Biological Investigation of *Jatropha gossypiifolia*: A Stiff Medicinal Plant in Bangladesh

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Abstract

This study describes a biological investigation of methanolic extract of *Jatropha gossypiifolia*, a plant belonging to the family *Euphorbiaceae*. The aim of this investigation is to expose the viable phytochemical constituents properly as their contribution towards the antioxidant, anthelmintic and membrane stabilizing activities. The anthelmintic test was conducted on earthworm *Pheritima phosthum*a using five unique concentrations of the extract and albendazole as standard drug. The extracts were used for the investigation of antioxidant activity followed by DPPH (Diphenylpicrylhydrazyl) assay and using BHT (tert-butyl-1-hydroxytoluene) as a standard. The crude methanolic stem and leaves extract of *J. gossypiifolia* (*Jatropha gossypiifolia*) was conducted to attempt for membrane stabilizing activities using standard protocols. The leaves and the stems portions considerably have the antioxidant property of 3.186 µg/ml and 10.56 µg/ml respectively. According to the absorbance values of the various extract solutions, results of the colorimetric analysis of total phenolic contents are that the leaves cover the highest Phenolic content (65.66mg/g in GAE (Galic Acid Equivalent)) rather than the stem portion (33.332mg/g in GAE). The leaves and stem methanolic extract was found both at hypotonic solution and heat induced conditions, inhibit 39.19±0.26%, 38.17±0.14 and 27.39±0.15%, 26.97±0.47% hemolysis of erythrocyte membrane respectively, when comparing with standard acetyl salicylic acid. The preliminary phytochemical evaluation of methanolic extract of *J. gossypiifolia* confirmed the presence of glycosides, tannins, phytosterols, triterpenoids, diterpenes, saponins and phenols both at its leaves and stem portions. The results of the study showed that the plant extract has potential membrane stabilizing, anthelmintic, antioxidant activities with significant phytochemicals.

*Keywords*: anthelmintic, antioxidant, Euphorbiaceae, *Jatropha gossypiifolia*, membrane stabilizing, phytochemicals.
1. Introduction

Nature has been a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from herbal sources and many of which is based on their use in conventional medicine [1]. Plant kingdom, the storehouse of thousands of unexplored compounds, possesses an exquisite potentiality for drug search even in the day of synthetic chemistry. The following fact shows how the plant kingdom enriches the cutting-edge medicinal practices. Approximately 33% of the drugs produced in the developed countries are derived from plants [2]. In the United States, in 1980 alone, consumers paid 8 billion dollars for prescription drugs wherein the active ingredients were nevertheless derived from plants. If microbes are added, 60% of the contemporary medicinal products are of natural foundation [2]. More than 47% of all drugs utilized in Russia are obtained from botanical sources. According to some generous estimates, nearly 80% of today’s medicines are directly or circuitously obtained from plant [3].

Today, the term ‘natural product’ is pretty generally understood to refer to herbs, herbal concoctions, dietary supplements, traditional Chinese medicine, or alternative medicine [4]. Thousands of year’s natural resources were in use for combating human ailments. Over the last fifteen years’ interest in drugs of plant origin has been reviving and developing steadily. Besides, the drug researchers are exploring the potential of natural products for the cures of nevertheless insurmountable diseases like cancer and AIDS (Acquired Immune Deficiency Syndrome) [5]. Current drug development trends are shifting towards ‘rationally designed drugs’, which involve the identification of novel targets and the subsequent design of small molecule inhibitors [6], examples consist of the tyrosine kinase inhibitors Glivec, Iressa and Herceptin [7]. Bearing in mind of these contemporary trends, there is still a niche for natural products in present drug discovery efforts. The structural diversity found in nature a long way surpasses that which can be synthesized at the bench.

*Jatropha gossypiifolia* (Local name: kerong, keron) belonging to the family *Euphorbiaceae* [8] is a shrub herb with 1.8-meter-high, clustered with palmate shaped 3-5 lobed leaves and dark red flowers. Its leaves’ stipules, petioles and margins, are covered with glandular hairs [9]. It is reported to be beneficial to dyscrasia, anemia, vertigo and dysphonia [8]. It is an antibiotic, insecticidal and used in toothache and act as blood purifier [10]. The leaves are employed in itches, carbuncles and eczema, as well as acting as purgative and tumefied. The extract of the leaves is practicable for stomachache, venereal disease and as blood purifier [11]. Leaves are
Biological Investigation of *Jatropha gossypiifolia*

Extracts of the plant are used as a cathartic and emetic, and to treat diarrhea, headache, skin and mouth sores [13].

The work is an attempt to characterize the chemical constituents of an indigenous medicinal plant, *Jatropha gossypiifolia* and to evaluate its possible biological, toxicological and membrane stabilizing potentialities.

2. Materials and Methods

2.1. List of Chemicals


2.2. Collection of Plant Materials

The whole plant of *J. gossypiifolia* were collected from Comilla, Bangladesh (The Geo position of the district is between 23°01’ to 24°11’ North latitudes and between 90°34’ to 91°22’ east longitude) during the month from March to April, 2015 and voucher specimens for each of the collections (DACB Accession no. 38200) have been deposited in Bangladesh National Herbarium (BNH), Dhaka for future references.

At first leaves were separated from the plants, washed carefully along with stem, then left for air drying. It takes about 2 weeks to make a dried one. After that leaves and stems were gone through grinding process followed by continuous sieving and finally the fine crashed powders were obtained.

2.3. Preparation of Extract

The air-dried and powdered leaves and stems of the plant (500 gm) were separately soaked in methanol (1.5 L each) for 15 days at room temperature with occasional shaking and invoking. Then filtered via a newly cotton plug and eventually with a Whatman No.1 filter paper. The intensity of the filtrate was then concentrated under reduced pressure using a rotary evaporator (HEYDOLPH, Germany) maintained at 45°C. The semi-dried methanolic extracts were further dried in a freeze drier (HETOSICC, Heto Lab Equipment, Denmark) at -55°C temperature and stored in a reagent bottle at -8°C in a freezer. The subsequent amount of leaves and stem extracts of *Jatropha gossypiifolia* were about 7.8 gm and 9.7 gm respectively.

2.4. Qualitative Phytochemical Screening

3 g methanolic leaves and stems extract of *Jatropha gossypiifolia* were boiled with 30 ml distilled water for 5 minutes in a water bath and were filtered separately while it was hot. Small quantity of freshly prepared extracts were used to prepare tea for constipation [12].
subjected to preliminary quantitative phytochemical analysis for the detection of phytochemicals such as alkaloids with Wagner’s and Hager’s reagent, carbohydrates with Benedict’s test and Milosh’s test, Glycosides with Legal test, flavonoids with alkaline reagent test and lead acetate test, tannins with FeCl$_3$ test, saponins with Froth test, phytosterol with Liberman test, phenols with ferric chloride test, Triterpenoid with Salkowski test, protein and amino acid with xanthotropic test and diterpenes with Cu acetate test [14] to evaluate the presence of bioactive compounds.

2.5. In Vitro Antioxidant Activity

2.5.1. Total Phenolic Content Determination

The total phenolic content was determined by Folin-Ciocalteu reagent using the method designed by Majhenic et al., 2007 [15]. The key roles of phenolic compounds as scavengers of free radicals are emphasized in several reports [16, 17]. The concentrations of total phenol in extract were measured by a UV (Ultra Violet) spectrophotometer based on a colorimetric oxidation/reduction reaction. During the whole operation, Gallic acid was used as standard. After incubation of the samples at room temperature, the absorbance was measured at 760nm. The results were expressed as mg of Gallic acid per gram of the extract (mg GA/g extract).

2.5.2. Free Radical Scavenging Activity by DPPH Method

The free radical scavenging activity of J. gossypifolia leaves and stems extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Diphenylpicrylhydrazyl) by the method of Brand-Williams et al., 1995, [18] at different concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.903, 1.953, 0.977 µg/ml). 2 ml of methanolic solution of sample (extract/standard) was mixed with 3.0 ml of a DPPH methanolic solution (20 µg/ml). The mixture was kept in a dark place at room temperature for 30 min and later absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

% scavenging activity = (1- A test sample/A control) × 100.

Here, A stands for Absorbance.

The graph plotted with inhibition percentage against extract/standard concentration; extract concentration providing 50% inhibition (IC$_{50}$) was calculated.
2.6. In Vitro Anthelmintic Activity

The anthelmintic test was carried out according to the method reported by Ajaiyeoba et al. (2001) [19] with some requisite moderations. Due to the anatomical and physiological similarities with intestinal round worm leech, adult earth worm (*Phertima posthuma*) was used to execute the test [20, 21]. The worms were collected from the damp soil of Noakhali Science and Technology University area. Methanolic extract of *J. gossypiifolia* leaves and stems were taken at different concentrations (10, 20, 40, 60 and 80 mg/ml) severally. To prepare the standard having a concentration of 10 mg/ml, 100 mg of albendazole was dissolved in 10 ml water. A control group was accomplished with distilled water for the test substantiation. Earthworms were placed into seven petri dishes in 7 groups, each containing five earthworms. Five dishes were used for the five different concentrations of methanolic extract of *J. gossypiifolia*, one for the reference standard and another for the control group. When there was no movement observed except that the worm was shaken vigorously, the paralyzing time was counted. After assuring that the worms moved neither when vigorously shaken nor when dipped in warm water (50°C), death time was recorded.

2.7. Membrane Stabilizing Activity

Membrane stabilizing activity was assessed by using hypotonic-solution and heat induced haemolysis of human erythrocyte by the method developed by Omale and Okafor [22].

2.7.1. Collection of Blood Samples

2 ml of Venus blood was collected from each of the healthy Bangladeshi human volunteers (aged 20 to 25 years) without a history of anticoagulant or oral contraceptive therapy including free from any harmful diseases. The protocol was in accordance with the Helsinki Declaration of 1975 (revised in 2008) and be approved by the institutional ethics committee. The participants were given a written informed consent and all the participants right to privacy were considered strictly. Under standard conditions including temperature (23±2°C) and relative humidity (55±10%), the blood was kept in a test tube with an anticoagulant Ethylene-diaminetetraacetic acid (EDTA).

2.7.2. Preparation of Erythrocyte Suspension

To prepare the erythroocyte suspension, blood was centrifuged for 10 min at 3000g to get RBC (Red Blood Cell) and then it was washed three times using isotonic solution (0.9% saline solution). The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained 1L of distilled water: NaH$_2$PO$_4$, 2H$_2$O, 0.26 g; Na$_2$HPO$_4$, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). Thus, the suspension finally amassed as the stock erythrocyte suspension.

2.7.3. Hypotonic Solution-Induced Hemolysis

0.5 ml stock erythrocyte suspension was mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered
saline (pH 7.4) containing either the leaves or stem extracts (2.0 mg/ml) or acetyl salicylic acid (0.1 mg/ml). The control sample was prepared by 0.5 ml of erythrocyte suspensions, mixed with hypotonic-buffered saline alone. The mixtures were incubated for 10 min at room temperature, centrifuged for 10 min at 3000g and the absorbance of the supernatant was measured at 540 nm using UV spectrometer [23]. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation:

% Inhibition of hemolysis = 100 × (OD1-OD2/OD1)

Where, OD1 = Optical density of hypotonic-buffered saline solution alone (control) and OD2 = Optical density of test sample in hypotonic solution.

2.7.4. Heat-induced Hemolysis

5 ml of the isotonic buffer along with 2.0 mg/ml of extracts of the plant were put into two duplicate sets of centrifuge tubes. The same amount of vehicle was added to another tube considered as control. Erythrocyte suspension (30 μl) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54°C for 20 min in a water bath. The other pair was maintained at 0 to 5°C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300g and the absorbance of the supernatant was measured at 540 nm using UV spectrometer [23]. The percentage inhibition or acceleration of hemolysis in tests was calculated using the following equation:

% Inhibition of hemolysis = 100 × [1 - (OD2-OD1/OD3-OD1)]

Where, OD1 = test sample unheated; OD2 = test sample heated and OD3 = control sample heated.

2.8. Statistical Analysis

All data were presented as mean ± standard deviation (SD) (standard deviation) and were analyzed by One-way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) for windows, version 18.0, IBM corporation, NY, USA and MS Excel for windows version 2010®. The values were considered significantly different at p<0.05.

3. Results and Discussion

3.1. Phytochemical Screening

The preliminary phytochemical evaluation of methanolic extract of *J. gossypiifolia* confirmed the presence of glycosides, tannins, phytosterols, triterpenoids, diterpenes, saponins and phenols both at its leaves and stem portions (Table 1).

3.2. Antioxidant Activity

3.2.1. Determination of Total Phenolic Content

Total phenolic compounds were reported as Gallic acid equivalents by reference to a standard curve (y = 0.012x + 0.036, R² = 0.992). According to the absorbance values of the various extract solution, react with Folin-ciocalteu reagent & compared to the standard solution of the Gallic acid equivalents, results of the colorimetric analysis of total Phenolic are given in Table 2. Here the leaves cover the
Biological Investigation of *Jatropha gossypiifolia*

3.2.1. Total Phenolic Content

The results of total phenolic content suggest that the plant may possess good antioxidant behavior. The leaves portion showed the highest phenolic content (65.66 mg of GAE/g) than the stems portion (33.332 mg of GAE/g).

3.2.2. Free Radical Scavenging Activity by DPPH Method

In this investigation, the crude methanolic extract of *J. gossypiifolia* leaves and stems showed the free radical scavenging activity with IC50 (50% inhibition Concentration) value of 3.186 µg/ml and 10.56 µg/ml respectively and the maximum inhibition was found as 89.06% and 74.37% respectively. On the other hand, the standard BHT showed maximum inhibition of 94.06 and 50% inhibitory concentration (IC50) was found as 20.73 µg/ml (Figure 1).

3.2.3. Anthelmintic Activity

From Table 3, it is observed that the gradual increase of sample concentration of methanolic extract of *J. gossypiifolia* demonstrates paralysis as well as death of worms in fewer times. At the concentration of 80 mg/ml, the methanolic extract of leaves and stems showed paralysis time of 5.38±1.58 and 5.00±1.58 min and death time of 8.53±1.57 min, 8.61±1.53 min.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Methanolic extraction</th>
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<tbody>
<tr>
<td></td>
<td>Leaf portion</td>
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<tr>
<td>Alkaloids</td>
<td>-</td>
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<tr>
<td>Carbohydrates</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
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<td>Tannins</td>
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<td>Phytosterols</td>
<td>+</td>
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<td>Triterpenoids</td>
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<td>Saponins</td>
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<td>Phenols</td>
<td>+</td>
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<tr>
<td>Flavonoid</td>
<td>-</td>
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<tr>
<td>Protein and amino acid</td>
<td>-</td>
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<tr>
<td>Diterpenoids</td>
<td>+</td>
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</table>

Present (+), Absent (-)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. (µg/ml)</th>
<th>Abs of extracts</th>
<th>Average Abs. (Y)</th>
<th>Total phenolic content (mg GA/gm extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>250</td>
<td>0.234</td>
<td>0.234 ± 0.05</td>
<td>65.66</td>
</tr>
<tr>
<td></td>
<td>0.233</td>
<td>0.137</td>
<td>0.136 ± 0.06</td>
<td>33.332</td>
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Data represent mean ± standard deviation (n=3) of duplicate analysis.
respectively. At the concentration of 60 mg/ml, methanolic extract of leaves and stem showed paralysis time of 9.83±1.58 and 10.23±1.00 min and death time of 12.68±1.00 min and 11.57±1.00 min respectively. These results were compared to the standard albendazole for which paralysis time was claimed at 8.20±0.53 min and death time 35.98±1.2 min at a concentration of 10 mg/ml.
3.2.4. Membrane Stabilizing Activity

The crude methanolic stem and leaves extract of *J. gossypiifolia* was conducted to attempt for membrane stabilizing activities using standard protocols and the results presented in Table 4. The results showed that both extracts were significantly potent on human erythrocyte, decently defending it against hypotonic solution and heat induced lyses, when compared to the standard drug acetyl salicylic acid. The leaves and stem methanolic extracts were found both at hypotonic solution and heat induced conditions, inhibit 39.19±0.26%, 38.17±0.14% and 27.39±0.15%, 26.97±0.47% hemolysis of erythrocyte membrane respectively. While at the same conditions, acetyl salicylic acid inhibited 50.36±0.26% and 41.28±0.39% hemolysis of erythrocyte respectively.

Table 4. Effect of *J. gossypiifolia* leaves and stem extract on hypotonic solution and heat induced hemolysis of erythrocyte membrane.

<table>
<thead>
<tr>
<th>Standard/sample</th>
<th>% inhibition on hemolysis</th>
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<tbody>
<tr>
<td></td>
<td>Hypotonic solution induced</td>
</tr>
<tr>
<td>MEL</td>
<td>39.19±0.26*</td>
</tr>
<tr>
<td>MES</td>
<td>38.17±0.14*</td>
</tr>
<tr>
<td>ASA</td>
<td>50.36±0.26</td>
</tr>
</tbody>
</table>

MEL= Methanolic extract of leaves, MES= methanolic extract of stem, ASA= Acetyl salicylic Acid, each value is presented as mean ± standard deviation (n = 5). Data are found to be significant by testing through one-way ANOVA at 5% level of significance * P<0.05 when compared to the standard.

3.3. Discussion

The methanolic extract of the stems and leaves of *Jatropha gossypiifolia* belonging to the family Euphorbiaceae were investigated for their biological activities. Investigation of *In-vitro* phytochemical screening of leaves and stems of *Jatropha gossypiifolia* showed high-quality end result. The both plant parts contain glycosides, tannins, phytosterols, triterpenoids, diterpenes, saponins and phenols. From the previous study, it was confirmed that phenolic compounds have anti-oxidative, antidiabetic, anticarcinogenic, antimutagenic and anti-inflammatory activity [24] and other phytochemicals present in extract are also evident for having active residences towards diverse diseases [25].

There is a considerable recent proof that free radicals induce oxidative damage to biomolecules. This damage causes atherosclerosis, aging, cancer and several other pathological events in living organisms [26]. Antioxidants which scavenge free radicals are regarded to possess an important role in preventing these free radical induced-diseases. There is an increasing interest in the antioxidants...
effects of compounds derived from plants sources. This could be applicable in relations to their nutritional prevalence and their role in health and diseases [27, 28]. A number of reviews on the isolation and checking out of plants derived antioxidants have been described during the past decade. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids [29-31]. The total phenolic content was determined by Folin-Ciocalteu reagent using the method designed by Majhenic et al., [15], reported that compared to the standard solution of the Gallic acid equivalents, the leaves cover the highest Phenolic content (65.66 mg GA/gm extract) than the stems portion (33.332 mg GA/gm extract). As former studies evidenced that higher the quantity of total phenolic contents in a plant extract is, the better is its antioxidant property [32, 33]. Consequently, it may be said that the presence of higher total phenolic components is accountable for demonstrating the antioxidant activity and free radical scavenging ability of the plant.

Lipid peroxidation is one of the main reasons for deterioration of food products at some point of processing and storage. Synthetic antioxidant such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are extensively used as food additives to increase self-life, especially lipid and lipid containing products by way of retarding the process of lipid peroxidation. However, BHT and BHA are recognized to have not only toxic and carcinogenic effects on humans [34-35], but anomalizing effects on enzyme systems [36]. Therefore, the interest in natural antioxidant, particularly plant origin, has greatly improved in recent years [37].

For testing radical scavenging activity of various plant extracts, most commonly DPPH assay is considered as a rapid approach [38]. Different part of methanolic extracts of Jatropha gossypiifolia were subjected to free radical scavenging activity developed by the method of Brand-Williams et al., [18]. Here, tert-butyl-1-hydroxytoluene (BHT) was used as standard. The crude methanolic extract of leaves and stems exhibit remarkable IC50 value, i.e. only 3.186 µg/ml and 10.56 µg/ml respectively (Figure 01).

The anthelmintic activity of the plant extract both at leaves and stems were in a dose dependent manner whilst comparing to the standard. The study showed that the extract had the potential of not only paralysis but also affirm demise of earthworms, relating to the concentrations of the extract. Studies showed that phenolic compounds have the ability that can interpose by uncoupling oxidative phosphorylation with the energy genesis in helminthic parasites [39]. At the concentration of 80 mg/ml, the methanolic extract of leaves and stem showed paralysis time of 5.38±1.58 and 5.00±1.58 min and death time of 8.53±1.57 min, 8.61±1.53 min respectively. These results were compared to the standard albendazole for
which paralysis time was claimed at 8.20±0.53 min and death time 35.98±1.2 min at a concentration of 10 mg/ml.

Previous reports showed that plants having the property of decreasing the risk of developing inflammatory and other related diseases, are rich in flavonoids and other phenolic compounds [40]. Compounds having membrane-stabilizing properties interfere with the early phase of inflammatory reaction [41]. Phytochemical screening confirms the presence of phenol and triterpenoid in the present plant. The extract at a concentration of 2 mg/ml promptly protected the lysis on human erythrocyte membrane hastened by hypotonic solution as well as heat induced solution at a significant level while comparing to the standard acetyl salicylic acid (0.1 mg/ml). This proposes that the plant extract both at leaves and stem section may possess good membrane stabilizing activity.

4. Conclusion

*Jatropha gossypiifolia* is a potential ethnopharmacological indigenous plant in Bangladesh. In view of development of new drugs from *Jatropha gossypiifolia* (Fam. Euphorbiaceae), several pharmacological and biological studies have been performed. Results of these studies are promising. Test results suggest that the crude methanolic extracts possess potentiality in antioxidant activity as well as anthelmintic and membrane stabilizing potentialities. Therefore, further chemical studies with methanol extract of *Jatropha gossypiifolia* for developing new bioactive compounds and evaluation of their exact mode of action and chronic toxicity profile may be the next approach to be followed.

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Conflict of interest

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

References


