CHAPTER 3

Anti-mutagenic activity of Phyllanthus amarus extract
3.1 INTRODUCTION

The human body is continuously and unavoidably exposed to a plethora of structurally diverse chemicals, of which many are established mutagens and carcinogens. For example poly aromatic hydrocarbons, aromatic and heterocyclic amines have been shown to induce carcinogenic activity in animal models and mutagenic activity in short term tests (Maron and Ames., 1983). The major sources for these chemicals are the air we breathe (polycyclic aromatic hydrocarbons), diet (heterocyclic amines) and in some cases, our occupational environment (aromatic amines).

One of the characteristics of the many chemical carcinogens is that in order to express their genotoxicity and carcinogenicity, they must be metabolized to reactive intermediates (ultimate carcinogens). These reactive intermediates, being formed intracellularly, can readily interact with DNA, but may also sufficiently stable to be distributed through the blood circulation to the other tissues of lower metabolic activity, where they can exert their genotoxic effect (Ioannides., 2000). A number of enzyme systems contribute to the metabolism and activation of chemical carcinogens, the most prominent being the cytochrome P450 mono-oxygenase system, which participates, at least to some extent, in the activation of almost every chemical carcinogen that finds its way into the human body (Guengerich and Shimada., 1998, Guengerich., 2000).
Mutation is the sudden heritable change formed as a result of genetic changes such as chromosomal aberrations, micronuclei (Nagalakshmi et al., 1995) and sister chromatid exchanges (Perera et al., 1992). The detection of mutagens and determination of the types of mutation induced are important in understanding the etiology of cancer and other degenerative diseases. Point mutations in human oncogenes or tumor suppressor genes (e.g. p53) may lead to cancer and the pattern of missense mutations can give clue as to the mutational events involved (Harris., 1993).

Tobacco smoke is one of the important sources of human exposure to mutagens and carcinogens (Bridges et al., 1979). It is known that both active and passive smokers excrete high amount of tobacco-derived mutagens in urine (Yamasaki and Ames., 1977). The development of cancer has been associated with DNA damage and defective DNA repair mechanism (Tornaletti and Pfeifer., 1994, Gao et al., 1994, Setlow., 1978).

In recent years, the influence of the non-nutritive dietary constituents, such as flavonoids, chlorophyllin, coumarins, dietary fibres, indoles, phytosterols and protease inhibitors abundantly present in fruits and vegetables, is receiving much attention as chemopreventive agents. One of the mechanisms by which these plant food protect against carcinogenesis is by their anti-oxidative property (Aruoma., 1994).
Several edible plant products were shown to be anti-mutagenic (Vinitketkumneun et al., 1994, Sripanidkulchai et al., 2001). Curcumin has been reported to have anti-mutagenic effect on bacteria *invitro* (Nagabhushan et al., 1987). Investigations carried out in this laboratory have found that spices (Soudamini et al., 1995), *Emblica officinalis* (Jose et al., 1997), and curcuminoids (Ruby et al., 1996) possessed significant anti-mutagenic property. *P. amarus* has been shown to be an effective medicine against viral hepatitis as it has been shown to suppress the mRNA transcription of hepatitis B virus (Lee et al.; 1996). It has been shown to be useful to reduce the HbsAg antigen found in human HIV carriers (Unander and Blumberg, 1992). *P. amarus* has been shown to reverse the chromosomal alterations induced by genotoxic agents (Gowrishankar and Vivekanandan., 1994).

Present investigation is to determine the anti-mutagenic activity of *P. amarus* extract to various environmental mutagens *in vitro* and *in vivo*.

**3.2 MATERIALS AND METHODS**

**3.2.1 Preparation of alcoholic extract**

Dried aerial parts of *P. amarus* were powdered and each time 50 grams of this powder was extracted twice in 5 volumes of 75% methanol by stirring overnight and centrifuged at room temperature. Supernatant was evaporated to dryness at 50°C under reduced pressure using a rotary evaporator. This was reconstituted in water and was used for all experiments unless and otherwise stated.
3.2.2 Salmonella / microsome test (Ames test)

The tester strains of *Salmonella typhimurium* bears a mutation in the histidine operon (his-) that prevent it to produce one of the enzyme required for the synthesis of histidine and hence it can not grow in a medium lacking histidine (Maron and Ames, 1983). When a reverse mutation occurs at the his -site by a mutagen, it can restore the original sequence (his -) and become independent of external supply of histidine. Such bacteria can be detected as observable and quantifiable colonies in agar plates.

Anti-mutagenicity experiments were performed by *Salmonella typhimurium* strains (TA 1535, TA 100 & TA 102), which were originally procured from Prof. B. N. Ames, University of California, Berkeley, USA. They were sub-cultured in nutrient broth for 12h and were stored in small vials and frozen in presence of dimethyl sulphoxide (9%) and kept at -70\(^0\) C. The bacterial culture was inoculated in fresh nutrient broth and grown for 12h at 37\(^0\)C before each experiment.

**Minimal agar plates**

Minimal agar plates were prepared by the method suggested by Ames.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>1.5 g for 100ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>85ml</td>
</tr>
<tr>
<td>10 X Spizizen’s salt</td>
<td>10ml</td>
</tr>
<tr>
<td>40% glucose</td>
<td>5ml</td>
</tr>
</tbody>
</table>

This solution was autoclaved for 20 minutes and 25ml poured into each petriplate, kept over night and used for the experiment.
Spizizen's salt solution (10 x)

MgSO$_4$ · 7 H$_2$O - 0.02g
Sodium citrate - 1 g
K$_2$ H PO$_4$ - 14g
KH$_2$PO$_4$ - 6g
(NH$_4$)$_2$SO$_4$ - 2g
Warm distilled water - 100ml

This solution was sterilized by autoclaving at 121°C for 20'.

Histidine / biotin solution (0.5 mM)

Biotin (12mg) was dissolved in 50ml of warm distilled water. To this solution L- histidine HCl (10mg) was dissolved and total volume was made up to 100ml with distilled water. The solution was autoclaved and stored in the refrigerator.

Top agar

Agar - 0.6g
Sodium chloride - 0.5g
Distilled water - 100ml

The agar was dissolved in distilled water by autoclaving and distributed into 2ml aliquots in sterile tubes.

3.2.3 Confirming genotype of tester strains

Plates containing histidine and biotin as well as plates with out histidine, as control plates were prepared aseptically. Cotton swab was dipped in the 12h broth culture and a single sweep was made across biotin control plate and then across the histidine/ biotin plate.
The strains were identified by labelling each streak with a marking pen on the bottom of the petriplates. Before swabbing the cultures on the plate, excess culture from the cotton swab was squeezed out on the inside of the culture tube. The plates were incubated overnight at 37\(^{0}\)C and the growth was examined on the next day.

**rfa mutation**

Strains having the deep rough (rfa) character were tested for crystal violet sensitivity (Ames et al., 1973). 0.1 ml of fresh overnight culture (TA 100, TA 102 and TA 1535) was added to a tube containing 2ml of molten agar held at 45\(^{0}\). The top agar tubes were vortexed for three seconds at low speed and then poured on nutrient agar plates. The plates were tilted and rotated for the even distribution of the top agar on the plates. The plates were placed on a level surface and allowed several minutes for agar to become firm. 10\(\mu\)l of 1mg/ml solution of crystal violet was pipetted to the center of sterile paper disc (1/4 inch) and discs were transferred to each of the inoculated plates using sterile forceps. The discs were tightly pressed with forceps to embed it slightly in the overlay. The plates were inverted and incubated at 37 \(^{0}\)C. After incubation for 24h the plates were observed for the zone of inhibition.

**uvrB mutation**

The uvrB mutation can be confirmed by demonstrating UV sensitivity in strains that contain this mutation (Ames et al., 1973).
The tester strain broth cultures (TA100, TA102, and TA1535) were streaked in parallel stripes across the nutrient agar plate with a sterile swab. A piece of cardboard was placed over the uncovered plate so that half of each of bacterial streak was covered. The plate was irradiated with a 15W germicidal lamp at a distance of 33cm. The non-R-factor strain TA 1535 was streaked on a separate plate and was half covered and irradiated for 6 sec. The R-factor strain TA 100 was irradiated for 8 sec. Strain TA 102, which possessed wild type excision repair enzyme, was tested on the same plate as a control. The irradiated plates were incubated at 37°C for 12-24h.

**R-factor**

The R-factor strains TA 100 and TA 102 were tested for the presence of the ampicillin resistance factor.

The ampicillin resistance was tested by streaking the cultures across the surface of ampicillin plates (0.8g ampicillin trihydrate in 100ml of 0.2N sodium hydroxide). A single sweep of culture was made with a cotton swab across the ampicillin plate. All the strains can be tested in a single plate. The strains were identified by labeling each streak with a marker pen on the bottom of the petriplate. The non-R-factor strain TA 1535 was tested on the same plate as a control for ampicillin activity. The plates were incubated at 37°C for 12-24h.

**Preparation of S9 fraction**

Many carcinogens and mutagens needed to be metabolized by the cytochrome P450 dependent monoxygenase systems before they elicit
mutagenic activity (Ames et al; 1975). Such chemicals have to pre – incubated with rat liver fraction (S9 fraction) in presence of the bacterium before placing to minimal agar plate. Male Wistar rats (150-200g) were given sodium phenobarbital (0.1%) in drinking water for 4 days to induce liver microsomal enzymes (Ames et al., 1973). Animals were killed on the 5th day livers removed, and homogenate was prepared aseptically. The homogenate was centrifuged in a cooling centrifuge at 8,600rpm for 10 min at 40°C. The supernatant was used as S9 fraction (Maron and Ames., 1983). The protein content of the S9 fraction was 40± 2mg /ml as determined.

**Artificial activation (S9) mixture was prepared in the following manner.**

- Sterile distilled water - 1.675 ml
- 0.2 M Phosphate buffer - 2.5 ml
- 0.1 M NADP solution - 0.2 ml
- 1 M glucose - 6 - phosphate solution - 0.025 ml
- 1.6 M KCl - 0.4 M MgCl₂ solution - 0.1 ml
- Rat liver S9 - 0.5 ml

### 3.2.4 Determination of anti- mutagenic activity of *P. amarus* using direct acting mutagens

Mutagenic agents used in the assay were MNNG (1μg/plate), NPD (20μg/plate) and sodium azide (2.5μg/plate).

On the start of the experiment placing the test tube in a water bath melted the agar. Each test tube contained 2ml of molten agar. 20μl
of sterilized Histidine- Biotin solution was added to the top agar tubes. Top agar tubes were held at 45° C and the appropriate concentration of mutagen (10μl) was added to the tubes. Different concentrations of *P. amarus* were added to different tubes and then 0.1ml of freshly grown 12h bacterial culture was added to each tube. The tube was vortexed for 3 seconds and the top agar was poured over minimal agar plates and spread evenly on the plates by rotating the plate gently. Control plates without mutagen and *P. amarus* were kept for checking spontaneous revertants. Triplicate plates were prepared for each dose of *P. amarus*. The plates were inverted and plates were incubated at 37° C for 48h. The plates were covered with brown paper or other suitable materials to avoid the effects of light on photosensitive chemicals.

3.2.5 Determination of anti-mutagenicity of *P. amarus* extract using mutagens which needed activation

Anti-mutagenic activity of *P. amarus* towards 2-acetamidofluorene (2-AAF) and aflatoxin B1, mutagens requiring metabolic activation was also studied.

Histidine and biotin were added to the tubes containing 2ml of top agar. The top agar tubes were melted and held at 45° C. S9 mix was prepared and 500μl of this mix was added to sterile 2ml vials. 0.1ml of fresh 12h culture of the tester strain and different concentrations of *P. amarus* were added to different vials along with mutagens (10μl). The vials were kept for incubation at 37° C for 30 min. After incubation contents of the vials were poured over the top agar tube held at 45° C.
The test components were mixed by vortexing the soft agar for 3 sec at low speed and then poured out to minimal glucose agar plate. To achieve uniform distribution of top agar on the surface of the plate, uncovered plates were quickly tilted and rotated and then placed covered on a level surface to harden. The mixing, pouring and distribution took less than 20 sec. Control plates kept without adding *P. amarus* and mutagen were kept for checking spontaneous revertants for each of the tester strains.

The plates were covered promptly with brown paper to avoid the effects of light on photosensitive chemicals. Within an hour after pouring, the plates were inverted and placed in dark incubator at 37°C for 48 h. The number of revertants formed was counted using a colony counter. Anti-mutagenicity of the *P. amarus* was determined by comparing the number of colonies in experimental plates to the number of spontaneous revertants produced in the control plates. Positive controls were kept for all experiments. Each experiment was performed twice with three plates per concentration. Values were expressed as total number of revertants minus that of spontaneous revertants.

### 3.2.6 Determination of inhibition of Urinary Mutagenicity in Rats by *P. amarus*.

Male wistar rats were divided into three groups (6 rats/group).

**Group I: Control (uncontrolled)**

**Group II:** Rats were given benzo [a]pyrene (10 mg/rat) in sun flower oil by i.p injection as a single dose.
Group II: Rats were given *P. amarus* extract (500mg/Kg. b. wt) for 12 days.

On the 13th day benzo [*a*] pyrene (10mg/rat) in sunflower oil was administered by i.p injection as single dose. All the rats were put into metabolic cages and urine samples were collected for 24hr after injection. The samples were frozen immediately and kept at -70°C until analysis.

The urine samples were filtered through Whatman No: 1 filter paper and passed through an XAD-2- Amberlite column to concentrate the mutagens (Yamasaki & Ames., 1977). 20ml of urine collected from the rat was loaded onto an XAD column (40m.m x 10m.m). The adsorbed components were eluted with 10ml acetone. The eluants were evaporated to dryness at 60°C and stored at -20°C and reconstituted in 1.5ml dimethyl sulfoxide just before they were used for the mutagenicity assay. To check the mutagenicity 0.1ml bacteria and 0.1ml urine extract were mixed with 2ml top agar containing traces of histidine and biotin and plated on minimal agar medium. The revertants were counted after incubation in the dark at 37°C for 48h.

**3.3 RESULT**

**3.3.1 Genotype testing**

**Histidine requirement**

Growth was observed only on the histidine biotin plates for tester strains TA100, TA102, and TA1535 after 24h incubation at 37°C. No growth was obtained on the control plates.
**rfa mutation**

A clear zone of inhibition (approximately 14mm) appeared around the crystal violet disc after 24h incubation at 37°C for all the tester strains. The appearance of a clear zone indicated the presence of rfa mutation, which permitted large molecules such as crystal violet to enter and kill the bacteria. This confirmed rfa mutation.

**uvrB mutation**

Growth was observed only on the unirradiated side of the plates for the strains TA1535, TA100 where as for TA102 growth was observed on the irradiated side also.

Strains with uvrB deletion grew only on the unirradiated side of the plate. TA102 grew even on the irradiated side of the plate as this strain possessed wild type excision repair enzymes. So TA 102 was treated as a control for UV irradiation.

**Rfactor**

Growth was seen in the ampicillin plates along the streaks made with TA100 and TA 102. No growth was observed for TA 1535. TA 100 and TA 102 were R factor strains. These strains possessed PKM 101 DNA essential for ampicillin résistance. No growth was seen on TA 1535 as it had no R factor and it was tested as a control for ampicillin sensitivity. The R factor served as a convenient marker that made it possible to test for the presence of plasmid.
3.3.2 Anti-mutagenicity of *P. amarus* extract using direct acting mutagens

**a). Sodium Azide (NaN₃)** - *P. amarus* extract was found to reduce the number of revertants produced by sodium azide (2.5µg/plate) to *Salmonella typhymurium* strains TA1535, TA100 and TA102. Anti-mutagenicity was found to be concentration dependent. The inhibition of mutagenicity was 44.9%, 34.8%, and 29.4% for *S. typhymurium* TA1535, TA100 and TA102 respectively at 1mg/plate of *P. amarus* extract (Table 3.1).

**b). N-Methyl-N’-nitro-N-nitrosoguanidine (MNNG)** - *P. amarus* extract was also found to inhibit mutagenicity produced by MNNG (1µg/plate) in a dose dependent manner in different *Salmonella* strains. Addition of *P. amarus* at 1mg/plate produced 40.0%, 24.6% and 27.2% inhibition of revertants produced by *S. typhymurium* TA 1535, TA100 and TA 102 strains respectively (Table 3.2).

**c) 4-Nitro-O-phenylenediamine (NPD)** - *P. amarus* extract was found to significantly inhibit the mutagenicity induced by NPD (20µg/plate) to *Salmonella* strains. The inhibition of mutagenicity to *S. typhymurium* strains TA1535, TA100 and TA 102 was 50%, 42.5% and 34.45 respectively at a concentration of 1mg/plate (Table 3.3).

**3.3.3 Anti-mutagenicity of *P. amarus* extract using mutagens, which needed activation**

Mutagenicity produced by 2-AAF and AFB₁ after their activation using S9 fractions was found to be significantly inhibited by *P. amarus*.
Concentration needed for 50% inhibition of mutagenicity to *Salmonella* strain1535 was found to be 2mg/plate in the case of 2-AAF and between 2-3 mg/plate in the case of AFB₁ (Table 3.4).

These results indicated that *P. amarus* extract could inhibit the mutagenicity induced by direct acting mutagens and mutagens, which needed activation at, low concentrations in several *S. typhimurium* strains.

### 3.3.4 Effect of *P. amarus* extract on urinary mutagenicity induced by Benzo [a] pyrene in rats.

*P. amarus* extract was found to inhibit the urinary mutagenicity induced by benzo [a] pyrene in rats. In this experiment urine collected from animals treated with benzo[a] pyrene in presence and absence of *P. amarus* extract was tested for mutagenicity in three different *Salmonella* strains. Urine from benzo[a]pyrene treated rats was found to exhibit high mutagenic potential as seen from the increase in number of revertants. Administration of *P. amarus* extract 500mg/kg body weight was found to inhibit urinary mutagenicity significantly which was 46% in the case of TA1535, 73% in the case of TA100 and 100% in the case of TA102 (Table 3.5).

### 3.4 DISCUSSION

It is well established that environmental factors such as diet and exposure to chemicals or physical agents play an important role in the development of human cancer (National Research council., 1982). The identification in the diet of naturally occurring inhibitors of
carcinogenesis could lead to important new strategies for cancer prevention by dietary intervention (Wattenberg, 1982). Such natural compounds are likely to be non-toxic, economically cheap and could be consumed in the diet over long periods.

In the present study we have evaluated the anti-mutagenicity of *P. amarus* extract, which is being used in hepatic diseases including acute viral hepatitis (Jayaram et al., 1997). Studies on the anti-mutagenic activity of the extract indicated that the extract inhibited the mutagenicity produced by direct acting mutagens such as NaN₃, MNNG and NPD and also inhibited the activation of the mutagens such as 2-AAF and AFB₁ by rat liver S9 fraction and there by inhibited the mutagenesis and possibly carcinogenesis. Inhibition of mutagenicity of 2-AAF and AFB₁ may be mainly due to inhibition of their activation mediated by P-450 enzymes. Activated metabolites form adducts with DNA and produce mutagenesis and carcinogenesis. In fact we have seen that *P. amarus* extract could inhibit the activation of P450 enzyme *in vitro* (Kumar et al., 2002). *P. amarus* extract also decreased the levels of benzo [*a*] pyrene induced mutagenesis in rats as seen from a decrease in urinary mutagens.

The major pharmacologically active compounds are gallotannins, (e.g. phyllanthusiin D, amariin, geraniin, corilagin and others) (Foo and Wong., 1992, Foo., 1993) and the lignans, phyllanthin and hypophyllanthin. Some of these compounds have been shown to have significant activity against experimental
carcinogenesis. The lignans were found to enhance the cytotoxic response mediators by vinblastin in multidrug-resistant cultured cells (Somanabandhu et al., 1993). The extract was found to inhibit the activity of topoisomerase I and II in Saccharomyces cerevisiae mutant cell cultures (Kumar et al., 2002) indicating that other than the inhibition of the activation of carcinogen the extract has multifocal activity to bring about a reduction in the neoplastic condition in animals and human.

This study indicates that *P. amarus* exerts strong anti-mutagenic activity against naturally occurring carcinogens *in vitro* and *in vivo*. After we have published this data, Sripanidkulchai et al., 2002 have also reported the anti-mutagenic activity of aqueous extract of *P. amarus* to *Salmonella typhimurium* strains. These studies are important in view of developing strategies in reducing the impact of exposure to environmental carcinogens.
Table 3.1 Anti-mutagenicity of *P. amarus* extract to *Salmonella typhimurium* strains against Sodium azide as mutagen

<table>
<thead>
<tr>
<th>Concentration of <em>P. amarus</em> extract (mg/plate)</th>
<th>Average number of revertants/ plate</th>
<th>Sodium azide (2.5µg/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TA1535</td>
</tr>
<tr>
<td>Nil</td>
<td>1039.3 ± 85.6</td>
<td>535.5 ± 40.3</td>
</tr>
<tr>
<td>1</td>
<td>571.7 ± 49.2*** (44.9%)</td>
<td>349.0 ± 15.1*** (34.8%)</td>
</tr>
<tr>
<td>0.5</td>
<td>693.3 ± 34.6*** (33.3%)</td>
<td>403.0 ± 11.3 (24.7%)</td>
</tr>
<tr>
<td>0.25</td>
<td>824.7 ± 67.6* (20.6%)</td>
<td>494.5 ± 24.7 (7.6%)</td>
</tr>
<tr>
<td>Spontaneous revertants</td>
<td>25.3 ± 3.1</td>
<td>150.5 ± 15.3</td>
</tr>
</tbody>
</table>

* P< 0.05; **P< 0.001
Values are after reducing spontaneous revertants
Values in the parenthesis are percent inhibition
Table 3.2 Anti-mutagenicity of *P. amarus* extract to *Salmonella typhimurium* strains against N-methyl N'-nitro N-nitrosoguanidine as mutagen

<table>
<thead>
<tr>
<th>Concentration of <em>P. amarus</em> extract (mg/plate)</th>
<th>Average number of revertants/plate</th>
<th>MNNG (1µg/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA1535</td>
<td>TA100</td>
</tr>
<tr>
<td>Nil</td>
<td>495.0 ± 21.2</td>
<td>487.5 ± 17.7</td>
</tr>
<tr>
<td>1 (40%)</td>
<td>297.0 ± 16.9***</td>
<td>367.5 ± 26.1***</td>
</tr>
<tr>
<td>0.5 (27.7%)</td>
<td>358.0 ± 18.4***</td>
<td>409.0 ± 12.7***</td>
</tr>
<tr>
<td>0.25 (15.9%)</td>
<td>416.5 ± 12.0***</td>
<td>442.0 ± 19.1*</td>
</tr>
<tr>
<td>Spontaneous revertants</td>
<td>26.6 ± 3.0</td>
<td>173.5 ± 24.8</td>
</tr>
</tbody>
</table>

* P< 0.05; **P< 0.01; ***P< 0.001

Values are after reducing spontaneous revertants
Values in the parenthesis are percent inhibition
Table 3.3 Anti-mutagenicity of *P. amarus* extract to *Salmonella typhimurium* strains against 4-Nitro-o-phenylenediamine as mutagen

<table>
<thead>
<tr>
<th>Concentration of <em>P. amarus</em> extract (mg/plate)</th>
<th>Average number of revertants/plate</th>
<th>NPD(20μg/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA1535</td>
<td>TA100</td>
</tr>
<tr>
<td>Nil</td>
<td>392.5±10.6</td>
<td>528±17.6</td>
</tr>
<tr>
<td>1 (50%)</td>
<td>196.0±15.7***</td>
<td>304.0±15.0</td>
</tr>
<tr>
<td>0.5 (33.5%)</td>
<td>253.0±23.3***</td>
<td>358.6±22.5</td>
</tr>
<tr>
<td>0.25 (25.6%)</td>
<td>292.0±12.8***</td>
<td>472.6±14.2**</td>
</tr>
<tr>
<td>Spontaneous revertants</td>
<td>26.3±7.2</td>
<td>167.6±17.2</td>
</tr>
</tbody>
</table>

* P< 0.05; **P< 0.01; ***P< 0.001

Values are after reducing spontaneous revertants
Values in the parenthesis are percent inhibition
Table 3.4 Anti mutagenicity of *P. amarus* extract to *Salmonella typhimurium* TA1535 against 2-Acetamidofluorene (2-AAF) and Aflatoxin B₁ as mutagens

<table>
<thead>
<tr>
<th>Concentration of <em>P. amarus</em> extract (mg/plate)</th>
<th>Average number of revertants per plate</th>
<th>2-AAF (50µg/plate)</th>
<th>Aflatoxin (5µg/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>376.0±12.7</td>
<td>261.1±13.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>190.0±14.5***</td>
<td>168.0±12.5***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(49.5%)</td>
<td>(35.6%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>219.5±17.8***</td>
<td>181.6±20.4***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(41.6%)</td>
<td>(30.4%)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>254.0±8.5***</td>
<td>205.3±18.5***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(32.4%)</td>
<td>(21.4%)</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>308.5±16.3***</td>
<td>223.0±17.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(17.9%)</td>
<td>(14.6%)</td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>33.6±7.7</td>
<td>37.0±11.1</td>
<td></td>
</tr>
</tbody>
</table>

* P< 0.05; ***P< 0.001

Values are after reducing spontaneous revertants
Values in the parenthesis are percent inhibition
Table 3.5 The effect of *P. amarus* extract on urinary mutagenicity induced by Benzo[a]pyrene in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Average number of revertants per plate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TA1535</td>
</tr>
<tr>
<td>I</td>
<td>Normal (Untreated)</td>
<td>56.0±11.3</td>
</tr>
<tr>
<td>II</td>
<td>Control B[a]P</td>
<td>336.7±45.1</td>
</tr>
<tr>
<td>III</td>
<td><em>P. amarus</em> extract 500mg + B[a]P</td>
<td>193.7±7.1*** (46%)</td>
</tr>
<tr>
<td></td>
<td>Spontaneous revertants</td>
<td>23.9±2.9</td>
</tr>
</tbody>
</table>

***P< 0.001

Values are without reducing spontaneous revertants

Values in the parenthesis are percent inhibition