

Research Article

# Effect of Methanolic Extract of *Justicia Flava* on Petroleum-Induced Reduced Cell Division and DNA Damage

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## Abstract

**The objective** of this research was to determine the import of *Justicia flava* methanolic extract against petroleum-stimulated reduced cell division and DNA fragmentation.

**Materials and Methods.** Ninety rats were distributed and treated as follows for thirty days: Group A - normal diet; Group B - normal feed + 100mg/kg bwt of *Justicia flava* methanolic extract; Group C - normal feed + 200mg/kg bwt of *Justicia flava* methanolic extract; Group D - normal feed + 300mg/kg bwt of *Justicia flava* methanolic extract; Group E - normal feed + 250 mg/kg bwt of standard ascorbic acid; Group F - crude oil-tainted feed; Group G - crude oil-tainted feed + 250 mg/kg bwt of standard ascorbic acid; Group H - crude oil-tainted feed + 100mg/kg bwt of *Justicia flava* methanolic extract; Group I - crude oil-tainted feed + 200mg/kg bwt of *Justicia flava* methanolic extract. After thirty days, the rats were sacrificed under chloroform sedation and the liver and kidney were collected for cellular analysis via standard protocols.

**Results.** The results showed that administration of *Justicia flava* methanolic extract decreased petroleum-induced reduced cell division and DNA damage in the liver and kidney of experimental rats. *Justicia flava* methanolic extract is potent antidote for petroleum-induced DNA damage in the liver and kidney of rats.

**Conclusions.** It is no doubt that ingestion of petroleum-tainted diet could culminate in DNA damage in organs and tissues of animals. However, the administration of *Justicia flava* methanolic extract was found to prevent this damage in renal and liver cells of experimental rats.

## Keywords

DNA; petroleum; *Justicia flava*; liver; kidney

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## Problem statement and analysis of the latest research

Across oil producing areas of the world, one negative consequence is the contamination of food and water [1, 2, 3, 4]. Exposure to crude oil and chemical derivatives has been reported to cause the impairment of hepatic and renal functions [5, 6]. The liver and kidneys are two important organs in animals. The liver is the principal organ of metabolism

and helps in the detoxification of chemical compounds, while the kidneys assist in the elimination of deactivated and solubilized toxicants from the body [7, 8, 9, 10]. Studies have shown a multiplicity of adversarial histopathological alterations due to exposure to crude petroleum [11]. Crude oil is also a nephrotoxicant. Previous studies have reported the nephrotoxicity effects of hydrocarbons [15]. Derangements in these two organs portend great danger to the body since toxicants cannot be de-

activated and eliminated from the body. This gives rise to the accumulation of metabolic waste in the blood and induction of myriads of toxicities and medical derangements in animals [4].

Polycyclic aromatic hydrocarbons are highly reactive substances that cause oxidative damage to DNA and induced micronucleated erythrocytes [16, 17, 18, 19]. The genotoxicity of petroleum in humans have been evaluated [20]. Moreover, various substances have been used as counter measures to crude oil toxicities. These substances include various vitamins [21, 22, 23, 24] organic and inorganic substances [9, 5, 25] and various plant extracts [9, 26, 27]. A review of literature shows that there is one important plant with a lot of curative ability [27]. This plant extract has not been tested against crude oil toxicity.

**The objective of this research** was to assess the protective potentials of *Justicia flava* methanol extract on crude oil-tainted diet-induced reduction in cell division and cell damage in Wistar albino rats.

## 1. Materials and Methods

The reagents used were of quality analytical grade, produced by British Drug House, Poole, England. Bonny Light crude oil was obtained from the Warri Refining and Petrochemical Company, Warri, Nigeria in May 2018. Crude oil was stored in the refrigerator to avoid evaporation of volatile components. The leaves of *Justicia flava* were collected from a fallow land in Obiaruku, Delta state, Nigeria and taken to Dr. H.A. Akinnibosun of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria for proper identification with a specimen number UBHJ0386 kept at the herbarium. Ninety Wistar male albino rats (weighing between 185–195 g) were obtained from the Animal house in the College of Medicine, Delta State University, Abraka, Nigeria. The rats were kept in plastic cages and fed with fish feed. This was to make room for acclimatization for two weeks on fish feed and laboratory conditions as fish feed is not the usual rat chow. The rats were managed following the Guide for the Care and Use of Laboratory Animals by the National

Research Council [15].

### 1.1 Preparation of plant extract

The leaves of *Justicia flava* were washed and air dried at laboratory of the Department of Biochemistry, Delta State University, Abraka, Nigeria at room temperature for one week. After drying, the leaves were separated from the stem and macerated to a smooth dry powder using a waring blender. The extracts of *Justicia flava* leaves were prepared using methanol extraction. One hundred gram of the ground leaves was dissolved in 400 mL of methanol by sonication for 10 min. The mixture was filtered with Whatman No.1 filter paper with vacuum pump. The extract was then concentrated using rotary evaporator at 40–50°C under reduced pressure to obtain the *Justicia flava* leaf methanol extract (JFME). The filtrate was evaporated to dryness using a water bath which produced a yield of 13.91 g/100 g of ground leaves. The extract was stored at -8°C until required [9].

### 1.2 Preparation of extracts for treatment

Exactly 2.5 g of JFME was dissolved in 25 mL of distilled water to make a concentration of the extract equivalent to 0.1g/mL. From this stock aliquots extract was administered by oral gavage in line with the rat body weight at 7 a.m. daily.

### 1.3 Experimental design

The rats were distributed into 9 groups per ten rats in each group and treated as follows:

- Group A - normal diet;
- Group B - normal feed + 100mg/kg bwt of JFME;
- Group C - normal feed + 200mg/kg bwt of JFME;
- Group D - normal feed + 300mg/kg bwt of JFME;
- Group E - normal feed + 250 mg/kg bwt of standard ascorbic acid;
- Group F - crude oil-tainted feed;
- Group G - crude oil-tainted feed + 250 mg/kg bwt of standard ascorbic acid;
- Group H - crude oil-tainted feed + 100mg/kg bwt of JFME;

- Group I - crude oil-tainted feed + 200mg/kg bwt of JFME.

The extracts were administered by dissolving the appropriate dose in 1 ml of distilled water and given by oral gavage. The contaminated feed was prepared by mixing 4 mL of crude oil with 100 g of feed. The concentration has been established to be tolerable by rats on prolong basis [6]. Each set of feed was prepared every day and drinking water was liberally provided.

#### 1.4 Tissue collection and analysis

After the expiration of the exposure period of thirty days, the rats were subjected to overnight fasting. This was to ensure the stabilization of animals [28]. The animals were sacrificed under chloroform sedation. The organs required, namely the liver and kidneys were collected, washed off blood with distilled water and stored at  $-4^{\circ}\text{C}$  in a deep freezer and used within 6 hours.

#### 1.5 Determination of DNA fragmentation and mitotic cell division

DNA fragmentation was determined using the method of Wu *et al.* [29]. To determine mitotic cell division, liver cells were isolated using the protocol described by Kegel *et al.* [30] while renal cells were isolated using the protocol described by Valente *et al.* [31]. Thereafter, methodology of Sharma AK, Sharma A. [32] was adopted for the analysis of mitotic cell activities using compound light microscope.

#### 1.6 Statistical Analysis

Data were reported as mean  $\pm$  SD and were analyzed with analysis of variance (ANOVA). Significant differences between all treatment means were set at  $p < 0.05$  confidence limit using Duncan's Multiple Range Test [19].

## 2. Results and Discussion

The use of enzyme activities and protein levels in assessing liver integrity and kidney status markers is widespread in scientific investigations [8, 24]. Therefore, there is a need to explore the harmful

import of petroleum hydrocarbon, especially at the cellular level. Based on this, the present investigation attempted the use of JFME against petroleum-mediated damages in hepatic and renal cells by way of genotoxic analyses.

In this study, ingestion of crude oil-tainted diet induced increase in DNA fragmentation in hepatic cells of the experimental animals. Table 1 showed a significant ( $p < 0.05$ ) increase in DNA fragmentation in rats fed crude petroleum-tainted diet as compared to rats fed normal diet. Similarly, a significant ( $p < 0.05$ ) decrease in mitotic cell division was caused by intake of petroleum-tainted feed. This observation coincided with the previous report on hydrocarbon stimulated DNA fragmentation and decreased cell division [34, 35, 36]. Nevertheless, the administration of JFME reduced petroleum-induced DNA damage in liver cells and improved mitotic cell division. This efficacy is comparable to the reduction in DNA damage and enhanced cell division in rats exposed to petroleum-tainted feed and administered with standard ascorbic acid. In fact, previous studies presented the medical ability of this plant [27]. Similarly, this observation is consistent with the papers where JFME was involved in reducing the damage caused by petroleum-tainted diet in the liver of rats [37].

The degree of DNA fragmentation and reduction in cell division by petroleum and the ameliorative ability of JFME in renal cells are shown in Table 2. Similar to liver cells, the action of the extracts prevented DNA fragmentation and enhanced cell division in the kidneys; this can be compared to the efficacy of standard ascorbic acid against petroleum-induced kidney damage (Table 2). The protective potential of this plant extract is in tandem with a variety of plant extracts against chemical toxicity [38, 39, 40].

Moreover, the efficacy of JFME was corroborated when the effects of the extracts and ascorbic acid on rats exposed to crude oil-tainted feed were compared (Table 1, 2). Previously, many authors used ascorbic acid as an antidote for petroleum toxicity [41, 42]. Therefore, semblances in the action of JFME and standard ascorbic acid against crude oil stimulated DNA damages in the liver and kidneys

**Table 1.** Effect of *Justicia flava* leaf treatment on liver cellular activities of rats exposed to crude oil adulterated diet.

| Groups | %DNA fragmentation       | Number of cells Mitotic activities |                           |
|--------|--------------------------|------------------------------------|---------------------------|
|        |                          | Interphase                         | Prophase                  |
| A      | 0.90 ± 0.10 <sup>a</sup> | 69.30 ± 4.01 <sup>a</sup>          | 13.80 ± 2.40 <sup>a</sup> |
| B      | 0.78 ± 0.31 <sup>a</sup> | 67.12 ± 5.30 <sup>ab</sup>         | 14.50 ± 1.51 <sup>a</sup> |
| C      | 0.85 ± 0.02 <sup>a</sup> | 66.01 ± 4.50 <sup>a</sup>          | 15.42 ± 2.21 <sup>a</sup> |
| D      | 0.71 ± 0.20 <sup>b</sup> | 66.01 ± 5.30 <sup>b</sup>          | 15.91 ± 2.70 <sup>b</sup> |
| E      | 0.61 ± 0.01 <sup>c</sup> | 67.75 ± 2.10 <sup>a</sup>          | 16.41 ± 1.70 <sup>a</sup> |
| F      | 1.05 ± 0.10 <sup>c</sup> | 63.02 ± 4.50 <sup>a</sup>          | 8.98 ± 1.33 <sup>a</sup>  |
| G      | 0.99 ± 0.20              | 64.39 ± 3.45                       | 10.56 ± 2.44              |
| H      | 0.93 ± 0.11              | 64.55 ± 3.52                       | 12.54 ± 1.90              |
| I      | 0.94 ± 0.03              | 64.05 ± 4.43                       | 11.66 ± 2.10              |

Notes: Values are expressed as Mean±SD. Values followed by different alphabet superscript in the same column indicates a significant difference.

**Table 2.** Effect of *Justicia flava* leaf treatment of diet on kidney cellular activities of rats exposed to crude oil adulterated diet.

| Groups | %DNA fragmentation       | Number of cells Mitotic activities |                           |
|--------|--------------------------|------------------------------------|---------------------------|
|        |                          | Interphase                         | Prophase                  |
| A      | 0.85 ± 0.20 <sup>a</sup> | 66.40 ± 3.51 <sup>a</sup>          | 11.30 ± 1.10 <sup>a</sup> |
| B      | 0.81 ± 0.41 <sup>a</sup> | 64.22 ± 6.0 <sup>ab</sup>          | 12.10 ± 1.01 <sup>a</sup> |
| C      | 0.82 ± 0.07 <sup>a</sup> | 65.53 ± 3.40 <sup>a</sup>          | 12.50 ± 2.51 <sup>a</sup> |
| D      | 0.76 ± 0.20 <sup>b</sup> | 64.21 ± 3.35 <sup>b</sup>          | 11.95 ± 1.80 <sup>b</sup> |
| E      | 0.74 ± 0.01 <sup>c</sup> | 63.40 ± 3.50 <sup>a</sup>          | 13.6 ± 1.40 <sup>a</sup>  |
| F      | 1.03 ± 0.40 <sup>c</sup> | 58.52 ± 2.57 <sup>a</sup>          | 8.08 ± 1.53 <sup>a</sup>  |
| G      | 0.88 ± 0.30              | 58.99 ± 2.55                       | 10.66 ± 2.64              |
| H      | 0.87 ± 0.21              | 60.50 ± 1.60                       | 11.54 ± 1.50              |
| I      | 0.88 ± 0.03              | 66.65 ± 2.40                       | 11.60 ± 2.60              |

Notes: Values are expressed as Mean±SD. Values followed by different alphabet superscript in the same column indicates a significant difference.

give an indication for the use of this plant to treat petroleum poisoning.

The medicinal ability of *Justicia flava* hinges on the presence of various phytochemicals with antioxidant properties [43]. This assertion is not surprising since earlier studies indicated that petroleum stimulated damages are preceded by the formation of free radicals that culminates in macromolecu-

lar damage [44, 45]. Therefore, the potency of petroleum and related chemicals are arrested in the presence of a sufficient amount of antioxidants [9, 46, 47]. The efficacy of JFME against DNA damage may be predicated on the rich bioactive compounds in Acanthaceae family, of which *Justicia flava* is a member [48]. These bioactive compounds include flavonoids which have been ascribed with antioxi-

dant capabilities [49].

### 3. Conclusions

It is concluded that ingestion of petroleum-tainted diet could culminate in reduced cell division and DNA damages in the organs and tissues of animals. However, the administration of JFME prevented this damage in renal and liver cells of experimental rats.

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