EFFECTS OF JAMBOLAN (SYZYGIUM CUMINI L. SKEELS) IN BIOCHEMICAL PARAMETERS AND IN HEMATOPOIETIC CELLS’ CHROMOSOME OF RODENTS

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ABSTRACT: The objective of this study was to investigate the jambolan acute effects in rodents. The jambolan lyophilized edible fraction (pulp and peel) was solubilized in drinking water and then administered orally to Wistar female rats for 28 days. It was evaluated the renal and hepatic functions by serum levels of urea, creatinine, alanine aminotransferase, aspartate aminotransferase. To make the micronucleus test, it was prepared a jambolan solution for administration orally, in a single dose, to female Swiss mice. After 24 hours was performed euthanasia by exsanguination under anesthesia to remove the marrow from the femurs of mice and to evaluate possible damage to the chromosomes of hematopoietic cells. According to the results, it can be stated that the oral ingestion of the peel and freeze-dried pulp jambolan showed no mutagenicity or no effect on biochemical markers of kidney and liver functions in rodents.

Keywords: Myrtaceae; biochemical markers, toxicity.

EFEITOS DOS FRUTOS DE SYZYGIUM CUMINI LAMARCK SKEELS EM PARÂMETROS BIOQUÍMICOS E EM CROMOSSOMA DE CÉLULAS HEMATOPOIÉTICAS DE RODORES FÊMEAS

RESUMO: A família Myrtaceae é amplamente diversificada. Entre seus exemplares está o jambolão (Syzygium cumini Skeels) que apresenta inúmeros estudos relacionando suas propriedades terapêuticas. No entanto, poucos estudos foram realizados para avaliar a segurança da ingestão do fruto pela população. Assim, o objetivo desse trabalho foi realizar uma investigação dos efeitos agudos do jambolão em roedores. A fração comestível liofilizada do jambolão foi solubilizada em água potável e administrada por via oral, durante 28 dias, nas concentrações de 100mg/kg, 200mg/kg e 400mg/kg em ratas Wistar, para avaliação das funções renal e hepática, por meio de dosagens séricas de ureia, creatinina, alanina aminotransferase (ALT), aspartato aminotransferase (AST). Para o teste de micronúcleo, foi preparada uma solução de jambolão de forma idêntica, nas mesmas concentrações, para administração, por via oral, em uma única dose aos camundongos fêmeas Swiss. Após 24h foi realizada a eutanásia por exsanguinhas e anestesiada para a retirada das medulas dos fêmures dos camundongos e avaliação de possíveis danos provocados nos cromossomas das células hematopoéticas. De acordo com os resultados obtidos, pode-se afirmar que a ingestão oral da casca e polpa liofilizadas do jambolão não

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apresentaram mutagenicidade ou efeitos sobre os marcadores bioquímicos das funções renal e hepática, em roedores, o que leva a pressupor segurança para o uso do fruto pelas indústrias alimentícias e farmacêuticas.

**Palavras-chave:** Myrtaceae, Marcadores Bioquímicos, Toxicidade.

**INTRODUÇÃO**

The use of medicinal plants for the treatment and prevention of diseases is as remote as the human species itself and often symbolizes the unique therapeutic resource in many communities and ethnic groups (SIMÃO, 2013).

Although medicinal plants contain xenobiotics, foreign substances to the human body, whose presence may cause unpredictable organic reactions (ROCHA et al., 2012; OLIVEIRA et al., 2014).

Scientifically, and contrary to common sense that "natural medicine does not hurt", research on medicinal plants involve complex investigations such as ethnobotany, phytochemistry, synthetic organic chemistry, pharmacology, toxicology and pharmaceutical technology, for the development of herbal medicines (MACIEL et al., 2002).

In Europe and the United States there is greater rigidity when referring to certification and quality control of plant preparations. In Brazil, the popular use of medicinal plants, often comes with no evidence of its pharmacological properties (VEIGA Jr et al., 2005).

Research on the assessment of the safe use of medicinal plants and herbal medicines in Brazil are still primitively disclosed. According to Capasso et al. (2000), there are chemical compounds present in plants, with potential to cause hepatotoxicity such as apiole, safrole, lignans and pyrrolizidine alkaloids; dermatitis situations such as sesquiterpene lactones and furanocoumarins, and renal toxic effects that may be caused by plant species that contains terpenes and saponins.

The Myrtaceae family is broadly diversified. There are numerous studies on a specimen of that family, *Syzygium cumini* (L.) Skeels, also known as *Eugenia jambolana* Lamark that is attributed to it various therapeutic applicability such as anti-diabetic action, astringent, carminative, stomacal, diuretic, anti-diarrheal, anti-oxidant, related to leaves, fruits, seeds and stems (Table 1) (LOGUERCIO et al., 2005; TEIXEIRA et al., 2006; MIGLIATO et al., 2007; BRASIL, 2008; SHARMA ; VISWANATH et al., 2008 ; LI; ADAMS et al., 2009; LI ; ZHANG et al., 2009).

According to Degani et al. (2011), the jambolan is among the plants most used by diabetic population interviewed in the city of Goioere, Paraná, just behind pata-de-vaca (*Bauhinia* sp). Tong et al. (2014) report on the plant tea the use of *Syzygium cumini* for the treatment of diabetes in South Asian population. According to the authors this species contains hydrolysable tannins (HT) monomeric and polymeric which are potent α-amylase inhibitors, which could justify the reduction of postprandial hyperglycemia in diabetic patients.
Although many pharmacological effects of *Syzygium cumini* (L.) Skeels have already been investigated and confirmed, no study has been conducted to evaluate the safety of consumption of the edible fruit fraction of the population. Therefore, the objective of this study was to evaluate whether oral administration of jambolan lyophilized edible fraction promotes changes in plasma glucose levels and liver and kidney function through serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) urea and creatinine in female Wistar rats and analyze in vivo mutagenicity induction in chromosome of hematopoietic cell of Swiss female mice.

**MATERIALS AND METHODS**

**ETHICAL ASPECTS**

The experimental protocol was submitted to the Federal University of Tocantins’s Ethics Committee on Animal Use (UFT-CEUA), and the opinion of approval convalidated under registration number 23101.003106 / 2013-55. All procedures adopted attended the Law No. 11,794, 2008 for use of rodents for scientific purposes and the rules for the Protection of Animals (BRASIL, 2008).

**BIOLOGICAL TEST FOR BIOCHEMICAL EVALUATION OF ACUTE JAMBOLAN EFFECTS IN RATS**

The biological assay was conducted at the Federal University of Tocantins’s Animal Experimentation Laboratory, with female Wistar rats, weighing 180g on average, from the Central Animal Facility of the Federal University of Goias, Brazil. To perform this test, it was used the adapted method of Mariz et al. (2006), during which the animals were housed in groups of three female in a polypropylene box, maintained on shelves vented to farmed animals, with controlled temperature (22 ± 2 °C) and wake cycles clear/dark 12 /12 hours. All animals were offered the standard commercial diet (solid diet) and drinking water *ad libitum*.

Females were divided randomly into 5 groups of 6 animals (*n* = 30), called: Group I (untreated control), group II (control receiving water via gavage) and groups III, IV and V receiving 100 mg/kg, 200 mg/kg and 400 mg/kg of jambolan lyophilized edible fraction (pulp and peel), respectively. The maximal dose administered (400 mg/kg) is equivalent to the human daily consumption of about 1kg of *in natura* jambolan fruits. The calculation was performed from the value found for moisture, in the study of the chemical composition of the fruit. The experiment lasted 28 days and jambolan freeze-dried edible fraction was solubilized daily in drinking mineral water, using a mixer.
From the stock solution (90 mg/ml), individual doses were aspirated into syringes and administered orally (gavage) to rats once a day, always at 5.00 pm. To carry out the calculation of individual doses, there were weekly weighing of females. On days 0 and 14 blood samples of 0.3 mL were collected via the tail tip amputation, for capillary blood glucose testing using a glucometer trademark Accu-chek Advantage II ® (Roche Diagnostics - Mannheim, Germany).

At the end of the clinical trial (day 28), the animals were sacrificed by exsanguination under anesthesia with sodium thiopental (50 mg/kg). For a third determination of glycemia it was repeated the procedure described above and then blood was immediately obtained by exsanguination for biochemical testing (AST, ALT, creatinine and urea).

For biochemical testing, blood was distributed in test tubes containing ethylenediamine tetraacetic acid (EDTA). The tubes were centrifuged for 10 minutes at 2500 rpm to separate the serum. Soon after and under refrigeration, samples were sent to the Clinical Quality Laboratory in Palmas, TO. For the creatinine, ALT and AST dosage was used the kinetic automated colorimetric method, and for the urea, enzyme-automated colorimetric method, either Brand A-15 Biosystens.

Carcasses of all animals were frozen in a freezer at -22 ± 2°C and subsequently incinerated.

BIOLOGICAL TEST FOR EVALUATION IN VIVO OF THE HARM IN HEMATOPOIETIC CELLS CHROMOSOME OF MICE FEMALES (MICRONUCLEUS TEST)

To perform this test, it was used the adapted method of Santos (2006). The test was conducted at the Animal Experimentation Laboratory, at Federal University of Tocantins, with Swiss female mice, weighing 35g, from the Central Animal Facility of the Federal University of Goias, Brazil. Females were accommodated in polypropylene cages and kept in ventilated shelves for animals, with controlled temperature (22 ± 2°C) and wake cycles clear/dark 12/12 hours. All animals were offered the standard commercial diet (solid diet) and drinking water ad libitum.

The female mice were divided randomly into 6 groups with 5 animals each (n = 30). Group A was composed of untreated animals with jambolan but the ones who received 250mg/kg cyclophosphamide intraperitoneally (positive control group); Group B, by untreated animals with jambolan, but received the solvent of cyclophosphamide (0.9% saline) intraperitoneally; Group C, for untreated (negative control group) and the Groups D, E and F, receiving 100 mg/kg, 200 mg/kg and 400 mg/kg of the jambolan lyophilized edible fraction (pulp and peel) orally administrated, respectively.

The experiment lasted 24 hours and the edible fraction was solubilized in drinking mineral water, using a mixer. From the stock solution (50 mg/ml), individual doses were aspirated into syringes and administered orally (gavage), the females only once. After 24h
euthanasia was performed by exsanguination under anesthesia with sodium thiopental (50mg/kg).

After performing euthanasia, each animal was placed separately on the bench for the removal of the femoral bones. Pelvic lower limbs were previously cleaned with 70% alcohol. Thereupon, incisions are made with surgical scissors to remove the skin tissue and bone (RIBEIRO et al., 2003).

To access the bone marrow canal, the two epiphysis were removed using aid a filled syringe with 3 ml of 0.9% saline solution. The needle was inserted into the opening of the femur and marrow was pushed by solution directly into test tubes identified with the number and the animal group. Then these tubes were placed in the centrifuge (Hettich Universal 320R®) at 1000 rpm for 5 minutes to separate the pellet. This one was removed using a micropipette for the preparation of smears. For each animal were prepared three blades.

The counting of micronuclei polychromatic erythrocytes was performed after staining with Leishmann (methyl alcohol associated with blue eosin methylene), in optical microscopy (100 x). 2,000 erythrocytes were observed per animal. Carcasses of all animals were frozen in a freezer at -22 ± 2°C and subsequently incinerated.

STATISTICAL ANALYSIS

The results were submitted to Analysis of Variance and averages were compared by Tukey test at the 5% level of significance using the SISVAR software. The results were expressed as the mean (M) ± standard deviation (SD). Linear regression analysis was also performed in order to observe possible effects of doses in biochemical parameters (AST, ALT, urea and creatinine). Such analyzes were performed using the Statistical Program Analysis System (SAS), version 9.2 (SAS, 2008).

RESULTS AND DISCUSSION

The test of lethal dose 50% (LD50) is to find a single dose of a substance that is sufficient to kill half of the animals in the group evaluated and is used widely as a basis of comparison and classification of the toxicity of substances (VALADARES, 2006; PLAZA, 2007).

Silva et al. (2012) conducted a study to determine the LD50 of the Syzygium cumini seed (L.) Skeels hydroethanolic extract. Male and female rats and mice were used. In mice was performed administration of the hydroethanolic extract powder, orally, at doses of 0.1 to 6 g/kg body weight and caused no deaths. However, when administered intraperitoneally at doses of 0.1 to 1g/kg of body weight, there was death of the animals, it was concluded that the LD50 was 0.489 g/kg. In rats, administered oral doses of 0.5; 1 and 2 g/kg of body...
weight also caused no deaths. Since intraperitoneally in dose of 2 g/kg body weight was lethal to 67% of the animals.

Kumar et al. (2008) also conducted a study using the acute toxicity classification method, according to the Organization of the Guideline Guidelines for Economic Co-operation and Development (OECD 2001). Were used in this study, Wistar rats (n = 6), of both sexes, selected by random sampling. The S. cumini seeds methanolic and ethyl acetate extracts, were administered orally at an initial dose of 5 mg/kg body weight through an intragastric tube, and the animals were observed for 14 days. The doses were gradually increased, in order to evaluate the toxicity of the extracts. There was no animal death registry to 2000 mg/kg body weight.

In this study there was no significant effect of doses administered in the biochemical parameters AST, ALT, urea and creatinine when carried out linear regression (p> 0.05). Furthermore, the biochemical parameters analyzed did not differ significantly between treatment groups and control groups, suggesting that there were no liver or renal disorders in animals treated with the jambolan lyophilized edible fraction.

According to Table 2 it can be seen that serum glucose levels of Wistar rats submitted to daily intake of lyophilized jambolan, they remained very close. The increase in values on the 28th day may be justified due to the stress of euthanasia procedure.

Mazzanti et al. (2003) found no hypoglycemic effect of jambolan bark extract in diabetic rats induced by alloxan, although several studies relate the high levels of flavonoids in Syzygium cumini seeds its antihyperglycemic action, anti-hyperlipidemic and antioxidant (BANERJEE; DASGUPTA, 2005; SHARMA; BALOMAJUMBER et al., 2008).

According to Feijó et al. (2012) studies contradict themselves about the reduction of glucose caused by Syzygium cumini, particularly as regards the plant parts used and ways of preparing the extracts, infusions and decoctions. Oliveira et al. (2005) reported reduction in blood glucose in non-diabetic mice after oral administration of Syzygium cumini leaves crude ethanol extract and butanol or aqueous fractions but assigned it to the effect on reduction of food intake and body weight of the animals.

Table 3 shows the serum levels of AST, ALT, urea and creatinine in female Wistar rats submitted to the ingestion of edible fraction of Syzygium cumini (L.) Skeels.

It is observed that AST, ALT, urea and creatinine were not significantly different between groups nor compared to the negative control group, which suggests that jambolan intake did not alter the normality of the biochemical parameters analyzed. The data from this study suggest the absence of renal and hepatic toxicity of edible fraction of jambolan for the tested doses.

Das and Sarma (2009) induced hepatotoxicity, through the use of paracetamol (2 mg/kg) in albino rats of both sexes, pretreated with Silymarin and Syzygium cumini pulp ethanolic extract. Liver function was assessed by measuring serum levels of ALT, AST, alkaline phosphatase, total bilirubin, total protein and albumin. It was observed that at the
dose of 200 mg/kg occurs reduction in the liver enzymes level and increase in total protein and albumin levels, showing a hepatoprotective effect.

Reactive oxygen species are substances resulting from the activation or reduction of molecular oxygen and are among the major internal mutagens, these mutations may be caused by errors during the DNA replication process. Most degenerative diseases and cancers is related to the accumulation of mutations (PFUHLER et al., 2009; DONYA; IBRAHIM, 2012).

Micronuclei is an additional corpuscle formed by chromatin and that was deleted during telophase of the new nuclei formed by cell division. It arises spontaneously or after experimentally induced by cytotoxic agents such as cyclophosphamide or doxorubicin, which promote alkylation of DNA (KIRSCH-VOLDERS et al., 2003; BRUNTON et al., 2012). According to Table 4, it is clear that there was no significant difference between the frequency of micronuclei in the blades of the negative control groups and other groups receiving doses of 100 mg/kg, 200 mg/kg and 400 mg/kg of the jambolan lyophilized edible fraction (pulp and peel).

Turatti (2008) evaluated the antimicrobial activity of Syzygium cumini and its possible use as a preservative in pharmaceutical or cosmetic preparations for external use. For that was made morpho-anatomical studies and cytotoxicity assays, mutagenicity and genotoxicity. The frequency of micronuclei in bone marrow cells of mice, showed no mutagenic effect in 24 to 48 hours after administration of dry extract of the fruit.

In another study Vicentini et al. (2001) used meristematic cells of Allium cepa L., and bone marrow cells of Wistar rats as cytotoxic and mutagenic assay systems in plants and animals, respectively. Tea plants were prepared of Averrhoa carambola L., Syzygium cumini (L.) Skeels and Cissus sicyoides L., as popular use in two different concentrations: the usual dose and one in a dose ten times more concentrated. After 24 h, there was no detection of damage to the chromosomes of plant and animal cells by any of the studied species.

According to Augustoni (2012), Syzygium cumini fruits crude extracts and hydroalcoholic fraction are capable of promoting the induction of quinone reductase enzyme in hepatocellular carcinoma cells (Hepa 1c1c7) and mutant (TAOr1BPrC1 and BPrC1) at various concentrations from 1.25 to 40 mg/mL, despite the potential genotoxic presented. When the author evaluated the antimutagenic action, extracts and fractions were able to significantly reduce the frequency of micronuclei in relation to the positive control, in both prophylactic and therapeutic manner.

There are numerous studies on the high levels of phenolic compounds, especially anthocyanins present in the pulp and peel Syzygium cumini. Antioxidants biological effects of anthocyanins depend on their chemical structure, such as degree of glycosylation and number of hydroxyl groups. Reviews in vitro and in vivo showed that anthocyanins are not toxic or mutagenic and can attenuate oxidative stress involved in atherosclerotic and neoplastic processes (BRIDLE; TIMBERLAKE, 1997; MAHMOUD et al., 2001; DAMETTO,
2010; CARDOSO et al., 2011).

CONCLUSIONS

The Syzygium cumini (L.) Skeels lyophilized edible fraction (pulp and peel) showed no mutagenicity or no effects on biochemical markers of renal and liver functions in rodents when administered orally. Also, there were no changes in glucose levels of treated animals compared to the control groups. So, it’s safe to assume security for jambolan consumption and for use by the food industry.

REFERENCES


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TABLES

Table 1. Popular use of jambolan (Syzygium cumini L. Skeels)

<table>
<thead>
<tr>
<th>Part of the plant</th>
<th>Popular use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>diabetes, hypotensive action, diuretic, astringent, constipation, leucorrhoea, stomach, poultice for skin diseases, soothe itching, anti-inflammatory.</td>
</tr>
<tr>
<td>Bark</td>
<td>antidiarrheal, inhibitory action activity against HIV -1 protease, hypoglycemic, astringency and constipation, anti-hemorrhagic, diabetes and venereal ulcers, indigestion and purification of blood, dysentery, dyspepsia, antiseptic, astringent in mouth ulcers, gums spongy and stomatitis, local inflammation, burns, cardiotonic, CNS stimulant, antipyretic.</td>
</tr>
<tr>
<td>Seeds</td>
<td>anticonvulsant, hypoglycaemic, astringent and constipation, euphhetic activity, anti-hemorrhagic, to changes in the stomach, anti-inflammatory, bactericidal, diarrhea, diabetes, dysentery and hypertension.</td>
</tr>
<tr>
<td>Fruits</td>
<td>hypoglycemia, astringency and constipation, diuretics and stomatal, gastrointestinal treatment, astringent and orally for stomach ulcer, diabetes, carminative, antiscorbutic and diuretic, acute and chronic diarrhea, urinary retention, gargle for throat irritation, peeling lotion scalp, anti-inflammatory, antipyretic, astringent, in the treatment of dysentery and diabetes.</td>
</tr>
<tr>
<td>Flowers</td>
<td>antibiotic activities</td>
</tr>
<tr>
<td>Root</td>
<td>Antiemetic, increase lactation in nursing mothers</td>
</tr>
</tbody>
</table>

Source: Adapted from Migliato et al., 2006.
Table 2. Dosages of glycaemia (mg/dl) in the serum of female rats submitted to oral ingestion of jambolam lyophilized pulp shell, on days 0, 14 and 28.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st dosage (Day 0)</th>
<th>(Day 0) 2nd dose</th>
<th>(Day14) 3rd dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>84.5 ± 5.3 (a)</td>
<td>93.0 ± 9.8 (b)</td>
<td>121.0 ± 23.7 (c)</td>
</tr>
<tr>
<td>II</td>
<td>83.5 ± 11.9 (a)</td>
<td>80.3 ± 5.0 (b)</td>
<td>130.6 ± 29.9 (c)</td>
</tr>
<tr>
<td>III</td>
<td>82.7 ± 7.8 (a)</td>
<td>99.0 ± 14.2 (b)</td>
<td>146.3 ± 11.2 (c)</td>
</tr>
<tr>
<td>IV</td>
<td>81.8 ± 11.5 (a)</td>
<td>80.4 ± 12.2 (b)</td>
<td>134.5 ± 14.9 (c)</td>
</tr>
<tr>
<td>V</td>
<td>80.5 ± 9.8 (a)</td>
<td>83.5 ± 16.3 (b)</td>
<td>139.2 ± 9.2 (c)</td>
</tr>
</tbody>
</table>

The figures represent the average ± standard error of the average (SEA); values followed by the same letter do not differ significantly (p>0.05). Analysis of Variance (ANOVA), Tukey Test.

Table 3. Serum levels of liver and kidney function markers in female Wistar rats submitted to the ingestion of edible fraction of *Syzygium cumini* (L.) Skeels

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ALT (IU)</th>
<th>AST (IU)</th>
<th>Urea (IU)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>76.93 ± 10.27 (a)</td>
<td>118.66 ± 15.76 (b)</td>
<td>44.6 ± 4.27 (c)</td>
<td>0.65 ± 0.23 (d)</td>
</tr>
<tr>
<td>II</td>
<td>67.60 ± 17.62 (a)</td>
<td>129.35 ± 23.91 (b)</td>
<td>49.9 ± 4.73 (c)</td>
<td>0.68 ± 0.11 (d)</td>
</tr>
<tr>
<td>III</td>
<td>84.85 ± 23.29 (a)</td>
<td>110.47 ± 11.99 (b)</td>
<td>47.3 ± 9.25 (c)</td>
<td>0.40 ±0.17 (d)</td>
</tr>
<tr>
<td>IV</td>
<td>65.88 ± 14.10 (a)</td>
<td>116.11 ± 32.60 (b)</td>
<td>46.7 ± 5.30 (c)</td>
<td>0.43 ± 0.24 (d)</td>
</tr>
<tr>
<td>V</td>
<td>72.50 ± 7.58 (a)</td>
<td>102.18 ± 36.49 (b)</td>
<td>49.7 ± 10.80 (c)</td>
<td>0.52 ± 0.31 (d)</td>
</tr>
</tbody>
</table>

The figures represent the average ± standard error of the average (SEA); values followed by the same letter do not differ significantly (p>0.05). Analysis of Variance (ANOVA), Tukey Test.

Table 4. *In vivo* evaluation of the damage on chromosome hematopoietic cells (mutagenicity) of female mice who ingested the edible fraction of *Syzygium cumini* (L.) Skeels

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average MNF (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: PC (Cyclophosphamide)</td>
<td>23.431 ± 5.89 (b)</td>
</tr>
<tr>
<td>B: NC (solvent - SS 0.9%)</td>
<td>1.915 ± 4.60 (a)</td>
</tr>
<tr>
<td>C: untreated</td>
<td>2.102 ± 3.14 (a)</td>
</tr>
<tr>
<td>D: treated females (100 mg/kg)</td>
<td>2.178 ± 1.68 (a)</td>
</tr>
<tr>
<td>E: treated females (200 mg/kg)</td>
<td>2.353 ± 2.65 (a)</td>
</tr>
<tr>
<td>F: treated females (400 mg/kg)</td>
<td>2.695 ± 4.79 (a)</td>
</tr>
</tbody>
</table>

PC (positive control); NC (negative control); SS (saline solution); MNF: micronucleus frequency in 2,000 polychromatic erythrocytes. The results were expressed as the average (A) and standard deviation (SD). Averages followed by different letters differ significantly (p < 0.05). Analysis of Variance (ANOVA), Tukey Test.