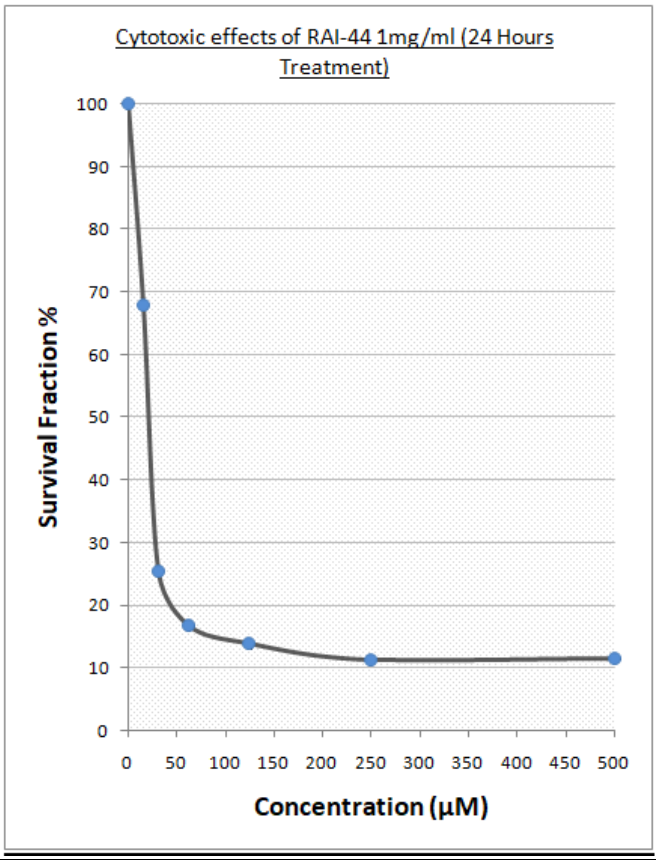
Drug assay was done using 96 well plates. Cells were plated in 96 well plates and after 24hr
of incubation. Albendazole derivatives compounds were applied in triplicate to each well with different concentrations. Compound were used in 500uM highest concentration ,then 250u M to 15.625u M being lowest. After drug application ,plate were left to incubate for 24hr. On next day cell were analyzed under phase contrast ,microscope and representative images were taken. After that cells were washed, fixed withformalin and then stained with crystal violet dye. Absorbance was taken by a 96 well platereader(Bio Tek) at 570Nm

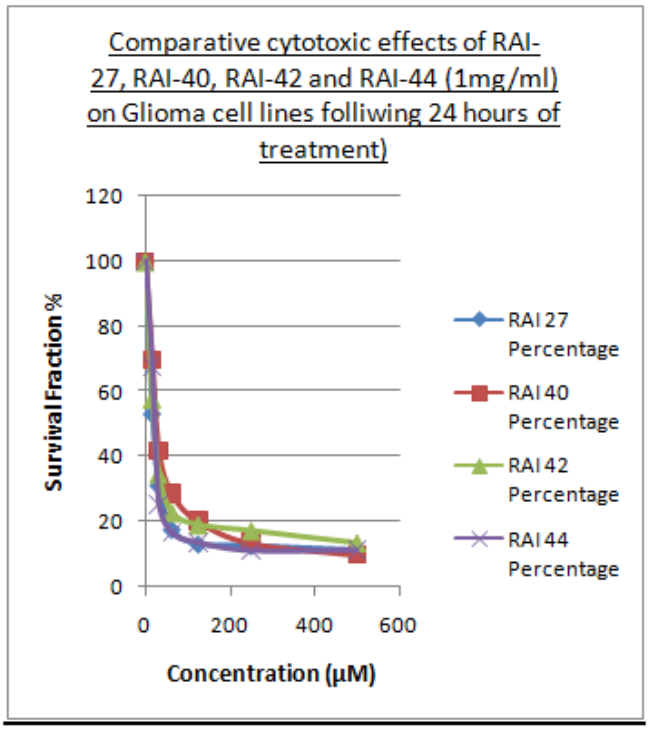
IV. RESULT











400micromolaris the highest dose concentration and lowest dose concentration is 15.65 micromolar .More drug dose leads to less c effect cell via liabilityso at 400 Micromolar there will be more killing.

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