**INTRODUCTION**

All chemicals that produce DNA damage leading to mutation or cancer are described as genotoxic. So far as we know, it was Herman Druckrey, at a conference in Sweden, who first used the word genotoxic for chemicals that can react with DNA, and thus have the potential of being mutagenic, cell transforming, and carcinogenic. Researchers have popularized the use of the term genotoxic as agents that were DNA-reactive, directly or after biochemical activation, with appropriate fractions from liver or other tissue of rodents or humans. In sharp contrast, there are other chemicals and agents that are clearly not mutagenic, but that have the ability of increasing the effectiveness or efficiency of a genotoxic carcinogen. One key property of this class of agents is that they may control the rate of DNA synthesis and thereof cell division. This is important ever since Cleaver discovered DNA repair. A cell containing mutated DNA will yield an abnormal fraudulent DNA only after such a DNA has served as a template in the synthesis of new DNA during cell cycling and mitosis. Thus, the rate of cell division is an important parameter. Nevertheless, simple screening bioassays can play very important role in pre-regulatory genotoxicity screening strategies because of their rapid genotoxicity detection requirement that is demanded in preliminary decision making process. Toxicological studies have undergone a significant evolution during the past decade, with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity. The mutations in somatic cells are not only involved in the carcinogenesis process but also play a role in the pathogenesis of other chronic degenerative diseases, such as atherosclerosis and heart diseases, which are the leading causes of death in the human population.

*Kigelia africana* (Lam Bignoniaceae) is widespread in India and Africa and is found in most wet savannah and riverine areas. Growing over 20 m high, it is semi-evergreen, with grey-brown smooth bark. The fruits are large grey-green “sausages” about 30 - 60 cm long which hang on stalks from the tree. In most parts of Africa, the Kigelia fruit has a long history of both consumption and topical application. It is valued as an aphrodisiac, a disinfectant and a cure for dermal complaints. The fruit is used effectively in dressing sores and wounds both for humans as well as animals and for a wide variety of skin afflictions, ranging from eczema, ulcers, acne, skin cancer and fungal infections. Scientific literature confirms the validity of many of these traditional uses due to the presence of numerous secondary metabolites. These compounds include iridoids, flavonoid, fatty acids, sterols, glycosides and naphthoquinones. So far only few studies have been carried out on this plant. Based on our preliminary findings, it was interested to assess the in vivo antimutagenic efficacy of *Kigelia africana*. Hence in the present study, author reported first time the effect of flavonoid molecule of *K. africana* on Swiss-Albino mice.

**MATERIALS AND METHODS**

**Plant Material**

Leaves of *K. africana* was collected in the month of June 2012 from Thirupathi and their identity was confirmed and authenticated by K. Madhava Chetty and the voucher...
A specimen was deposited in the Department of Pharmacology, Karnataka College of Pharmacy, Bangalore India. The shade dried healthy leaves were powdered separately using mechanical grinder and then passed through sieve in order to maintain uniform powder size.

**Preparation of Extracts**

About 250 g of dry leaf powder was extracted using methanol solution in soxhlet apparatus. After about 2 h, the materials were concentrated by evaporation. The concentrated extract was shade dried to remove the solvent for further use. The yield of extract was calculated and stored for further use. The concentrated methanolic extract was then subjected to column chromatography, from which a flavonoid was isolated and it was confirmed through spectroscopy and structural elucidation.

**Experimental animals and treatment**

Eight to ten weeks old Swiss albino mice of either sex, weighing 25-30 g maintained under standard environmental conditions (25 ±2°C, relative humidity 45 ± 10%, light and dark cycle of 12 h) and fed with standard pellet diet and water *ad libitum*, were used for the present study. The experimental protocol, which is in accordance with the OECD (Organization of Environmental Carcinogen Detection) guidelines No.470 and WHO guidelines for mutagenicity studies in animals, was approved by the Institutional Animal Ethics Committee before starting the experiments. The animals were divided into five groups consisting of six animals each. Group one served as normal control, group two was treated with clastogen, cyclophosphamide 100mg/kg, i.p. and bone marrow was collected after 24 h of clastogen administration. Group three was treated with *K.africana* (200 mg/kg, p.o.) for seven days. Groups four and five were treated with Flavonoid of *K.africana* 200 mg/kg, p.o. and 100 mg/kg, p.o. respectively for seven days followed by Cyclophosphamide as a challenge. The dose for mice was calculated based on LD$_{50}$ (2000mg/kg) values of *K.africana*. On seventh day, bone marrow was collected at 24h after clastogen administration, respectively.

**Chromosomal aberrations test**

As shown from Table 1 and figure 1 & 2, there was a statistically significant increase in chromosomal aberrations in response to cyclophosphamide (100 mg/kg). Flavonoid of *K.africana* significantly inhibited the frequency of various chromosomal aberrations and the decrease in mitotic index induced by clastogen.

**Bone Marrow Micronucleus Assay**

On seventh day, the animals were anesthetized and the bone marrow was aspirated from femur and tibia into one ml of 5%bovine albumin in phosphate buffered saline (pH 7.2). The cell suspension was centrifuged (1000 rpm for 5 min) and the smears were prepared from the pellet on chemically cleaned glass slides and stained with May-Grunwald’s and followed by Giemsa stain. The smears were analysed under oil immersion using Labomed Model Digi 2 microscope for the presence of micronuclei (MN) in polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). P/N (Polychromatic erythrocyte/ Normochromatic erythrocyte) ratio was determined by counting a total of about 500 erythrocytes per animal and 2000 erythrocytes were examined for the presence of micronuclei.

**RESULTS**

### Chromosomal aberrations test

As shown from Table 1 and figure 1 & 2, there was a statistically significant increase in chromosomal aberrations in response to cyclophosphamide (100 mg/kg). Flavonoid of *K.africana* significantly inhibited the frequency of various chromosomal aberrations and the decrease in mitotic index induced by clastogen.

**Figure 1:** Total aberrations of different groups in Chromosomal aberration test

**Figure 2:** Mitotic index of different groups in Chromosomal aberration test
Table 1: Effect of flavonoid of *K. africana* on chromosomal aberrations and mitotic index

<table>
<thead>
<tr>
<th>Groups</th>
<th>Types of aberrations</th>
<th>Total number of aberrations</th>
<th>Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rings</td>
<td>Exchanges</td>
<td>Breaks</td>
</tr>
<tr>
<td>Normal</td>
<td>1.00±0.26</td>
<td>0.667±0.33</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>CYP treated</td>
<td>5.16±0.54</td>
<td>2.00±0.36</td>
<td>4.67±0.71</td>
</tr>
<tr>
<td>KA (200 mg/kg) alone</td>
<td>1.00±0.45</td>
<td>0.83±0.31</td>
<td>1.67±0.56</td>
</tr>
<tr>
<td>KA (200 mg/kg)</td>
<td>2.50±0.76</td>
<td>1.33±0.42</td>
<td>2.17±0.65</td>
</tr>
<tr>
<td>KA (100 mg/kg)</td>
<td>1.00±0.26</td>
<td>0.67±0.33</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN±SD n=6  ***p<0.0001, ** p<0.001, ns=non-significant; when compared to CYP control group CYP- Cyclophosphamide, KA- *Kigelia africana*

Table 2: Effect of flavonoid of *K. africana* after 24 h of clastogenic challenge

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Groups</th>
<th>PCE</th>
<th>MNPCE</th>
<th>%MNPCESEM</th>
<th>NCE</th>
<th>MNNCE</th>
<th>%MNNEC</th>
<th>P/N RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>6033</td>
<td>28</td>
<td>0.46±0.05</td>
<td>6048</td>
<td>15</td>
<td>0.24±0.042</td>
<td>0.91±0.007</td>
</tr>
<tr>
<td>2</td>
<td>CYP Treated</td>
<td>6109</td>
<td>162</td>
<td>2.58±0.055</td>
<td>6045</td>
<td>54</td>
<td>0.88±0.056</td>
<td>0.63±0.010</td>
</tr>
<tr>
<td>3</td>
<td>KA(200mg/kg) alone</td>
<td>6035</td>
<td>61</td>
<td>1.01±0.059***</td>
<td>6053</td>
<td>26</td>
<td>0.42±0.048***</td>
<td>0.85±0.010***</td>
</tr>
<tr>
<td>4</td>
<td>KA(200mg/kg)+CYP</td>
<td>6039</td>
<td>117</td>
<td>1.90±0.072***</td>
<td>6020</td>
<td>38</td>
<td>0.62±0.032**</td>
<td>0.76±0.013***</td>
</tr>
<tr>
<td>5</td>
<td>KA(100mg/kg)+CYP</td>
<td>6060</td>
<td>35</td>
<td>0.57±0.030***</td>
<td>6025</td>
<td>19</td>
<td>0.31±0.059***</td>
<td>0.98±0.011***</td>
</tr>
</tbody>
</table>

Values Are Expressed as Mean ± SEM, n=6, **P<0.001, ***P<0.0001 when Compared with CYP treated Group KA- Kigelia africana CYP- Cyclophosphamide

DISCUSSION

Flavonoids act as an important class of antimutagens and anticarcinogens with high potential. Distinct structure activity relationship were detected when 56 flavonoids, 32 coumarins, 5 naphthoquinones and 12 anthraquinones were tested for their antimutagenic potencies, with respect to mutagenesis induced by 2-nitrofluoro 3-nitro fluoranthene and 1-nitropyrene in *S. typhimurium* TA98. Among flavonoids, all flavones and many flavonoids with phenolic hydroxyl group like leuteolin, kaempherol etc., exerted antimutagenicity; chalcones and dihydrochaleones were potent antimutagens.

A number of known flavonoids including flavonoid glycosides and isoflavones were reported to possess significant antimutagenic activity. Citrus juice flavonoids are reported to possess anticarcinogenic and antimutagenic properties. Heo et al. (1992) tested 14
flavonoids including flavones and flavonol derivatives for their antimutagenic effect against induction of micronuclei by benzo(α)pyrene (Bap) in polychromatic erythrocytes (PCEs) of mice.11

In the Chromosomal aberration test, the administration of flavonoid of Kigelia africana at the dose of 100 and 200mg/kg body weight prior to the administration of cyclophosphamide have significantly prevented the chromosomal aberrations in dose dependent manner. The different types of chromosomal aberration such as chromatid rings, chromatid exchanges, chromatid breaks and minutes were prevented in challenge (Kigelia africana + cyclophosphamide) group as compared to control (cyclophosphamide) group. The % protection was increased in dose dependent manner. These results showed that flavonoid of Kigelia africana exhibited effective degree of antimutagenic activity.

The micronucleus study showed that the administration of isolated pure compound of Kigelia africana at the dose of 100 and 200 mg/kg body weight prior to the administration of cyclophosphamide have significantly inhibited the micronucleus formation in the erythrocytes.

CONCLUSION

Plants having flavonoids has showed antimutagenic activity and therefore conducted study on flavonoid of Kigelia africana for its antimutagenicity. By the results obtained, it is confirmed that, flavonoid of Kigelia africana caused antimutagenicity in micronucleus and chromosomal aberration tests in bone marrow cells of mice. Although a variety of work has been done to explore the various potential of the different parts of Kigelia africana tree, but very few works are reported on the leaves of Kigelia africana. Thus, the present study on leaves of Kigelia africana will provide a lot of scope for further research in this regard and isolation of chemicals from it may lead to the production of a variety of potent drugs.

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