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Original Research Article

Assessment of Carcinogenic/Mutagenic Potential of Different Series of Synthetic Compounds

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Abstract

The new drug research is usually based on synthesis medicine. The use of these medications has created problems such as tolerance in humans, for a long time and due to legitimate use of anti-infection, microbial defense against branded medication is growing. A mutagensis study by Ames in the early 1970's, used worldwide by drug and chemicals companies to diagnose mutagens carcinogenes, making it possible for them to be detected, and to be added to the mutagenic synthesis portion or radiation source triggering irreversible changes, and to the genetic material transmitted from the parent. deoxyribonucleic acid (DNA). The purpose of this study was to assessment of carcinogenicity of synthetic compounds series by hemolytic, Ames and Damaged DNA protection assay. The cytotoxicity was determined with hemolytic assay and DNA Damage protection assay while mutagenicity was resolute by using S. typhimurium TA100 and TA98 strains. It is concluded that the compounds with less hemolytic compounds are good for uses in drugs. Synthetic compounds were determined to be non- mutagenic in nature. Analysis of variance (ANOVA) was applied to compare the hemolysis percent between different concentrations.

Keywords: Biological activity, Biochemical activity, synthetic compound, DNA damage, Ames test.

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INTRODUCTION

The detection of mutations-capable compounds has become an important safety evaluation technique. Mutational chemicals that potentially harm the germ line that contributes to fertility issues and mutations in future generations. Mutagenic chemical products can also cause cancer, and most mutagenicity research projects have motivated this concern (Mortelmans and Zeiger, 2000). An important safety testing tool has become the identification of mutation-capable substances. Mutational chemicals which may damage the germ line and lead in future generations to fertility problems and mutations. Mutagenic chemicals can cause cancer, and this concern has been motivated by most mutagenic projects (Ames *et al.*, 1975).

Mutagenicity means a chemical's ability to cause genetic damage through several mechanisms involving DNA interactions (i.e., adduct formation, basic substitutions, frame-shift deletions, intercalations), DNA and other cellular objectives, for instance protein (i.e.,

Specific Salmonella typhimurium genetically diverse types are used in mutagenic research. TA97, TA98, TA100, TA102, TA1537 are the most commonly used varieties. Opera mutations make his ability to synthesize histidine weaker. These bacteria are reversed to their normal functionality in a deficient histidine medium. The reverse mutation in which bacteria can synthesize histidine is called reversal to normal bacterial activity (Mortelmans and Zeiger 2000).

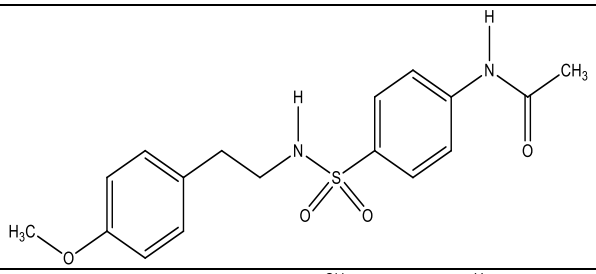
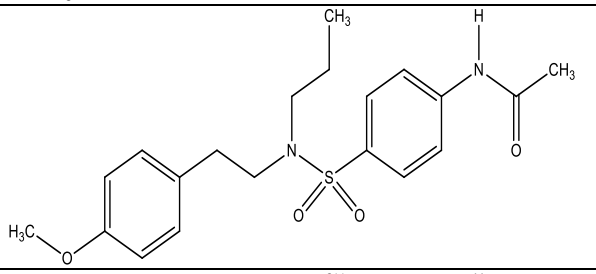
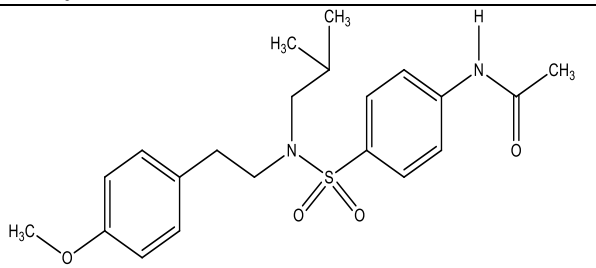
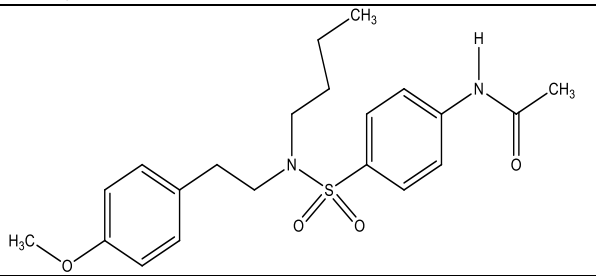
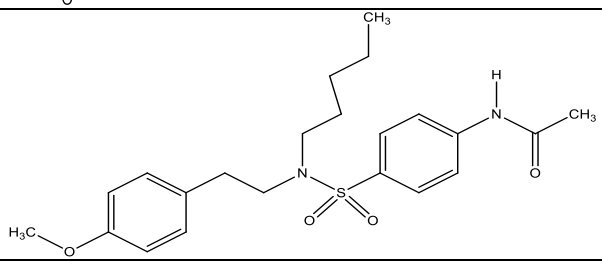
Hemolysis is the disintegration by the loss of a hemoglobin in red blood corpuscles. At about 120 days of exposure, in vivo red cells are normally destroyed. Hemolysis is rare before this. Clinically speaking anemia

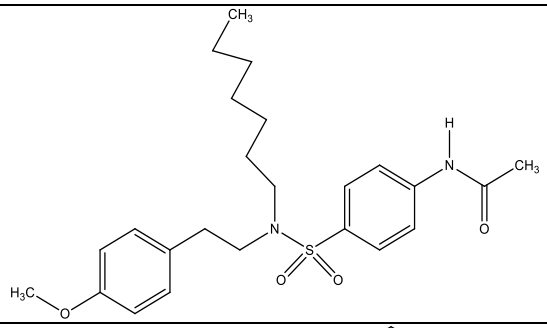
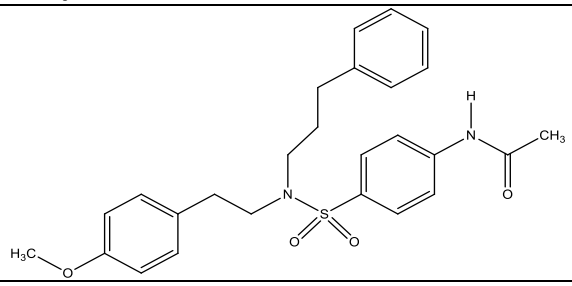
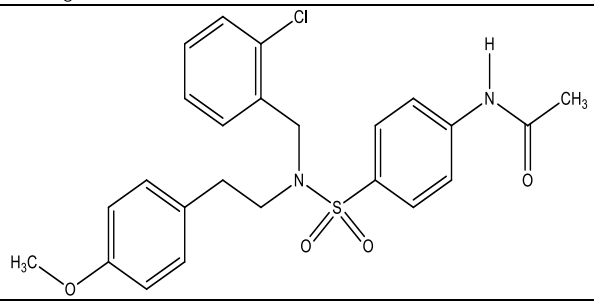
becomes apparent when the amount of red cell death isn't balanced by the resulting production of new cells (Santos-Filho, 2016).

Oxidation, methylation, purification, and defamination are the endogenous responses that can lead to continuing DNA damage. Cytosine methylation in DNA is essential for gene expression regulation, and standard forms of methylation can be enhanced during carcinogenesis. Guanine has been transformed to 8-hydroxyguanine; a specific effect of the ROS occurrence has been shown to modify adjacent cytosine's enzyme-catalyzed methylation to relate oxidative DNA damage to altered patterns of methylisation. The loss and the alteration in mitochondrial DNA that accumulates through the time greater than nuclear DNA could be induced by the oxidative damage triggered by any of the above processes. Harm to in neurodegenerative diseases, mitochondrial DNA may have a role: deletions from mitochondrium and increased stable mitochondrial-oxidative DNA damage have been documented in Alzhondria (Wiseman and Halliwell, 1996).

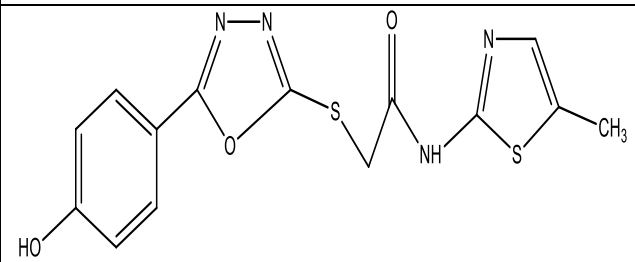
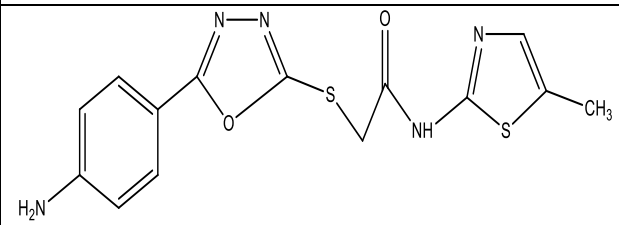
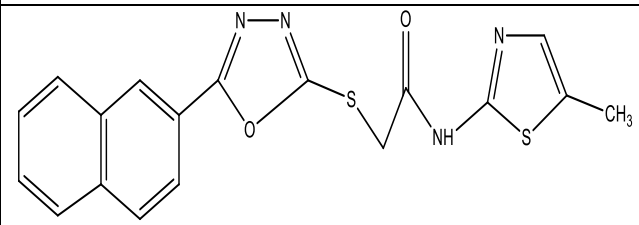
MATERIALS AND METHODS

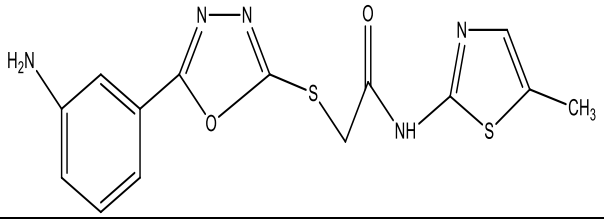
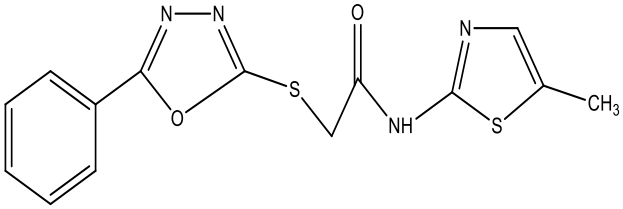
1) AMA SERIES

Code	Yield	Structure	Solvent	Molecular Weight	Molecular Formula
AMA1	70% / 80%ºC		DMSO	376.5	$C_{19}H_{24}N_2O_4S$
AMA2	79% / 101%ºC		DMSO	390.51	$C_{20}H_{26}N_2O_4S$
AMA3	86% / 146.2%ºC		DMSO	390.51	$C_{20}H_{26}N_2O_4S$
AMA4	63% / 72%ºC		DMSO	404.6	$C_{21}H_{28}N_2O_4S$
AMA5	81.5% / 69%ºC		DMSO	419.1	$C_{21}H_{30}N_2O_4S$

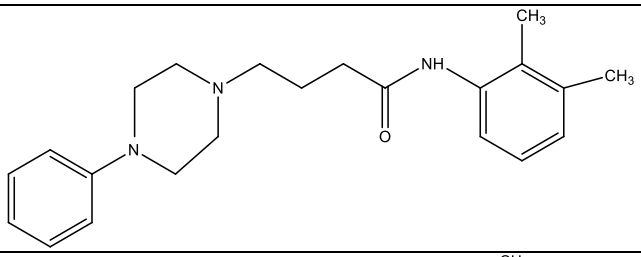
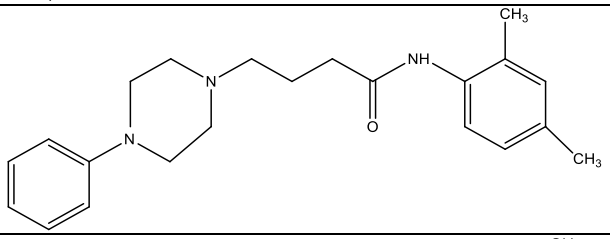
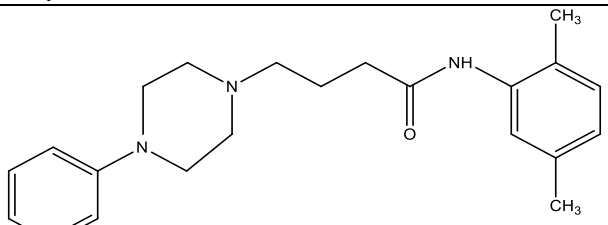
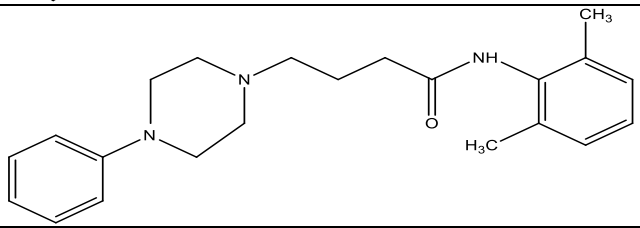
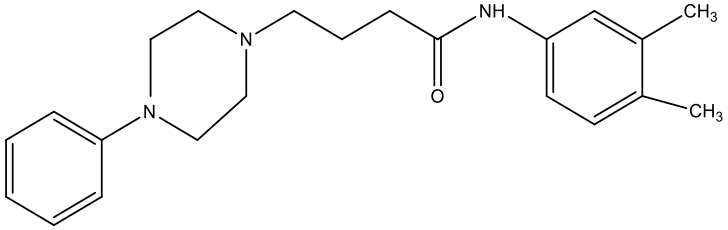
Code	Yield	Structure	Solvent	Molecular Weight	Molecular Formula
AMA6	66% / 84%^{O}C		DMSO	447.10	$C_{24}H_{34}N_2O_4S$
AMA7	60% / 84%^{O}C		DMSO	439.2	$C_{24}H_{26}N_2O_4S$
AMA8	73% / 80%^{O}C		DMSO	473.2	$C_{24}H_{25}ClN_2O_4$

5MB Series

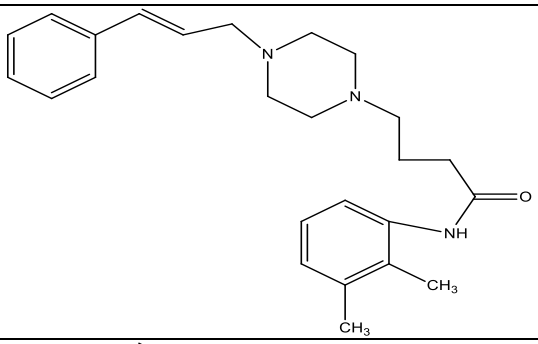
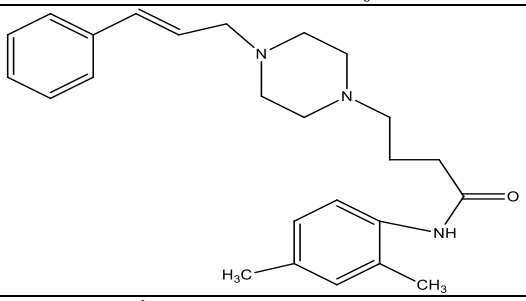
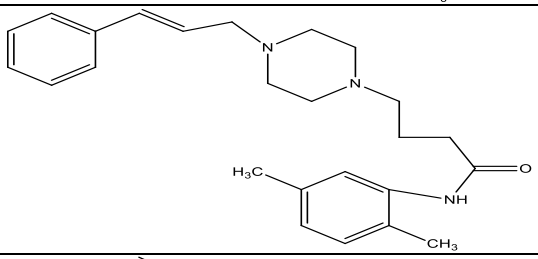
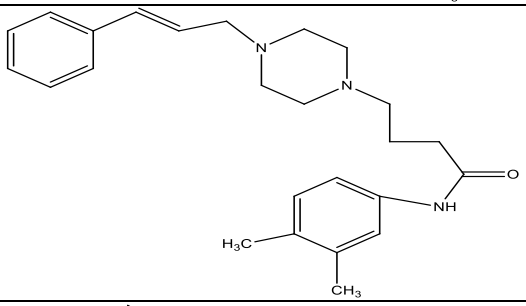
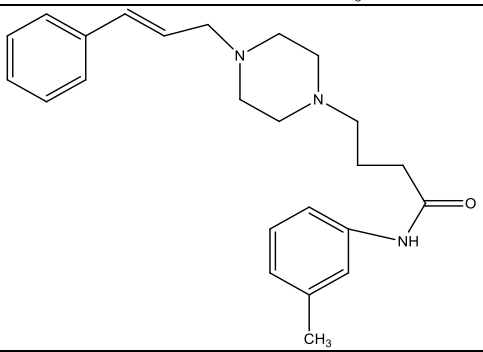
Code	Structure	Solvent	Molecular Weight	Molecular Formula
5MB1		Choroform/ DMSO	339.10 g/mol	$C_{14}H_{12}N_4O_3S_2$
5MB2		Choroform/ DMSO	348.2 g/mol	$C_{19}H_{16}N_4O_2S_2$
5MB3		Choroform/ DMSO	396.2 g/mol	$C_{19}H_{16}N_4O_2S_2$

Code	Structure	Solvent	Molecular Weight	Molecular Formula
5MB4		Choroform/ DMSO	348.1 g/mol	$C_{14}H_{13}N_5O_2S_2$
5MB5		Choroform/ DMSO	333.4 g/mol	$C_{14}H_{12}N_5O_2S_2$

SMI Series

Code/ Mp/ Yield	STRUCTURE	Coloure/ Molecular weight	Solvent	Molecular Formula
SMI 1 81%		Reddish or light redsticky 352.38	Chloroform/ DMSO	$C_{22}H_{29}N_3O$
SMI 2 104°C 84%		OFF White 352.56	Chloroform/ DMSO	$C_{22}H_{29}N_3O$
SMI 3 66% 113°C		Light purple 352.37	Chloroform/ DMSO	$C_{22}H_{29}N_3O$
SMI 4 71% 274°C		Dark brown 352.56	Chloroform/ DMSO	$C_{22}H_{29}N_3O$
SMI 5 73% 75°C		Light yellow 352.7	Chloroform/ DMSO	$C_{22}H_{29}N_3O$

SAC Series

Code	Yield/ M.p.	Structure	Solvent	Molecular Formula	Molecular Weight
SAC1	73%/105^{O}C		DMSO	$C_{25}H_{33}N_3O$	392.3
SAC2	86%/122^{O}C		DMSO	$C_{25}H_{33}N_3O$	392.3
SAC3	75%/111^{O}C			$C_{25}H_{33}N_3O$	392.3
SAC4	71%/128^{O}C		DMSO	$C_{25}H_{33}N_3O$	392.3
SAC5	Semi solid		DMSO	$C_{25}H_{33}N_3O$	392.3

Preparation of Bacterial Culture

Nutrient broth 1.3 g was dissolved into 100ml of distilled water and well mixed for homogenous mixture. Made volumes up to 1000ml. Autoclaving was used to clean growth media in ordrer to free it from all sorts of contaminants and even spores. The loop was applied to the whole bacterial culture and shakered 24 hours at 37oC.

3.7 Determination of cytotoxic potential

Hemolytic assay were performed to check the cytotoxicity of different series of synthetic copmpounds.

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The assay was performed in Bioassay Section. Medicinal Biochemistry Research Laboratory Department of Biochemistry of University of Agriculture Faisalabad.

3.7.1 Hemolytic Activity

Powell *et al*., method was used to determine hemolytic activity of various synthetic compounds According to this process, centrifuged 3 mL of heparinized blood in 15mL falcon tube at standard conditions of rotation and time (850 rpm for 30seconds).Plasma layer was appeared from rotation is discarded.5 mL phosphate buffer (pH 7.4) saline (PBS) was added to blood and again centrifuged at standard conditions of rotation and time (850 rpm for 300 seconds).Adjusted pH of mixture in falcon tube ~ 7.4 and stayed 30 min at 25oC.Erythrocytes concentration 108 cells/ mL should be maintained and calculated by Hemacytometer.100 µL each sample (derivative) was taken in appendorf tubes separately mixed with erythrocytes. Triton X-100 were taken as positive and phosphate buffer saline were used for negative control. Take about 100 µl of supernatant from each above appendorf tube and create up to 10 mL with a chilled (4oC) PBS. Take up to 1310 rpm for 5 minutes and then coole in an ice bath at 37oC for 35 mints. Absorption of all these dilutions at 576 nm. Blood lysis was performed by %

% Hemolysis = sample absorbance- blank absorbance/positive control absorbance×100

3.8 Anti-Mutagenic Assay

Two bacterial strains were tested with the mutagenic and non-mutagenic ability of the specified variety of synthetic compounds, i.e. The TA-98 and TA-100.Ames's research was performed by Maron and Ames process (Alabi *et al.*, 2014).

Study of DNA Damage

Protective effect of extracts against DNA damage was studied by agarose gel electrophoresis (Kumar *et al.*, 2013).

Preparation of Gel

1% agarose gel was prepared by mixing 1 gram of agarose in 100 ml of 1X TAE buffer. The solution was microwaved for 30 seconds until it became clear. It was left to cool down until the temperature was bearable. It was then poured in gel casting tray with combs already affixed. It was allowed to solidify.

Preparing DNA solution

DNA solution was prepared by dissolving 2 mg of calf thymus DNA in 4 ml of potassium phosphate buffer. It resulted in 500 micro grams per mL solution of DNA. 10 microliters of DNA, 10 microliters of dye and 10 microliters of 30% H_2O_2 and 10 microliter of sample was added in Eppendorf tube. PBS instead of sample was added as positive control. A negative control in which only DNA, dye and H_2O_2 were added was also run. It was incubated for 20 minutes and then loaded in gel.

Loading of Samples

The combs were removed from gel. 1X TAE buffer was added up to maximum fill mark of electrophoresis tank. 10-15 microliter of the above-mentioned mixture was poured into each well carefully using a micropipette. After sample loading the electrophoresis tank was covered and connected with electrical supply. It was kept connected for almost 45 min until the DNA has travelled ¾ of the gel casting tray.

After removing electrical supply, the gel was gently slid from the tray and placed in the container containing ethidium bromide solution. It was kept in it for almost 20 minutes. Gel was then removed from it and placed on UV-illuminator to visualize the DNA. The DNA appears orange in colour when viewed in UV light.

RESULTS AND DISCUSSION

In the present study following series of synthetic compounds had been evaluated

1. Acetamide derivatives
2. Prodigine derivatives
3. Piperadine derivatives
4. Carboxmide derivaties
5. Pyrimidine Acetamide Derivatives

The main structural elements in medical chemistry have been found to be heterocycles, and in biomolecules, such as antifungal, anti-inflammatory, antibacterial, antioxidants, anti-convulsants, anti-allergics, inhibitors of the encimic activity, anti-HIV, antidiabetic, anti-cancer activity, insect diseases, and other biomolecules, they are frequently found in large percentages.

The heterocyclic ring in the main skeleton includes biological molecules such as DNA and RNA, chlorophyll, hemoglobin, vitamins and more. There are numerous heterocyclic components used as an antimicrobial herbicide, urinary antiseptic products and counterinflammatory agents for many common diseases, for example triazine derivatives. A wide range of biological activities including antibacterial, antifungal, antiviral and anthelmintic have been reported in Benzimidazole derivatives.

Layout of the results and discussion

- DNA damage protection assay
- In vitro cytotoxicity (hemolytic assay)
- Mutagenic assay/ Ames test

DNA damage protection assay
AMA Series

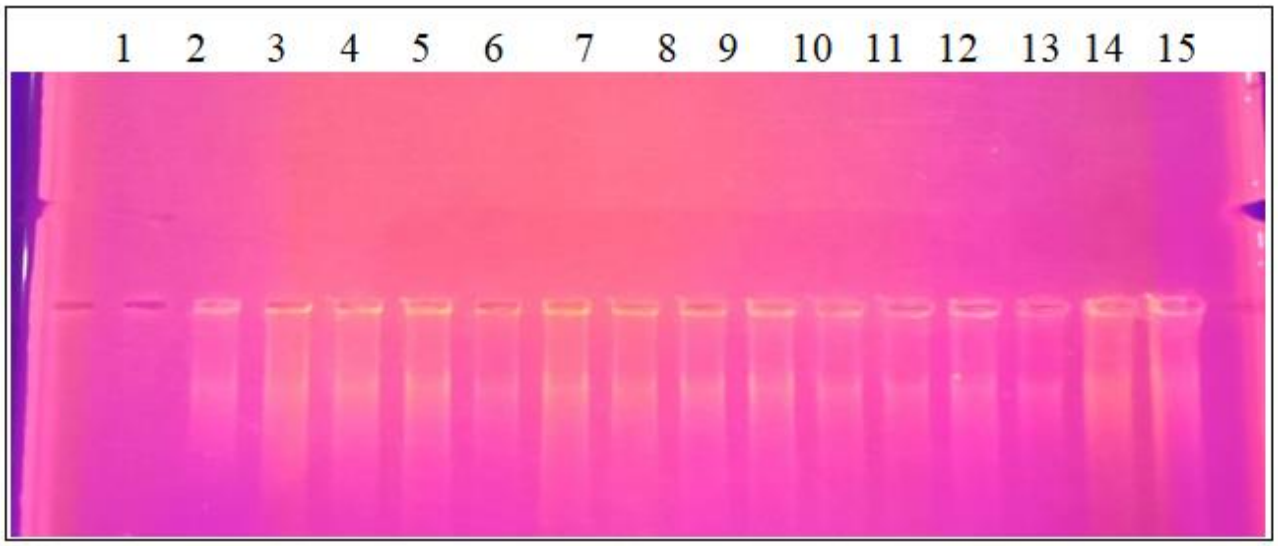

Fig 4.1: DNA damage protection effect of AMA series with H_2O_2

Lane 1= 10µl DNA with bromophenol blue dye: Lane 2 = DNA with H_2O_2 Lane 3= DNA treated with H_2O_2, Lane 4 = DNA; treated with H_2O_2 + ; Lane 5 = DNA treated with AMA1+ H_2O_2; Lane 6 = DNA treated with AMA 2+ H_2O_2; Lane 7 = DNA treated with AMA 3+H_2O_2; Lane 8 = DNA treated AMA4 fraction + H_2O_2; Lane 9 = DNA treated with AMA5 fraction + H_2O_2; Lane 10 = DNA treated with AMA6 + H_2O_2,Lane 11 = DNA treated with AMA7 fraction + H_2O_2; Lane 12= DNA treated with AMA8 + H2O2 +UV; Lane 13= DNA treated with AMA9 +H_2O_2 +UV; Lane 14= DNA treated with AMA10 +H_2O_2 +UV; Lane 15= DNA treated with AMA11 +H_2O_2 +UV

SAC Series

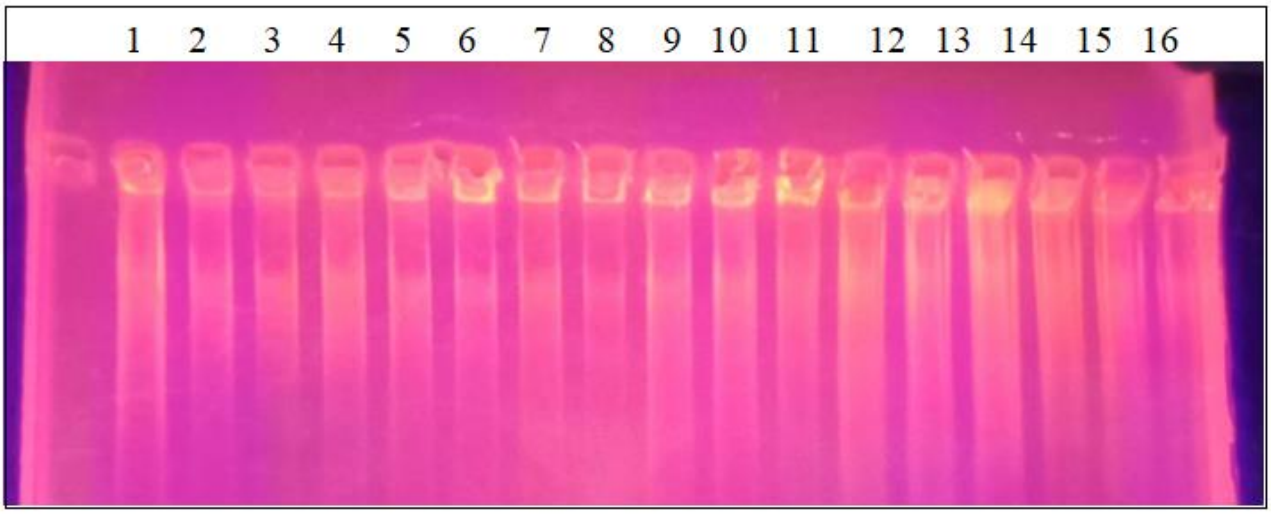

Fig 4.2: DNA damge protection effect of SAC series with H_2O_2

Lane 1= 10µl DNA with bromophenol blue dye: Lane 2 = DNA with H_2O_2 Lane 3= DNA treated with H_2O_2, Lane 4 = DNA; treated with H_2O_2 + ; Lane 5 = DNA treated with SAC1+ H_2O_2; Lane 6 = DNA treated with SAC 2+ H_2O_2; Lane 7 = DNA treated with SAC 3+H_2O_2; Lane 8 = DNA treated SAC 4 fraction + H_2O_2; Lane 9 = DNA treated with SAC 5 fraction + H_2O_2; Lane 10 = DNA treated with SR 6 + H_2O_2,Lane 11 = DNA treated with SAC 7 fraction + H_2O_2; Lane 12= DNA treated with SAC 8 + H_2O_2 +UV; Lane 13= DNA treated with SAC 9 +H_2O_2 +UV; Lane 13= DNA treated with SAC 10 +H_2O_2 +UV; Lane 13= DNA treated with SAC 11 +H_2O_2 +UV

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SR Series

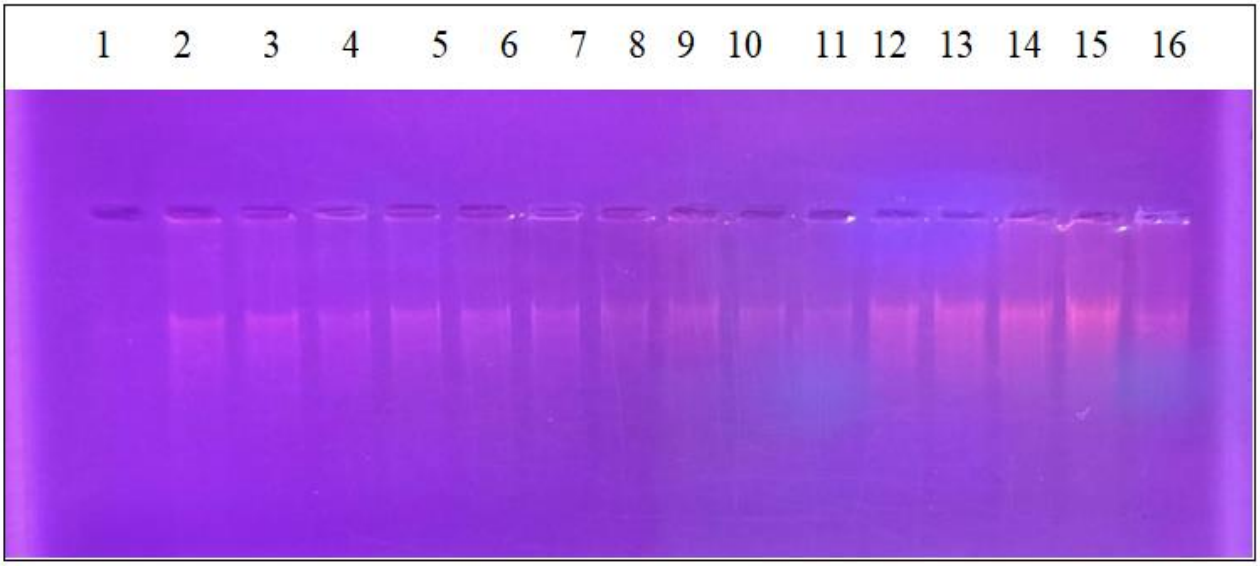

Fig 4.3: DNA damge protection effect of SR series with H_2O_2

Lane 1= 10µl DNA with bromophenol blue dye: Lane 2 = DNA with H_2O_2 Lane 3= DNA treated with H_2O_2, Lane 4 = DNA; treated with H_2O_2 + ; Lane 5 = DNA treated with SR1+ H2O2; Lane 6 = DNA treated with SR 2+ H_2O_2; Lane 7 = DNA treated with SR 3+H_2O_2; Lane 8 = DNA treated SR 4 fraction + H_2O_2; Lane 9 = DNA treated with SR 5 fraction + H_2O_2; Lane 10 = DNA treated with SR 6 + H_2O_2,Lane 11 = DNA treated with SR 7 fraction + H_2O_2; Lane 12= DNA treated with SR 8 + H_2O_2 +UV; Lane 13= DNA treated with SR 9 +H_2O_2 +UV; Lane 13= DNA treated with SR 10 +H_2O_2 +UV; Lane 13= DNA treated with SR 11 +H_2O_2 +UV

5MB Series

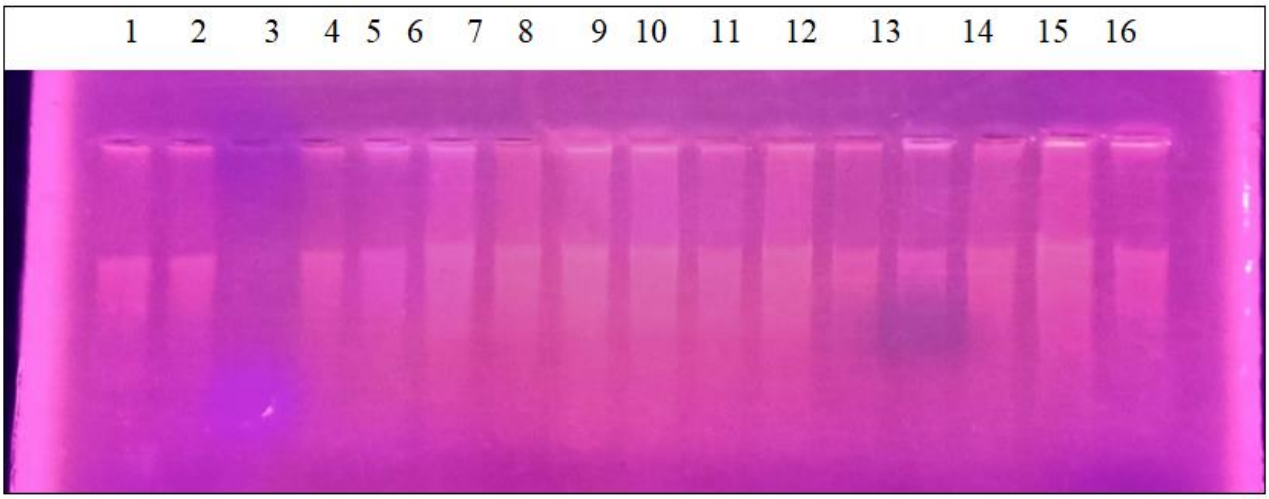

Fig 4.4: DNA damge protection effect of 5MB series with H2O2

Lane 1= 10µl DNA with bromophenol blue dye: Lane 2 = DNA with H_2O_2 Lane 3= DNA treated with H_2O_2, Lane 4 = DNA; treated with H_2O_2 + ; Lane 5 = DNA treated with 5MB 1+ H_2O_2; Lane 6 = DNA treated with 5MB 2+ H_2O_2; Lane 7 = DNA treated with 5MB 3+H_2O_2; Lane 8 = DNA treated 5MB 4 fraction + H_2O_2; Lane 9 = DNA treated with 5MB 5 fraction + H_2O_2; Lane 10 = DNA treated with 5MB 6 + H2O2,Lane 11 = DNA treated with 5MB 7 fraction + H_2O_2; Lane 12= Plasmid pBR322 DNA treated with 5MB 8 + H_2O_2 +UV; Lane 13= DNA treated with 5MB 9 +H_2O_2 +UV; Lane 13= DNA treated with 5MB 10 +H_2O_2 +UV; Lane 13= DNA treated with 5MB 11 +H_2O_2 +UV

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SMI Series

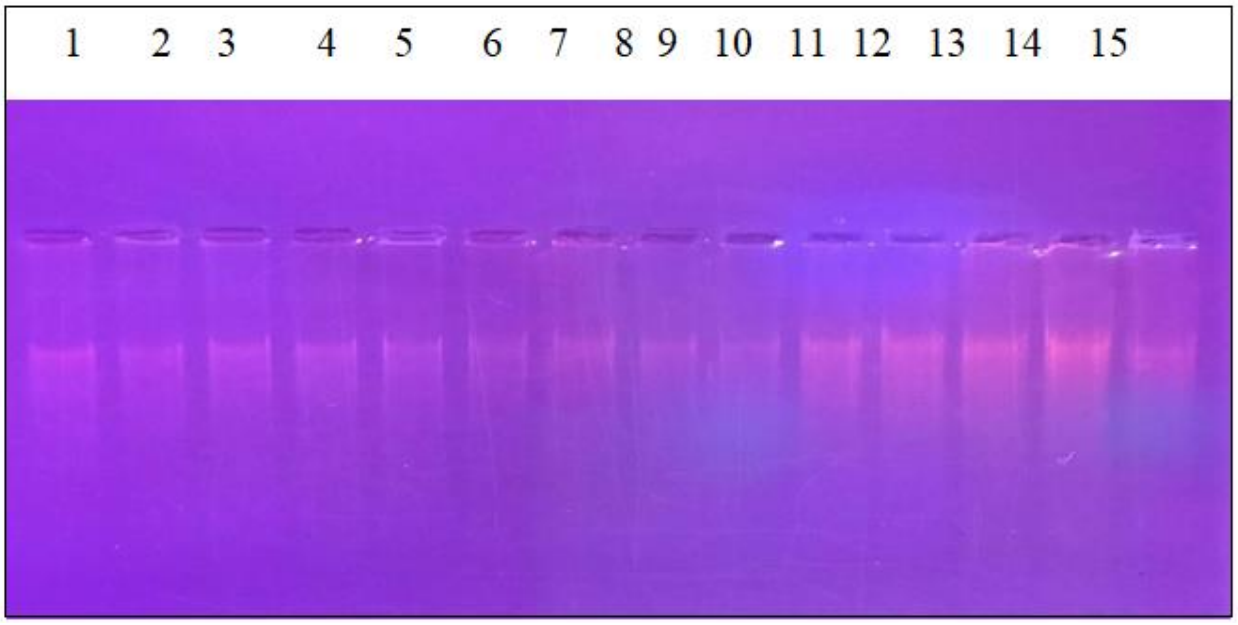

Fig 4.5: DNA damge protection effect of SMI series with H2O2

Lane 1= 10µl DNA with bromophenol blue dye: Lane 2 = DNA with H_2O_2 Lane 3= DNA treated with H_2O_2, Lane 4 = DNA; treated with H_2O_2 + ; Lane 5 = DNA treated with SMI 1 + H_2O_2; Lane 6 = DNA treated with SMI 2+ H_2O_2; Lane 7 = DNA treated with SMI 3+H_2O_2; Lane 8 = DNA treated SMI 4 fraction + H_2O2; Lane 9 = DNA treated with SMI 5 fraction + H_2O_2; Lane 10 = DNA treated with SMI 6 + H_2O_2, Lane 11 = DNA treated with SMI 7 fraction + H_2O_2; Lane 12= DNA treated with + H_2O_2

Relative to other pathways, alone DNA treated with H_2O_2, and UV showed a harm to the DNA and a band protecting impact nearly equal to the pure plasmid DNAs of the extract in conjunction with the H_2O_2 and UV. There was no protective effect on the 5 MB 3 portion. The band of DNA was distinctly like the weakened 5 MB fraction of plasmid DNA. The safety effect on DNA triggered by H_2O_2 and UV radiation was, however, demonstrated to all other fractions and total methanol extracts. Nonetheless, total organic substances which could provide more quantities of bioactive compounds such as antioxidants have shown the most effective protection against DNA.

It has been shown that in the mineral oxidation products that destroy DNA are scavenged by bioactive compounds. The active components are also an inhibition of the H_2O_2 action on plasmid DNA bobbles, and so the extracts demonstrated protection against both H_2O_2 and UV plasmid DNA damage. DNA damage is caused by H_2O_2 and Ultraviolet. Therefore, it is likely to protect the genetic damage as a result of herbal extract therapy. A higher concentration of phenolic, flavomoid and other bioactive compounds that itch free radicals and oxidation products could be the protective effect offered by total methanol extract.

So, sufficient cheap and renewable formulations must be produced that can avoid photo-induced and other oxidation reactions with biological systems.

To this end, the exploration of compounds may be useful as synthetic compounds have a variety of antioxidants and related compounds, and these will be modified for stress over time. In this study, 5 series synthetic coomounds have been used to assess the effect on H_2O_2-induced DNA damage. DNA harm could be preserved from the operation of H_2O_2 by synthetic compounds. Thus, a promising solution for DNA defense seems to be the organic compounds. In fact, there is an improved DNA safety capacity used for medicinal and cosmetical uses in the future for the nature of the lead molecules from these compounds.

4.2 Hemolytic activity

This study included a set of planned I vitro and in vivo toxicological tests that examined the toxic potential of selected plants. In the first part of the in-vitro research, the erythrocyte has produced elevated polyunsaturated fatty acid, molecular oxygen and iron ions thought to be highly vulnerable to oxygen radical development. RBCs (RBCs) have been used in the in vitro analysis. Erythrocytes are highly oxidation susceptible; therefore, erythrocytes are a suitable cell model for the investigation of biomembrane oxidative damage. Cellular oxidant damage that can result from H_2O_2 can lead to erythrocyte hemolysis (Kumar and Xagoraraki, 2010).

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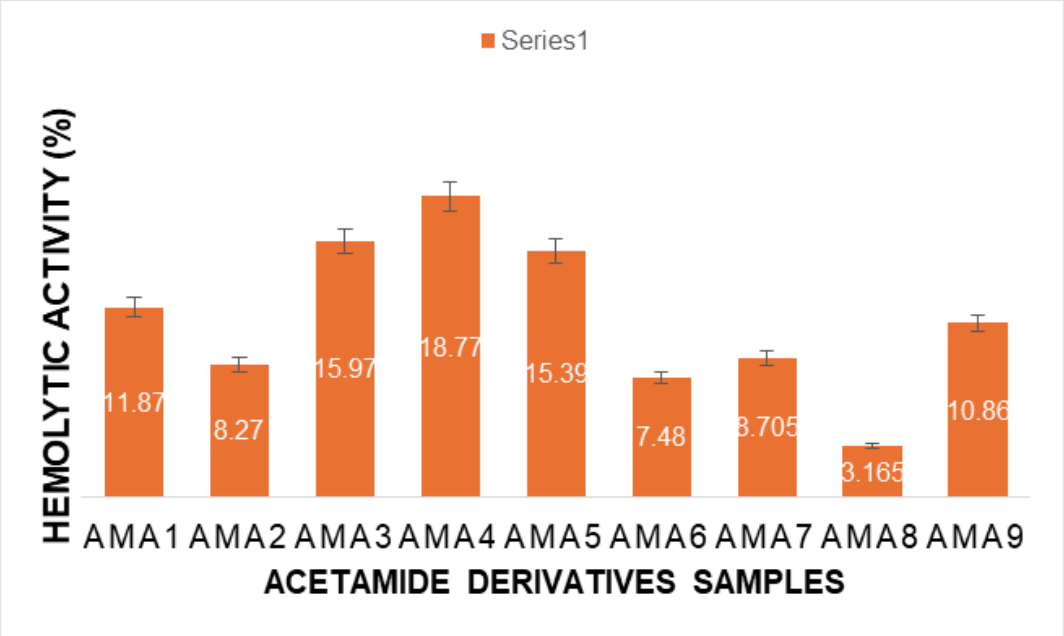
Percentage Hemolysis= (Asample-Anegative control)/Apositive control×100

Hemolytic Activity of Acetamide Derivatives Compounds

Samples	Absorbance	Negative control	Positive control	Final cytotoxicity
AMA 1	0.0845	0.068	0.139	11.87
AMA2	0.0795	0.068	0.139	8.27
AMA3	0.0941	0.068	0.139	15.92
AMA4	0.0894	0.068	0.139	7.43
AMA5	0.0784	0.068	0.139	8.705
AMA6	0.0801	0.068	0.139	3.165
AMA7	0.0724	0.068	0.139	10.86
AMA8	0.091	0.068	0.139	16.54
AMA9	0.0793	0.068	0.139	5.97



4.2-a Graphical representation of the percentage hemolytic activity of Acetamide Derivatives (AMA Series)

Cytotoxicity of AMA derivatives was evaluated by (Powell *et al.*, 2012). In which compounds was analyzed by hemolytic assay and their results are documented in the form of graph. In the result AMA 4 show the highest activity among all the other compounds of the series. But in case of other compounds, it nontoxic toward red blood cell membrane. Minimum activity show by AMA8. The whole mechanism was undiscovered. It is concluded that methyl group on ortho para position of benzene ring greatly affect the cytotoxicity.

Hemolytic Activity of Poridigine Derivatives Compounds

Sample	Absorbance	Negative control	Positive control	Final cytotoxicity
SAC1	0.0793	0.069	0.138	5.97
SAC2	0.0854	0.069	0.138	12.51
SAC3	0.0841	0.069	0.138	10.86
SAC4	0.0952	0.069	0.138	19.56
SAC5	0.092	0.069	0.138	15.82
SAC6	0.0789	0.069	0.138	7.12

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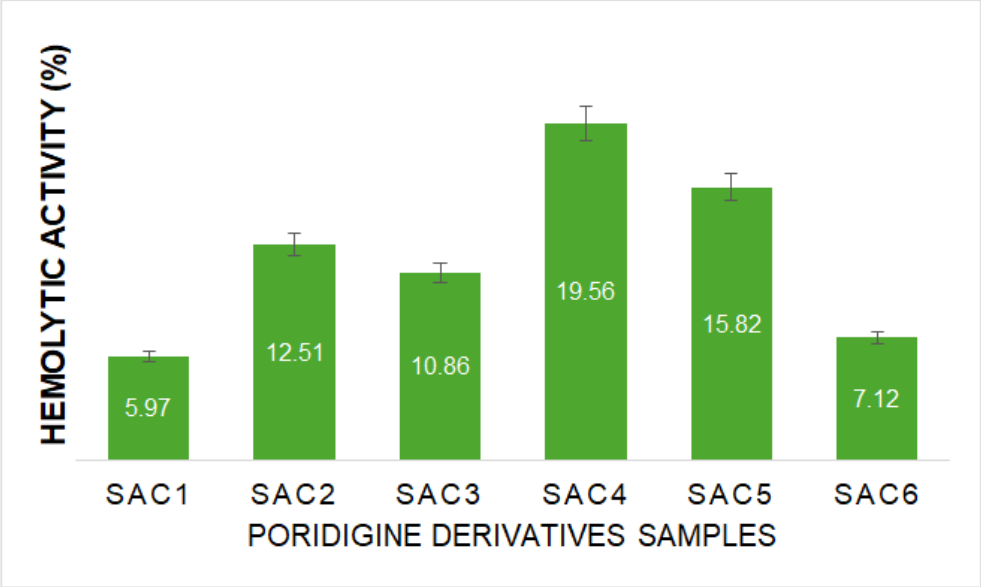
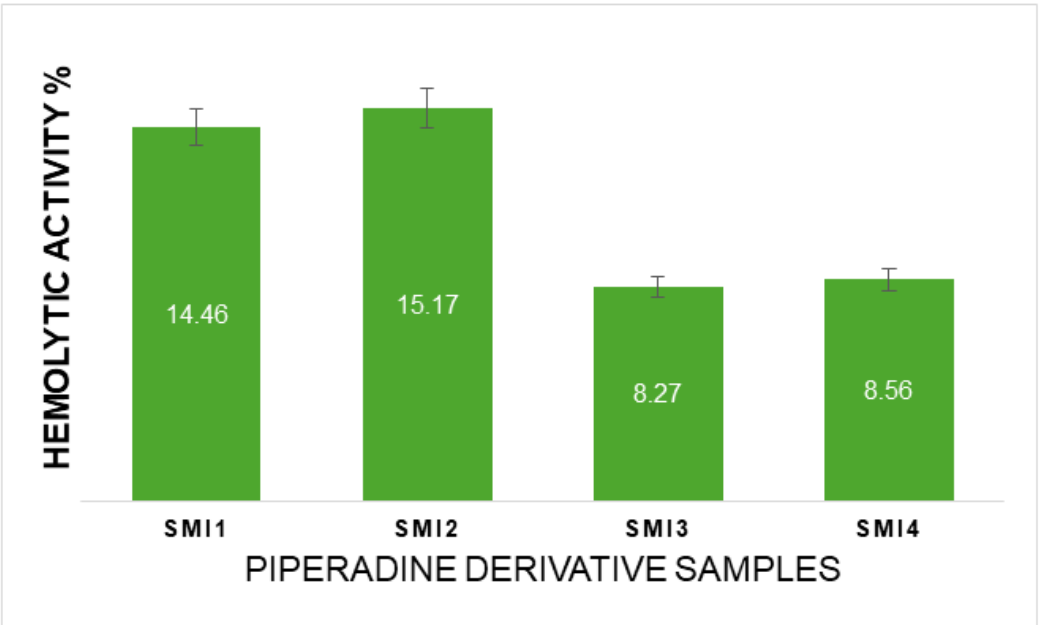


4.2-b Graphical representation of the percentage hemolytic activity of Pordigine Derivatives (SAC Series)

The compounds of SAC series were analyzed by hemolytic activity and results are in the form of graph. In the research of that, SAC4 show the highest activity among all other compounds. Through results we concluded that which compounds are less toxic and used in drugs.

Hemolytic Activity of Piperadine Derivative Compounds

Samples	Absorbance	Negative control	Positive control	Final cytotoxicity
SMI1	0.0891	0.069	0.139	14.46
SMI2	0.0854	0.069	0.139	15.17
SMI3	0.0795	0.069	0.139	8.27
SMI4	0.0799	0.069	0.139	8.56

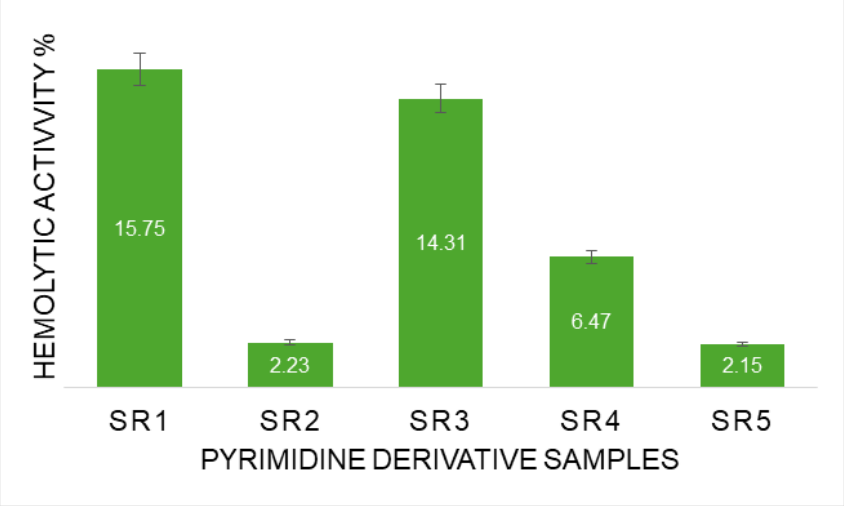


4.2-c Graphical representation of the percentage hemolytic activity of Piperadine Derivatives (SMI Series)

In the hemolytic activity of SMI derivatives, SMI2 show the highest hemolysis of red blood cells and not good for use in drugs.

Hemolytic Activity of Pyrimidine Acetamide Derivative Compounds

Sample	Absorbance	Negative control	Positive control	Final cytotoxicity
SR1	0.0899	0.068	0.139	15.75
SR2	0.0721	0.068	0.139	2.23
SR3	0.0879	0.068	0.139	14.31
SR4	0.076	0.068	0.139	6.47
SR5	0.071	0.068	0.139	2.15

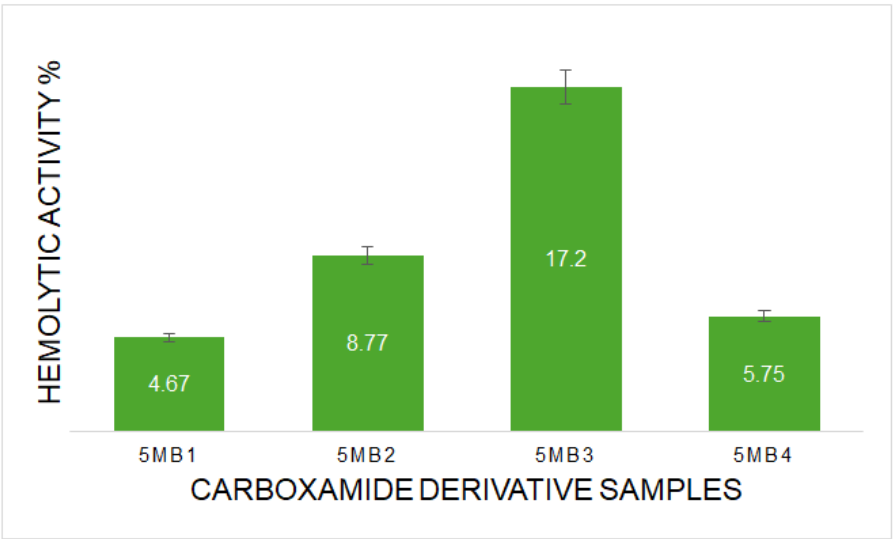


4.2-d Graphical representation of the percentage hemolytic activity of Pyrimidine Acetamide Derivatives (SR Series)

In pyrimidine Acetamide derivatives, the SR1 show the highest activity and SR2 and SR5 show the hemolysis of red blood cell and good for used in grugs.

Hemolytic Activity of Carboxamide Derivative Compounds

Samples	Absorbance	Negative control	Positive control	Final cytotoxicity
5MB1	0.0745	0.068	0.139	4.67
5MB2	0.0802	0.068	0.139	8.77
5MB3	0.092	0.068	0.139	17.2
5MB4	0.076	0.068	0.139	5.75



4.2-e Graphical representation of the percentage hemolytic activity of Carboxamide Derivatives (5MB Series)

In carboxamide derivatives, the 5MB3 show the highest activity among all the compounds of series.5MB1 show the less hemolysis of red blood cells.

So all the discussion ended that the para and meta position are decreased the cytotoxicity of selected series of compounds. And the compound with less hemolytic activity are good for used in drugs.

Statistical analysis of hemolytic activity of selected series of synthetic compounds

The results of hemolytic activity of synthetic compounds were analysed by one way ANOVA using statistix 8.1.

4.6 Treatments

1	2	3	4	5	5	Total
N	5	5	5	5	5	25
∑X	52.295	65.45	62.21	31.67	51.275	262.9
Mean	10.459	13.09	12.442	6.334	10.255	10.516
$\sum X^2$	596.0581	966.4961	828.9495	275.3357	633.6997	3300.5392
Std.Dev.	3.5037	5.2382	3.7058	4.3226	5.1931	4.7253

RESULTS

Source	df	SS	MS	
Between treatments	139.4772	4	34.8693	F = 0.05
Within treatments	396.4055	20	19.8203	
Total	535.8828	24		

Mutagenic/NON-Mutagenic test

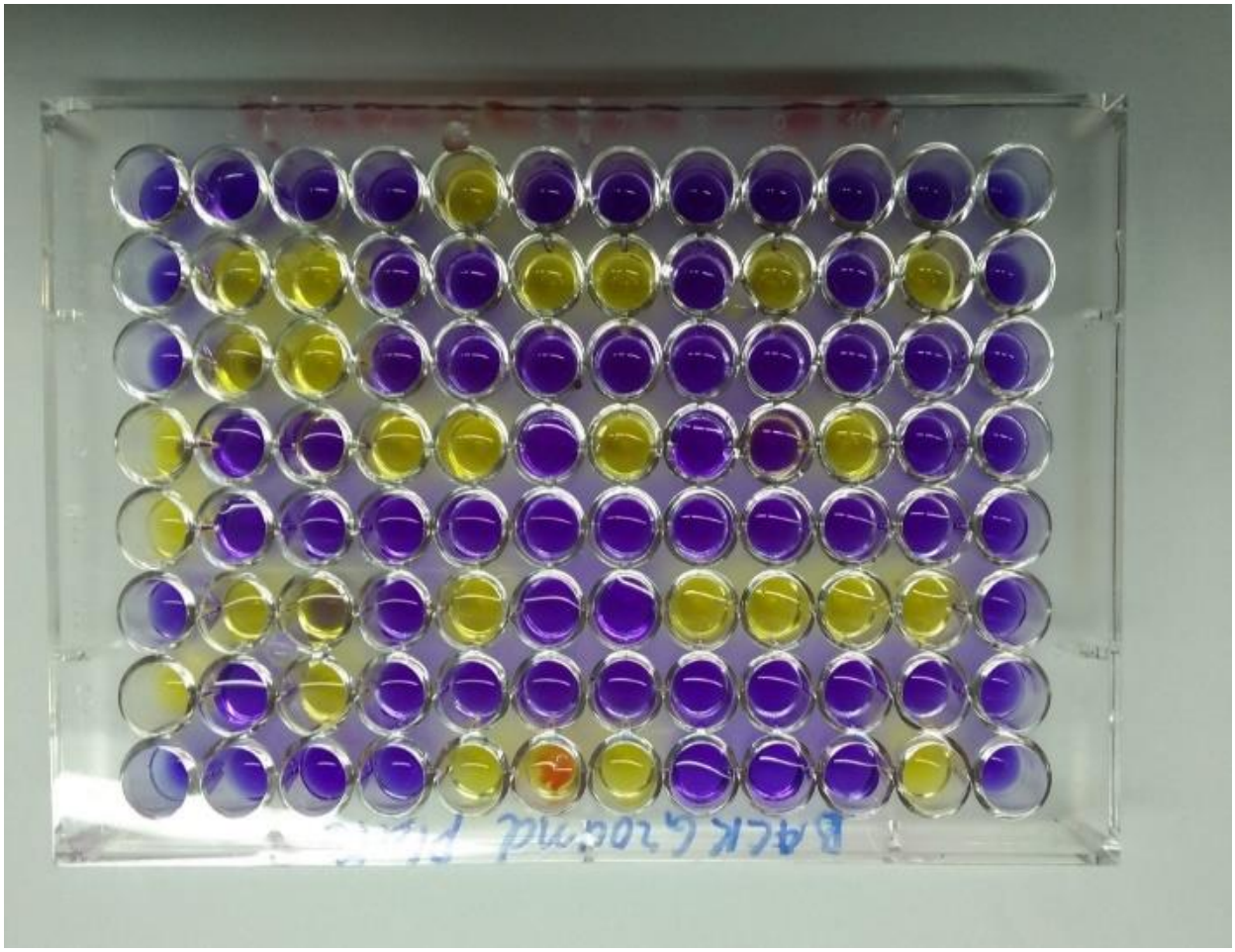


Fig 4.6: (a) Background TA98

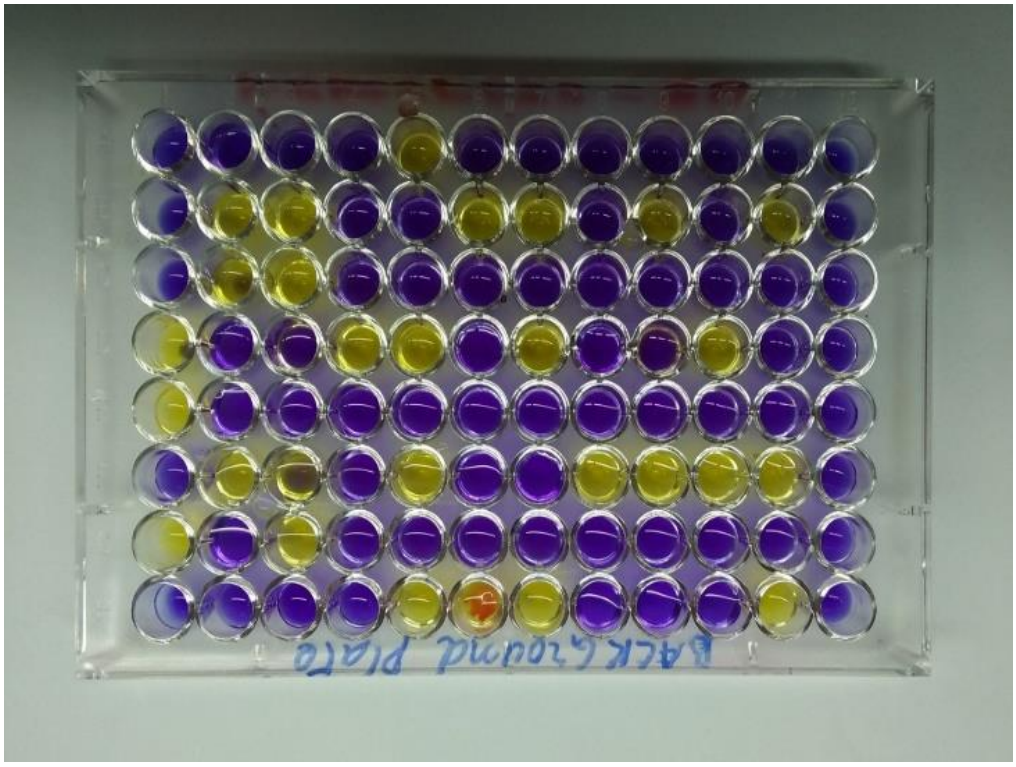


Fig 4.6: (b) Background TA100

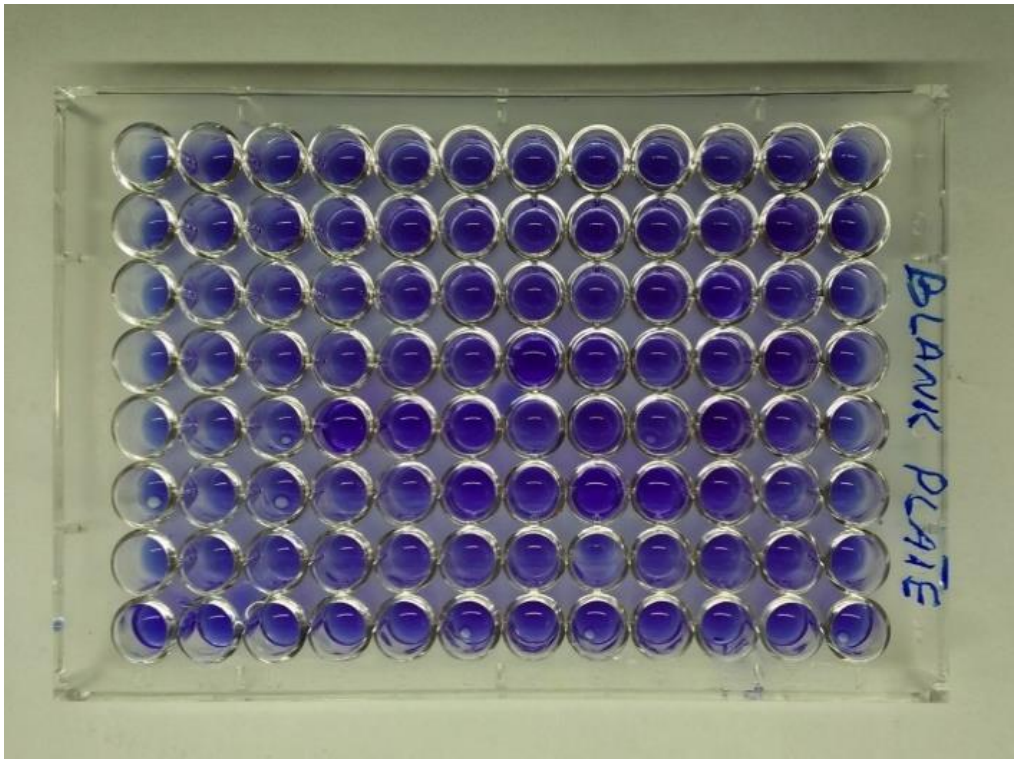


Fig 4.7: Sterility Plate

Sterility Plate

In sterility only reagent mixture and water was added. No colour change that showed no contamination occuered in this plate.

Two plates prepared for background plates .1 for TA98 and 1 for TA100. In TA98 22 well turned into yellow and in TA100 23 wells turned into yellow showed the mutagenesis. Both TA98 and TA100 revealed the mutagenic nature.

AMA Series

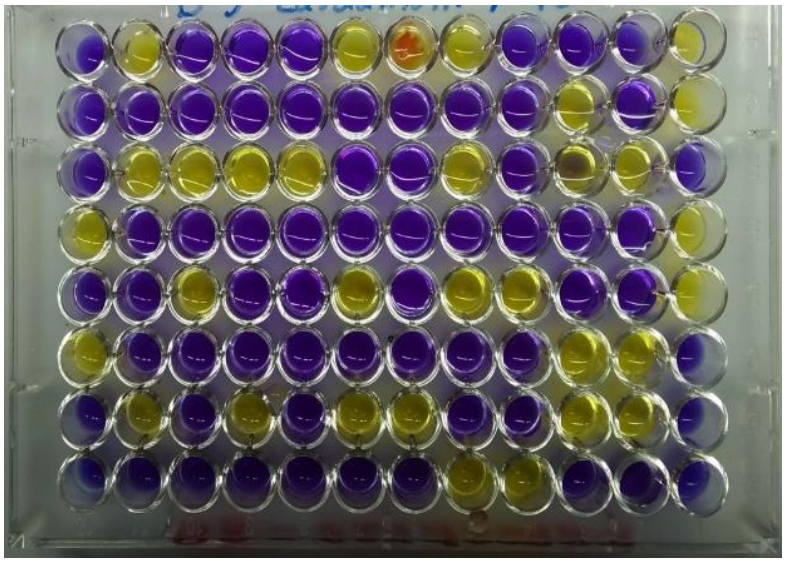


Fig 4.8: (a) AMA 1 TA-98

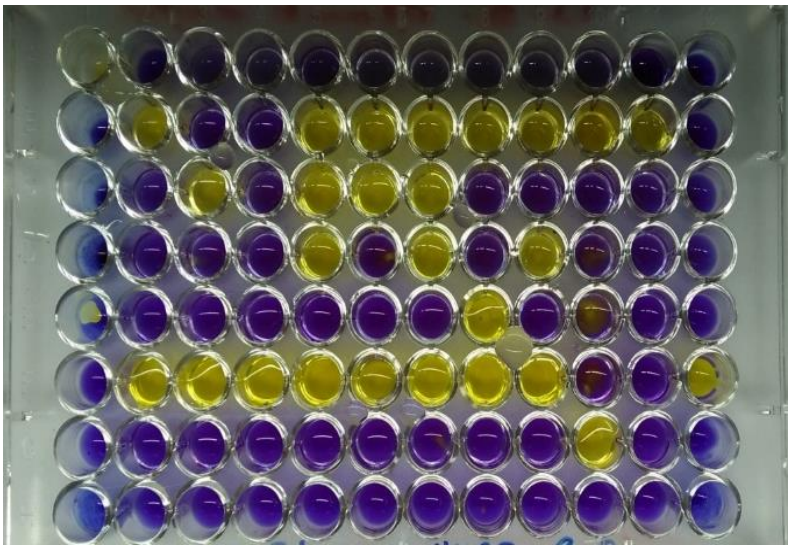


Fig 4.8: (b) AMA 1 TA-100

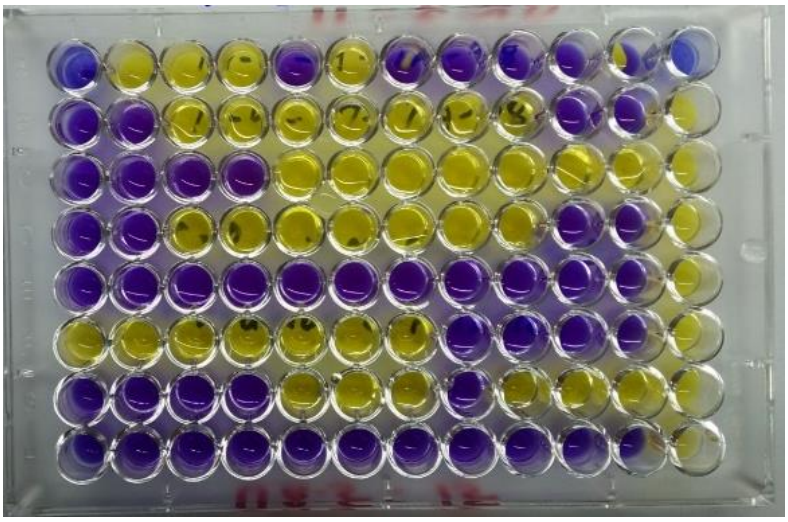


Fig 4.9: (a) AMA4 TA-98

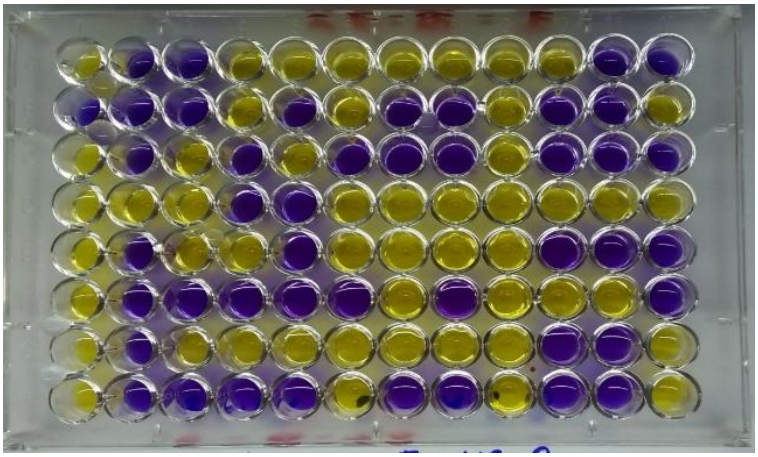


Fig 4.9: (b) AMA4 TA-100

Mutagenic/Non mutagenic test of AMA series

Synthetic Compound	$K_2Cr_2O_7$	NaN_3	TA-98	TA-100	Wells
AMA 1	NO	NO	YES	NO	22/96
AMA 1	NO	NO	NO	YES	23/96
AMA 2	NO	NO	YES	NO	20/96
AMA 2	NO	NO	NO	YES	23/96
AMA 3	NO	NO	YES	NO	25/96
AMA 3	NO	NO	NO	YES	23/96
AMA 4	NO	NO	YES	NO	45/96
AMA 4	NO	NO	NO	YES	41/96
AMA 5	NO	NO	YES	NO	24/96
AMA 5	NO	NO	NO	YES	20/96
AMA 6	NO	NO	YES	NO	23/96
AMA 6	NO	NO	NO	YES	18/96
AMA 7	NO	NO	YES	NO	22/96
AMA 7	NO	NO	NO	YES	25/96

Ame's test was performed by Maron and Ames process (Alabi *et al*., 2014). AMA 4 showed the high mutagenic effect among all the compounds in this series. The methyl group on ortho para position greatly affect the mutagenesis of selected series. It substitution increased the mutagenesis of AMA 4. AMA4 show the mutagenic effect among all the compounds of this series.

Table 4.3: Muttagenic/Non mutagenic test of SR series

Synthetic Compound	$K_2Cr_2O_7$	NaN_3	TA-98	TA-98	TA-100	Wells
SR 1	NO	NO	YES	YES	NO	21/96
SR 1	NO	NO	NO	NO	YES	23/96
SR 2	NO	NO	YES	YES	NO	24/96
SR 2	NO	NO	NO	NO	YES	27/96
SR 3	NO	NO	YES	YES	NO	20/96
SR 3	NO	NO	NO	NO	YES	22/96
SR 4	NO	NO	YES	YES	NO	19/96
SR 4	NO	NO	NO	NO	YES	23/96
SR 5	NO	NO	YES	YES	NO	26/96
SR 5	NO	NO	NO	NO	YES	29/96
SR 6	NO	NO	YES	YES	NO	23/96
SR 6	NO	NO	NO	NO	YES	25/96
SR 7	NO	NO	YES	YES	NO	30/96
SR 7	NO	NO	NO	NO	YES	32/96

In the research, SR7 show the mutagenicity of comparison to all other compounds of pyrimidine acetamide derivative. SR4 show less mutagenic effect only few wells are defected.

Muttagenic/Non mutagenic test of SAC series

Synthetic Compound	$K_2Cr_2O_7$	NaN_3	TA-98	TA-100	Wells
SAC 1	NO	NO	YES	NO	20/96
SAC 1	NO	NO	NO	YES	25/96
SAC 2	NO	NO	YES	NO	30/96
SAC 2	NO	NO	NO	YES	27/96
SAC 3	NO	NO	YES	NO	32/96
SAC 3	NO	NO	NO	YES	17/96
SAC 4	NO	NO	YES	NO	19/96
SAC 4	NO	NO	NO	YES	20/96
SAC 5	NO	NO	YES	NO	24/96
SAC 5	NO	NO	NO	YES	26/96
SAC 6	NO	NO	YES	NO	25/96
SAC 6	NO	NO	NO	YES	29/96
SAC 7	NO	NO	YES	NO	32/96
SAC 7	NO	NO	NO	YES	36/96

In the research of that, SAC7 show the mutagenic effect towards both TA-98 and TA-100.Through research we concluded that which compound are non-mutagenic and good for u used in drugs.

Muttagenic/Non mutagenic test of SMI series

Synthetic Compound	$K_2Cr_2O_7$	NaN_3	TA-98	TA-100	Wells
SMI 1	NO	NO	YES	NO	17/96
SMI 1	NO	NO	NO	YES	25/96
SMI 2	NO	NO	YES	NO	29/96
SMI 2	NO	NO	NO	YES	27/96
SMI 3	NO	NO	YES	NO	22/96
SMI 3	NO	NO	NO	YES	20/96
SMI 4	NO	NO	YES	NO	18/96
SMI 4	NO	NO	NO	YES	20/96
SMI 5	NO	NO	YES	NO	23/96
SMI 5	NO	NO	NO	YES	26/96
SMI 6	NO	NO	YES	NO	27/96
SMI 6	NO	NO	NO	YES	19/96
SMI 7	NO	NO	YES	NO	24/96
SMI 7	NO	NO	NO	YES	32/96

In Ames assay, Piperadine derivatives show the less mutagenic effect towards both bacterial strins TA-98 and TA-100.

Table 4.6: Muttagenic/Non mutagenic test of 5MB series

Synthetic Compound	$K_2Cr_2O_7$	NaN_3	TA-98	TA-100	Wells
5MB 1	NO	NO	YES	NO	20/96
5MB 1	NO	NO	NO	YES	25/96
5MB 2	NO	NO	YES	NO	30/96
5MB 2	NO	NO	NO	YES	27/96
5MB 3	NO	NO	YES	NO	32/96
5MB 3	NO	NO	NO	YES	39/96
5MB 4	NO	NO	YES	NO	35/96
5MB 4	NO	NO	NO	YES	20/96
5MB 5	NO	NO	YES	NO	24/96
5MB 5	NO	NO	NO	YES	26/96
5MB 6	NO	NO	YES	NO	25/96
5MB 6	NO	NO	NO	YES	29/96
5MB 7	NO	NO	YES	NO	22/96
5MB 7	NO	NO	NO	YES	17/96

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The mutagenic/non mutagenic test of 5MB showed that the synthetic compounds of this series are less toxic.5MB 3 show the toxic effect due to the presence of amino group at ortho position.39 wells appeared to be defected. Synthetic compounds were determined to be non-mutagenic in nature. The mutagenicity has been measured using 96 micro-plates and the examination of a sample is mutagenic in nature whether > 40 well or doubles as context plates are affected. Both TA98 and TA100 revealed the mutagenic nature. AMA 4 showed mutagenesis due to presence of methyl group at ortho or para positions.

Nonetheless, more recent (and expensive) pharmacological and clinical studies (Halliwell, 2008) would suggest the compounds collected and subsequently tested in vitro. Chemical extract considered to be non-toxic and widely used as a histological and vital stain or pH indicator in many biological test systems. It is assumed, in other cytotoxicities, that all organic or bioactive compounds may or may not be found to be non-toxic in the Ames research. Because the sensitivity of mutagenicity is better considered than another in vitro cytotoxicity test, it is therefore essential to screen for their mutagenicity test before using newly synthetic animal tests and a purified compound (Guerard *et al.*, 2013).

The in vivo genotoxicity agent, either synthetically or derived from medicinal plants, has a different nature before being used in medication, before it has to be checked. by (Kirkland *et al.*, 2011). Ames test (detects gene mutations) were often conducted for testing their toxic and mutagenic ability.

CONCLUSION

The new medical scientific research typically is focused on Synthetic medication. The lengthy use of these medicines produced the issues of addiction in people. Synthetic drug products contain compounds pharmacologically similar to those found in conventional illicit drugs. Due to the legal use of anti-infection, microbial resistance against branded medications is growing. There are different bioactive blends that will provide food with the possibility of growth, in addition to traditional scientific information.

In the present study following series of synthetic compounds had been evaluated:

1. Acetamide derivatives
2. Prodigine derivatives
3. Piperadine derivatives
4. Carboxmide derivaties
5. Pyrimidine Acetamide Derivatives

Different in vitro test were done to find the toxixcty of synthetic compounds.in vitro cytoxicity of synthetic compounds by hemolytic activity, in vitro mutagenesis of synthetic compounds by DNA damage protection assay and Ames test.The present work was aimed to appraise the carcinogenic and toxic potential of different series of synthetic compounds. The protective effect of synthetic compounds induced by H2O2 in plasmid DNA were evaluated and found that sample tested protected the DNA damage which is attributed to the presence of antioxidant compounds.

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