ABSTRACT

Cape gooseberry (Cg) has been an abundant source of highly effective phytochemicals which offer great potential in the fight against cancer. The goal of the present study to investigate genotoxicity of potassium bromate (KBrO₃) in mice and treatment with Cg and interferon-α. Ninety male Swiss albino mice were divided into six groups, 15 animals for each group. Mice were received KBrO₃, Cg orally and interferon-α subcutaneously. Molecular changes were detected using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). The most important changes after using primer 5’AGGGGTCTTG3` that was quantitative mutation 3 times between control mice and mice treated with KBrO₃ at R_F 0.68. Also, one common band appeared in control group and all treated groups at R_F 0.68 with M.wt. 300 bps. In addition, similarity index (S.I.) between control mice and mice treated with 1g/kg Cg and interferon together is one as compared to mice treated with KBrO₃ (S.I.= 0.50). Using primer 5’GAAACGGGTG3` the similarity index between control mice and mice treated with 500mg/kg Cg was 0.80 as compared to mice treated with KBrO₃ (S.I.= 0.00). Using six different primers showed that potassium bromate produced complete and partial disturbance in the DNA sequence (S.I.= 0.0 or 0.67). In addition, treatment with Cg decreased alterations occurred in the DNA sequence which was complementary to primers as compared to mice treated with potassium bromate alone. Treatment with interferon-α and 1g/kg Cg together reduced damage to DNA more than treatment with interferon-α alone. From these findings it
is to be suggested that Cape gooseberry as new source of bioactive phytochemicals and functional foods.

Keywords: Potassium bromate; Cape gooseberry; random amplified polymorphic DNA polymerase chain reaction and interferon-α.

INTRODUCTION
Potassium bromate (KBrO₃) is used as in bread making a flour improver and in the production of fish-pastes. KBrO₃ causes tumors, especially in kidney of rats and mice after long-term oral administration in drinking water [1, 2]. Also, [3] demonstrated that KBrO₃ is genotoxic. It is positive in vitro genotoxicity tests including the bacterial reverse mutation assay, the chromosomal aberration test conducted in Chinese hamster cells, and the mouse lymphoma assay and in vivo in the micronucleus test [4, 5]. While it induces chromosome aberrations strongly both in vivo and in vitro [6, 7].

Over the last few years, much attention has been paid to the research of naturally occurring agents that are able to stimulate defense mechanisms of the organism. Among these agents is Cape gooseberry (as natural treatment) and interferon-α (as chemotherapy treatment). Interferons (IFNs) are an important treatment for a number of solid tumors and hematological malignancies. In addition, IFN therapy is associated with significant side effects which have an impact on the patient’s quality of life and the physician’s ability to optimally treat the patient [8]. The IFNs are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, or parasites or tumor cells. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to "interfere" with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages. Interferon α-2a is one of the types I of interferon that bind to a specific cell receptors [9].

The Cape gooseberry (Physalis peruviana) is the second highest fresh fruit export in Colombia. Because of its nutritional and medicinal attributes, it is attractive for international markets. Cape gooseberry has been grown in Egypt, New Zealand, Australia and Great Britain [10-13]. The fruits, the part of the plant that is commercialized, are contained in a calyx and are characterized for their nutritional value. The Cape gooseberry have been shown to contain several phytochemicals that are thought to be protective: these include carotenoids, flavonoids, isoflavonoids, provitamin A, minerals, vitamin C, vitamin B-complex and many
classes of phenolic compounds \cite{14}. Therefore, the main object of the present work was to study the protective and the treative effects of Cape gooseberry and interferon-α against genotoxic effects of potassium bromate under in vivo conditions in mice.

**MATERIALS AND METHODS**

**Animals**

Male Swiss albino mice (*Mus musculus*) aged 9-11 weeks and weighing 30-35g was used in all experiments. Mice were housed in plastic cages with stainless steel grill tops, bedded with rice husk. Ninety mice were divided into six experimental groups of 15 animals in different cages. They were maintained under proper environmental conditions. Mice were provided with standard diet and tap water. They were acclimatized to laboratory conditions for at least 7 days before the experiment.

**Experimental doses**

**A-Cape gooseberry (Physalis peruviana)**

Cape gooseberry juice yield is 72.6% of the berry weight. Good amounts of phenolics and β-carotene were detected in Cape gooseberry juice. Fruit juice was found to contain 0.2% oil \cite{15}.

**Selection of Cape gooseberry doses**

Cape gooseberry (Cg) was administered to mice by gavage, at doses 500 mg and 1 g/kg daily according to \cite{16} for 2 weeks (14 days) according to \cite{17}.

**Preparation of the doses for treatment**

Cape gooseberry was purchased from local markets. Fruits were first washed thoroughly to remove impurities. After washing the fruits were cut into small pieces. Pieces placed in the blender to make a juice (500g Cg juice up to 500ml dist. water) where each 1 ml juice contain 1g Cg. The stock was distributed into small tubes and preserved in the refrigerator for the time of use. The juice was shaken well just before oral administration by oral tube.

**B- Potassium bromate**

Potassium bromate (KBrO₃) is a white crystalline powder, which is colourless, odourless, and tasteless with a molecular mass of 167 g. Potassium bromate is also found in drinking-water samples as a by-product of ozone disinfection \cite{18}.
Selection of doses of potassium bromate
The recommended dose was 1.6g/liter (196 mg/kg) daily according to \[1\] for 15 weeks according to \([19, 20]\).

Preparation of doses for treatment
Potassium bromate present in the form of white crystal powder. The bottle was contained 500 g and was purchased from EL-Gamhuria Company, Egypt. We used 1.6g and were dissolved in liter of distilled water. Mice were received the potassium bromate orally by oral tube.

C- Reiferon (interferon α-2a)
Reiferon (interferon α-2a) was purchased from EL-Esaph pharmacy, Egypt. The box was contained six ampoules, each one contain 3 M.IU/1ml. It was administered to mice subcutaneous injection (s.c./i) at dose 6.5 \(\times 10^5\)/kg three time weekly for 6 weeks (42 days) according to \[21\].

E- Experimental groups
Group one as control group, group two mice treated with 1.6g/liter (196 mg/kg) for 15 weeks, group three and four including pre-treatment with 500mg/kg and 1g/kg Cape gooseberry for 2 weeks before KBrO\(_3\) and continuous with it, group five and six containing post-treatment with 6.5 \(\times 10^5\)u/kg interferon-α alone and interferon-α & Cape gooseberry together for 6 weeks.

Genomic DNA extraction and RAPD-PCR technique
Genomic DNA was extracted from the liver tissue isolated from six groups. DNA was extracted by using GF-1 DNA extraction Kit instructions of the user's guide. DNA polymerase chain reaction technique was performed by using six primers with sequences 5`GAAACGGGTG3`, 5`TTCCGAACCC3`, 5`TCTGTGCTGG3`, 5`GACCGCTTGT3`, 5`CAATCGCCGT3` and 5`AGGGGTCTTG3`.

The procedure of DNA polymerase chain reaction technique
To 0.2 ml PCR reaction tube, 12.5\(\mu\)l of Green \(Taq\) Ready Mix added and followed with 1\(\mu\)l (25 pmol.) primer and \(X\)\(\mu\)l DNA template (25 ng/ml). The total volume was completed to 25\(\mu\)l with PCR water. All the contents were mixed gently, the 20\(\mu\)l of mineral oil added to top of each tube to prevent evaporation. The tubes were placed on thermal cycler and the
reaction series was performed as follows: 95°C for 5 minutes (Initial temp.). 35 cycles of 95°C for 30 second (Denaturation), 36°C for 2 minutes (Annealing) and 72°C for 2 minutes (Extension or Polymerization). At end of the reaction, the temperature fixed at 72°C for 10 minutes followed by keeping the amplicon at 4°C.

PCR products were subjected to electrophoresis through 1% agarose gel containing ethidium bromide. After electrophoresis process, gels were visualized using U.V-transilluminator. Gel plates were photographed and the analyzed using a gel pro Analyzer (Version 3.1). The similarity index (S.I) compares between control and treated groups using the formula: S.I. = (2 Nab/Na + Nb)\(^{[22]}\).

RESULTS AND DISCUSSION

The present study describes molecular processes which may be involved in the toxicity and carcinogenicity of potassium bromate and treatment with Cape gooseberry and interferon. The genomic DNA pattern carried out in liver of control and treated groups using six different primers 5´GAAACGGGTG3´ 5´TTCCGAACCC3´, 5´TCTGTGCTGG3´, 5´GACCGCTTGT3´, 5´CAATCGCCGT3´, 5´AGGGGTCTTG3´.

DNA pattern after using primer 5´ GAAACGGGTG 3´

Mice treated with 196 mg/kg potassium bromate for 15 weeks appeared one characteristic band with \(R_F\) 0.67 and M.wt. 210 bps. In addition, mice treated with 1g/kg Cape gooseberry showed one characteristic band with \(R_F\) 0.54 (M.wt. 295 bps).

On the other hand, there are quantitative mutation appeared between control mice and mice treated with 1g/kg Cape gooseberry and interferon together at \(R_F\) 0.8 and band density percentage 48.03 & 24.38 respectively Table 1. Also, control mice and mice treated with 1g/kg Cape gooseberry at \(R_F\) 0.8 and band density percentage 48.03 & 24.38 respectively Figure 1. This indicated that 1g/kg Cape gooseberry alone or with interferon does not protect this band against damaged effect of KBrO\(_3\).

It is important to notice that similarity index between control mice and mice treated with KBrO\(_3\) (S.I.= 0.0). Therefore, the genetic distance equal one (G.d.=1) between mice treated to KBrO\(_3\) alone and control mice. This showed that there is complete disturbance in the DNA sequence. This may be due to metabolism of potassium bromate involves increased lipid peroxidation and the generation of intermediates and bromine oxide-radicals that attack
DNA; oxidative damage to sugars or bases (guanine base) in DNA induced 8-oxoguanonine resulting in transversions mutation which typically arise after replication of DNA.

These results were in agreement with [23], who investigated that molecular analysis of potassium bromate-induced mutations indicated a high portion of deletion mutations. Three out of four point mutations were G to T transversions which typically arise after replication of 8-oxoguanine (8-hydroxyguanine). Also, [24] reported that KBrO₃ resulted in the generation of intermediates that can react with DNA and cause single and double strand breaks. Bromine radicals (Br•) or oxides (BrO•, BrO₂•) are the species directly responsible for the observed cellular and cell free DNA damage.

In addition, [25, 26] showed that potassium bromate exposure led to an increase in both DNA damage and frequency of micronucleated cells. Also, they indicated that potassium bromate would induce DNA damage by several mechanisms besides oxidative stress.

On the other hand, similarity index between control mice and mice treated with 500 mg/kg Cape gooseberry (S.I.= 0.8) indicated that Cape gooseberry decrease alterations the same DNA sequence which was complementary to primer 5′GAAACGGGTG3′, as compared to mice exposure to potassium bromate (S.I.= 0.0). The protective effect of Cape gooseberry may be the possible involvement of its antioxidant and scavenging properties.

To the author's opinion, phenolic compounds, withanolides, ascorbic acid and other components in Cg have antioxidant activities and their ability to scavenge free radicals, break radical chain reaction and chelate metals. This result was in agreement with [27], who demonstrated that various flavonoids such as quercetin could protect DNA both by reducing oxidative DNA damage and by enhancing DNA repair through modulation of DNA repair enzymes expression.

In addition, [28] showed that the ability of ascorbic acid to inhibit reactive oxygen species production, but not alter Bro₃⁻ (bromate) induced nuclear fragmentation or 8-hydroxy-deoxyguanosine (8-OHdG) formation, is some what in contrast to studies demonstrating that ascorbic acid reduces bromate induced formation of 8-OHdG in vivo [29].

**DNA pattern after using primer 5′ TTCCGAACCC 3′**

In the present study, it is observed that control group has one characteristic band at RF 0.41 and M.wt. 829 bps Table 2. This indicated that potassium bromate induced disappearance of this band and different treatment could not appear it.
Our finding that mice treated with potassium bromate produced one characteristic band with RF 0.15 and M.wt. 1184 bps. Also, similarity index between control mice and mice treated with potassium bromate (S.I. = 0.25). This showed that KBrO3 caused alterations the same DNA sequence which complementary to primer 5′TTCGGACCC3′. This result was in agreement with [2], they reported that bromate is mutagenic and that this activity is mediated by the formation of oxidative damage to the DNA. Not only does bromate induce genetic damage in vitro.

Also, [20] revealed that treatment with KBrO3 significantly increased the mutant and total mutation frequencies and frequency of GC to TA transversion of the lacI gene in the kidney compared to non-treatment control group. In addition, [30, 31] suggested that KBrO3 exerts oxidative stress and genotoxic effects in human hepatoma cell line (HepG2) cells, possibly through the mechanisms of lysosomal damage, an earlier event preceding the oxidative DNA damage.

In the present work, there are quantitative mutation appeared between control mice and mice treated with 500 mg/kg Cape gooseberry at Rf 0.62 and band density percentage 24.68 and 49.02 respectively Figure 2.

On the other hand, similarity index between control mice and mice treated with 1g/kg Cape gooseberry (S.I. = 0.75) indicated that Cape gooseberry may decrease alterations the same DNA sequence which was complementary to primer 5′TTCGGACCC3′, as compared to mice treated with potassium bromate (S.I. = 0.25). This finding was in accordance with [32] demonstrated that Indian gooseberry and galangal extracts showed good activity against the growth of food poisoning bacteria, both extracts were found to possess high antioxidant activities. However, Indian gooseberry extract was found to have higher total phenolic contents than did the galangal extract. Furthermore, [33] demonstrated that golden berry-derived 4β-Hydroxywithanolide (4β-HWE) is a potential DNA damaging and chemotherapeutic agent against lung cancer.

In the present work, mice treated with interferon alone or interferon with Cape gooseberry did not produce any DNA bands. This indicated that this primer did not anneal with DNA of these groups.
DNA pattern after using primer 5’TCTGTGCTGG 3’
The data demonstrated that DNA fractionation of the control sample, mice post-treated with interferon alone or interferon with Cape gooseberry did not produce any DNA bands. This indicated that this primer did not anneal with DNA of these groups.

DNA pattern after using primer 5’ GACCGCTTGT 3’
In the current work, potassium bromate induced disappearance of all normal DNA bands as compared with control and appearance of one abnormal band at R_F 0.06 and M.wt 1480 bps Table 3.

In addition, similarity index between control mice and mice treated with potassium bromate (S.I.= 0.0). This showed that KBrO_3 caused complete alterations in the same DNA sequence which complementary to primer 5’GACCGCTTGT 3’. These results were in agreement with [5], who revealed that treatment with KBrO_3 produced a measurable increase of 8-oxoG in the kidney. It can induce oxidative DNA damage.

Furthermore, [34] decided that rather bromine radicals (Br•) or oxides (BrO•, BrO2•) might be responsible for oxidative DNA damage and the induction of mutations and micronuclei are easily detectable at non-cytotoxic concentrations of bromate. On the other hand, it is observed that there are quantitative mutation appeared between control mice and mice treated with 1g/kg Cape gooseberry at R_F 0.59 and band density percentage 32.27 and 16.68 respectively.

Also, quantitative mutation between control mice and mice treated with 500 mg/kg Cape gooseberry at R_F 0.62 and band density percentage 25.32 & 53.9 respectively Figure 3. This may be due to oxidative stress of potassium bromate on these DNA bands. Fortunately, Cape gooseberry protected these bands from disappearance.

In addition, similarity index between control mice and mice treated with 1g/kg Cape gooseberry (S.I.= 0.5) indicated that Cape gooseberry may decrease alterations the same DNA sequence which was complementary to primer 5’GACCGCTTGT3’, as compared to mice treated with potassium bromate (S.I.= 0.0). These results were coincided with [35, 36], they cleared that withanolides exhibited a broad spectrum of biological properties and significant pharmacological activities, including antibacterial, antitumor and immunomodulatory.
DNA pattern after using primer 5’ CAATCGCCGT 3’
In the present study, control group appeared 3 DNA bands at $R_F$ 0.26, 0.33 and 0.75. It is observed that control mice induced one characteristic band at $R_F$ 0.26 (M.wt. 889 bps) did not appear in other groups. This indicated that potassium bromate induced disappearance of this band and different treatment could not appear it.

On the other hand, potassium bromate appeared one abnormal band at $R_F$ 0.29 and M.wt 833 Figure 4. Also, it showed two common bands with control group at $R_F$ 0.36 and 0.76 Table 4. In addition, similarity index between control mice and mice treated with $\text{KBrO}_3$ (S.I. = 0.67). These revealed that $\text{KBrO}_3$ induced damage to this sequence of DNA which complementary to primer 5’CAATCGCCGT3’. This result was in agreement with [37], who showed that $\text{KBrO}_3$ decrease antioxidant status permits further oxidation of cellular DNA resulting in the formation of the mutagenic lesion 8-oxodeoxyguanosine (8-oxodG) and an increase in cellular proliferation in the target tissue.

Furthermore, there are quantitative mutation appeared between control mice and mice treated with interferon alone at $R_F$ 0.35 and band density percentage 23.3 and 10.11 respectively. Also, qualitative mutation between control mice and mice treated with 1g/kg Cape gooseberry and interferon together at $R_F$ 0.34 and band density percentage 23.3 & 49.31 respectively.

It is important to noticed that similarity index between control mice and mice treated with 1g/kg Cape gooseberry and interferon together (S.I. = 0.8) indicated that Cape gooseberry with interferon decrease alterations the same DNA sequence which was complementary to primer 5’CAATCGCCGT 3’, as compared to mice treated with potassium bromate (S.I. = 0.67). This result was in accordance with [38], who demonstrated that interferon stimulates DNA synthesis and repair. Also, [39] interferon - α alone is active against hairy cell leukemia. Moreover, [40] suggested that interferon-α can disrupt DNA synthesis by direct inhibition of DNA polymerases alpha and beta.

On the other hand, [26] revealed that the activities of antioxidant enzymes were significantly reduced with intoxication of KBrO$_3$ in rats which might be due to the presence of catechin, phenolic and polyphenolic compounds (kaempferol, rutin and quercetin) which, propagating free radicals like peroxyl radicals and converting the reactive free radicals to inactive products.
DNA pattern after using primer 5’ AGGGGTCTTG 3’

In the current work, control mice appeared one DNA band at RF 0.68 M. wt. 300 bps. It is important to notice that one common band appeared in all groups at RF 0.68 with M.wt. 300 bps. This showed that all treatments did not affect on this band.

It is observed that mice treated with potassium bromate alone induced one characteristic band at RF 0.62 (M.wt. 420 bps). This indicated qualitative mutation. Furthermore, there are quantitative mutation appeared between control mice and mice treated with potassium bromate at RF 0.68 and band density percentage 100 and 28.63 respectively Table 5.

Similarity index between control mice and mice treated potassium bromate (S.I.= 0.50). These results revealed that KBrO₃ produced damage to this sequence of DNA which complementary to primer 5’AGGGGTCTTG 3’, DNA sequence from alterations which was complementary to primer 5’AGGGGTCTTG 3’. These results were in accordance with [6], they revealed that bromate can damage DNA both in vitro and in vivo. Additionally, [41] demonstrated that potassium Bromate, effectively induced mismatch repair is involved in the suppression of oxidative stress-induced intestinal tumorigenesis in mice.

It is one of the important results that similarity index between control mice and mice treated with 1g/kg Cape gooseberry and interferon (S.I.= 1). Genetic distance is zero Figure 5. This demonstrated that Cape gooseberry and interferon together protected the same DNA sequence from alterations which was complementary to primer 5’AGGGGTCTTG 3’, as compared to mice treated with potassium bromate (S.I.= 0.50).

To the author's opinion, post treatment with 1g/kg Cape gooseberry and interferon-α might increase the intracellular content of antioxidant compounds, thus intensifying the protection against damage induced by free radicals (bromine oxide or radical), reactive oxygen species (ROS), which may react with DNA. Also, Cg and interferon may stimulate mechanism defense of the immune system of mice. This result was in agreement with [42] concluded that ethanolic extract of Physalis peruviana (EEPP) possessed potent antihepatoma activity and its effect on apoptosis is associated with mitochondrial dysfunction. In addition, [43] showed that Physalis peruviana L. (Solanaceae) aqueous extract possesses antioxidant activity and potent hepatoprotective effect against acetaminophen induced liver injury in rats.
Also, [44] demonstrated that the supercritical extraction carbon dioxide of *Physalis peruviana* possessed the highest polyphenol content and exhibited the most potent antiproliferative effect in human lung cancer H661 cells. Its treatment caused cell cycle arrest at S phase, increased expression of the DNA fragmentation, and the accumulation of p53, as well as inducing cytochrome c release, which further activated pro-caspase-3 and consequently caused H661 cell death. Moreover, [45] showed that the chloroform extract of Physalis minima exerted anticancer effect due to a combination of apoptotic and autophagic cell death mechanisms on Caov-3 (human ovarian carcinoma) cells. The induction of these programmed cell deaths was mediated via c-myc, p53 and caspase-3 dependent pathway [46].

On the other hand, [47] concluded that recombinant interferon alpha-2a (rIFN-α2a) enhances 3’-azidothymidine (AZT) induced tumor cell growth inhibition by increasing AZT metabolism, or inhibiting DNA repair and p53-mediated cell cycle control processes. [48] showed that injection of mice with a neutralizing antibody to interferon α/β demonstrated the essential role of endogenous interferon in the defense of the mouse against the development of syngeneic, allogeneic and xenogeneic tumors.
Table (1): DNA pattern through random amplified polymerase chain reaction (RAPD-PCR) in liver of inducted mice with potassium bromate and treated mice with cape gooseberry or interferon using primer 5’GAAACGGGTG 3’.

<table>
<thead>
<tr>
<th>Rows</th>
<th>Control group</th>
<th>Potassium bromate (KBro₃) group</th>
<th>500mg/kg cape gooseberry (Cg)+KBro₃</th>
<th>1g/kg cape gooseberry+ KBro₃</th>
<th>Potassium bromate + Interferon α-2a</th>
<th>Potassium bromate + Interferon α-2a + Cg</th>
</tr>
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<tr>
<td></td>
<td>R</td>
<td>M.w .</td>
<td>B.d</td>
<td>R</td>
<td>M.w t.</td>
<td>B.d</td>
</tr>
<tr>
<td>R1</td>
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<td>-</td>
<td>-</td>
<td>0.37</td>
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</tr>
<tr>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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</tr>
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<td>51.97</td>
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<td>-</td>
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</tr>
<tr>
<td>R6</td>
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<tr>
<td>R7</td>
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<td>120</td>
<td>48.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R8</td>
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Table (2): DNA pattern through random amplified polymerase chain reaction (RAPD-PCR) in the inducted mice with potassium bromate and treated mice with cape gooseberry or interferon using primer 5’ TTCCGAACCC 3’.

<table>
<thead>
<tr>
<th>Rows</th>
<th>Control group</th>
<th>Potassium bromate (KBro3) group</th>
<th>500mg/kg cape gooseberry (Cg)+KBro3</th>
<th>1g/kg cape gooseberry+ KBro3</th>
<th>Potassium bromate + Interferon α-2a</th>
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<tr>
<td></td>
<td>R_f</td>
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<td>B.d%</td>
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<tr>
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<td>-</td>
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<td>0.77</td>
<td>298</td>
<td>29.12</td>
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Table (3): DNA pattern through random amplified polymerase chain reaction (RAPD-PCR) in the inducted mice with potassium bromate and treated mice with cape gooseberry or interferon using primer 5’ GACCGCTTGT3’.

<table>
<thead>
<tr>
<th>Rows</th>
<th>Control group</th>
<th>Potassium bromate (KBro3) group</th>
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<th>1g/kg cape gooseberry+ KBro3</th>
<th>Potassium bromate + Interferon α-2a</th>
<th>Potassium bromate + Interferon α-2a + Cg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R_f</td>
<td>M.wt.</td>
<td>B.d%</td>
<td>R_f</td>
<td>M.wt.</td>
<td>B.d%</td>
</tr>
<tr>
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<td>-</td>
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<td>1480</td>
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<tr>
<td>R2</td>
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<tr>
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<td>0.62</td>
<td>530</td>
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<tr>
<td>R4</td>
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</tr>
<tr>
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<td>292</td>
<td>42.41</td>
<td>-</td>
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Table (4): DNA pattern through random amplified polymerase chain reaction (RAPD-PCR) in the inducted mice with potassium bromate and treated mice with cape gooseberry or interferon using primer 5’CAATCGCCGT3’.

<table>
<thead>
<tr>
<th>Rows</th>
<th>Control group</th>
<th>Potassium bromate (KBro₃) group</th>
<th>500mg/kg cape gooseberry (Cg)+KBro₃</th>
<th>1g/kg cape gooseberry+ KBro₃</th>
<th>Potassium bromate + Interferon α-2a</th>
<th>Potassium bromate + Interferon α-2a + Cg</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.26</td>
<td>889</td>
<td>22.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.29</td>
<td>833</td>
<td>25.87</td>
</tr>
<tr>
<td>R3</td>
<td>0.33</td>
<td>676</td>
<td>23.30</td>
<td>0.36</td>
<td>722</td>
<td>32.38</td>
</tr>
<tr>
<td>R4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R9</td>
<td>0.75</td>
<td>200</td>
<td>54.45</td>
<td>0.76</td>
<td>178</td>
<td>41.75</td>
</tr>
</tbody>
</table>

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Table (5): DNA pattern through random amplified polymerase chain reaction (RAPD-PCR) in the inducted mice with potassium bromate and treated mice with cape gooseberry or interferon using primer 5’ AGGGGTCTTG3’.

<table>
<thead>
<tr>
<th>Rows</th>
<th>Control group</th>
<th>Potassium bromate (KBro₃) group</th>
<th>500mg/kg cape gooseberry (Cg)+KBro₃</th>
<th>1g/kg cape gooseberry+ KBro₃</th>
<th>Potassium bromate + Interferon α-2a</th>
<th>Potassium bromate + Interferon α-2a + Cg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R_f</td>
<td>M.wt.</td>
<td>B.d.%</td>
<td>R_f</td>
<td>M.wt.</td>
<td>B.d.%</td>
</tr>
<tr>
<td>R1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.62</td>
<td>420</td>
<td>32.50</td>
</tr>
<tr>
<td>R2</td>
<td>0.68</td>
<td>300</td>
<td>100</td>
<td>0.69</td>
<td>288</td>
<td>28.63</td>
</tr>
<tr>
<td>R3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.77</td>
<td>179</td>
<td>38.87</td>
</tr>
<tr>
<td>R5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**R_f** = Rate of flow  
**M.wt.** = Molecular weight  
**B.d. %** = Band density percentage

500mg/kg cape gooseberry (Cg)+KBro₃ = Mice were protected 500mg/kg cape gooseberry for 2 weeks then continuously with injection of KBro₃ for 15 weeks.

1g/kg cape gooseberry+ KBro₃ = Mice were protected 1g/kg cape gooseberry for 2 weeks then continuously with injection of KBro₃ for 15 weeks.

Potassium bromate + Interferon α-2a= Mice were injected with KBro₃ for 15 weeks then treated with Interferon α-2a alone for 6 weeks.

Potassium bromate + Interferon α-2a + Cg= Mice were injected with KBro₃ for 15 weeks then treated with Interferon α-2a and cape gooseberry for 6 weeks.
Fig. (1): The RAPD-PCR detection of DNA pattern using primer 5´GAAACGGGTG 3´.

Fig. (2): The RAPD-PCR detection of DNA pattern using primer 5´TTCCGAACCC3´.

Fig. (3): The RAPD-PCR detection of DNA pattern using primer 5´GACCGCTTGT3´.
CONCLUSIONS AND RECOMMENDATION

In the present study, using six different primers showed that potassium bromate produced abnormal DNA bands and suppression of some normal bands as compared with control group. Also, KBrO₃ induced complete or partial disturbance in the DNA sequence which was complementary to primers as compared to control mice (S.I.= 0.0 or 0.67). Therefore, molecular analysis indicated that KBrO₃ can react with DNA and cause single or double strand breaks resulting in damage of DNA.

In our study, low or high dose of Cape gooseberry decreased alterations occurred in the DNA sequence which was complementary to primers as compared to mice treated with potassium bromate alone. The protective effects of Cape gooseberry may be the possible involvement of
its antioxidant contents. Also, Cg could protect DNA both by reducing oxidative DNA damage and by enhancing DNA repair through modulation of DNA repair enzymes expression. In the same time, treatment with 1g/kg Cape gooseberry and interferon-α together reduced damage to DNA (high similarity index) more than treatment with Cg or interferon-α alone. This suggested that post treatment with 1g/kg Cape gooseberry and interferon-α might increase the intracellular content of antioxidant compounds, thus intensifying the protection against damage induced by free radicals, reactive oxygen species (ROS) of KBrO3, which may react with DNA. In addition, Cape gooseberry and interferon-α may modulate cell metabolism and DNA repair. From these findings it is to be suggested that Cape gooseberry as new source of bioactive phytochemicals and functional foods. The present study recommended that, the given of Cape gooseberry was effective in preventing genetic damage. Cape gooseberry could be used as new source of bioactive phytochemicals and functional foods, which are low cost and are available for a major part of the population.

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