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DETOXIFIED *JATROPHA CURCAS* KERNEL MEAL IMPACT AGAINST BENZENE-INDUCED GENETIC TOXICITY IN MALE RATS

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ABSTRACT

Pollution by crude oil is wide spread and a common problem, and particularly endemic in countries whose economies are dependent on the oil industry. Such pollution arises either accidentally or operationally wherever oil is produced, transported, stored, processed or used. Benzene contamination of soil may also occur due to oil production facilities and coastal refineries. This problem arises because most of petrochemicals including benzene and its derivatives are carcinogens and mutagens with the capacity to affect both the structural integrity of DNA and the fidelity of its biologic expression. *Jatropha curcas* (L.) is a multipurpose and drought resistant tree, widespread throughout the tropics and subtropics. Its seeds are rich in oil and protein. *J. curcas* can also be used for human food, animal feed, fertilizer, fuel and traditional medicine. Therefore, the objective of this work is to evaluate protective effect of detoxified *J. curcas* kernel meal (DJKM) against benzene-induced clastogenicity in male rats. Eighty adult male rats were allocated in several groups treated with benzene and/or several concentration of DJKM. The results revealed that low, medium and high doses of DJKM did not increase the DNA fragmentation, incidence of MnPCEs and alterations in the stress related genes (Hsp70a, MT and CYP450 genes) compared with benzene induced high levels of these parameters. The protection effects of DJKM on the genetic toxicity were attributed to its imact on the glutathione peroxidase activity which was increased significantly with DJKM treatment compared to benzene exposure.

Keywords: DNA damage, Micronucleus formation, Gene expression, Glutathione peroxidase activity, Rats

INTRODUCTION

Millions of workers in various occupational settings have the potential to be exposed to hazardous substances. These substances, including dusts, fibers, and organic or inorganic chemicals, are used as raw materials, intermediates, by-products or end products in industrial processes. They can exist in the form of gases, vapors, fumes, mists or particles. Inhalation is the primary route of exposure, however, exposure can also take place through dermal absorption or ingestion [1–2].

Many of these substances are now known to be genotoxic and have the potential to cause genetic alterations in the target tissues of exposed workers. Such alterations, if they occur in proto-oncogenes or tumor suppressor genes, which are involved in controlling cell growth or differentiation, may lead to the development of cancer in the target organs [1–2].

A large number of industrial workers from petroleum, rubber, paint, shoe making, printing, solvent and other chemical industries are occupationally exposed to benzene [1–2].

They are primarily exposed during the production of petrochemicals, petroleum refining, coke and coal chemical manufacturing, rubber tire manufacturing, and storage or transport of benzene and petroleum products containing benzene. Steel workers, printers, rubber workers, shoe makers, laboratory technicians, firefighters and gas station employees may also be exposed to benzene to certain extent. Studies on genotoxicity show that benzene is clastogenic in humans and induces CAs in peripheral lymphocytes [3]. In another study, workers exposed to benzene for

various time periods showed a significantly higher proportion of chromosome breaks [4]. Some studies involving individuals with long term occupational exposure to benzene also suggests that benzene damage chromosomes in hematopoietic cells. A slight increase in SCEs was observed in peripheral lymphocytes from workers exposed to benzene [5]. Other evidence of genotoxicity induced by benzene is the inhibition of DNA synthesis in certain cell types [6]. Epidemiological studies have shown acute myeloid leukemia in benzene exposed workers [7].

Jatropha curcas (Linnaeus) belongs to the family Euphorbiaceae and is thus closely related to other important cultivated plants like rubber tree and caster etc. The plant is believed to be a native of South America and Africa but later spread to other continents of the world by the Portuguese settlers [8]. The Arabs have been using this plant for medicine purpose. Today it is found in almost all the tropical and sub-tropical regions of the world. There are more than 200 different names for its great significance to man and the various possibilities of its uses. When the botanist Carl Von Linne first classified the plants in 1753, gave it the botanical name "Jatropha curcas" from the Greek word "Jatros" meaning a "Doctor" and "trophe" meaning "nutrition". Even Linne had realised the potential of this plant for medicinal purposes [8]. The plant is regarded as a shrubs/small tree with height generally ranging from 3-5 metre (m). It has been estimated that the life of the plant is up to 50 years. Different varieties of the plant can be found which are generally Cape Verde, Nicaragua, Ife-Nigeria, non-toxic Mexico [9].

The latex of *Jatropha curcas* contains an alkaloid known as "Jatrophine" which is believed to be having anti-cancerous properties. Curcas oil possess purgative properties (urging does 0.3 to 0.6 cc or 5 to 10ml). It differs from castor oil in that it has a low viscosity. It is used as castor oil in that it has a low viscosity. It is used as an external application for skin diseases and rheumatism, it is reported to be abortificient and also efficacious in dropsy, sciatic and paralysis. It can also be found that the Tender twigs of the plant are used for cleaning teeth. The juice is reported to relive toothache and strengthen gums. The leaf juice is used as an external application for piles. It is also applied for inflammations of the tongue in babies. The twig sap is considered stypic and used for dressing wounds and ulcers. An emulsion of the sap with benzyl benzoate is said to be effective against scabies, wet eczema and dermaties. A decoction of leaves and roots is given for diarrhea. The root is reported to contain yellow oil with strong antelmintic action. The root bank is used to external application for sores. A decoction of the bark is given for rheumatism and leprosy. Similarly, roots are also reported to be used as antidote for snakebite [8].

Reactive oxygen species (ROS) occur naturally in most eukaryotic cells because energy metabolism depends on oxygen, though stresses enhance these molecules [10]. Due to high levels of phospholipid unsaturation in mitochondria and chloroplasts, these organelles are considered likely targets of damage induced by abiotic stresses [10]. In an attempt to alleviate such damage, plants developed two antioxidant systems, an enzymatic and a nonenzymatic one, to protect plant cells [10]. Two examples of the enzymatic scavenger system are catalase (CAT), (EC 1.11.1.6) and ascorbate peroxidase (APX) (EC 1.11.1.11). CAT is an enzyme present in all aerobic eukaryotes that participate in the dismutation of hydrogen peroxide (H2O2) into oxygen and water [10]. APX promotes dismutation of H2O2 and uses the oxidized ascorbate as electron acceptor, resulting in production of H2O, O2, and reduced ascorbate [10].

The main objective of the present study is to evaluate the efficacy of detoxified *Jatropha curcas* seed meal on petrochemicals such as benzene induced genetic toxicity. Also, the toxicological effects of the treatment at supplementation doses of *Jatropha curcas* were determined. The aim of work was performed using analysis at the DNA fragmentation assay, micronucleus test and gene expression levels for several stress genes were evaluated. Moreover, biochemical markers for antioxidants using animal models were compared to normal features.

MATERIALS AND METHODS

Preparation of the Jatropha meal: Jatropha seeds were obtained from local farm in Saudi Arabia and deshelled manually to obtain kernels. Defatting of Jatropha kernals was performed using petroleum benzene (b.p. 40–60 ºC) in a Soxhlet apparatus according to Kumar et al. [11]. Organic solvents (alkaline methanol) were used to detoxify defatted Jatropha kernel meal [11]. After removal of phorbol esters (PEs), the meal was autoclaved (121 ºC) to remove heat-labile antinutrients, trypsin inhibitor and lectin.

Experimental animals: One eighty adult male rats with an average body weight of 110.5 ± 4.7 g (range 100–120 g), from the Biology Department, College of Science, King Abdul Aziz University, Saudi Arabia, were used in this study. The animals were housed in plastic cages, ten per cage. They were maintained on standard laboratory diet (protein,

16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum* at the Animal House Laboratory, Biology Department, College of Science, King Abdul Aziz University, Saudi Arabia. All animals were left to acclimatize into the new place for 1 week before the treatment started. They were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the College of Science, King Abdul Aziz University, Saudi Arabia.

Experimental design: The animals were assigned to several groups (n=10) as follows: Group 1: Normal healthy animals were serving as the control group. Group 2: Animals were injected orally with benzene (10 ppm, [12]) daily for 8 weeks. Group 3: Animals were fed on standard diet replaced by 25% of detoxified jatropha kern meal (DJKM25) daily for 8 weeks. Group 4: Animals were fed on standard diet replaced by 50% of detoxified jatropha kern meal (DJKM50) daily for 8 weeks. Group 5: Animals were fed on standard diet replaced by 75% of detoxified jatropha kern meal (DJKM75) daily for 8 weeks. Groups 6, 7 and 8: Animals were fed as in groups 3, 4 and, respectively, plus injection orally with benzene (10 ppm) daily for 8 weeks. The dose of detoxified jatropha kern meal (25, 50 and 75%) was chosen according to Kumar et al. [11], while the dose of benzene (10 ppm) was chosen according to Al-Sabt [12].

DNA fragmentation analysis using diphenylamine reaction procedure: Liver tissues of male rats were used to determine the quantitative profile of the DNA fragmentation. Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer containing, 10 mM tris-HCl (pH 8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10 000 rpm (Eppendorf) for 20 min at 4°C. The pellets were re-suspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 0.5 ml of 25% tri-chloroacetic acid (TCA) were added and incubated at 4°C for 24 h. The samples were then centrifuged for 20 min at 10 000 rpm (Eppendorf) at 4°C and the pellets weresuspended in 80 ml of 5% TCA, followed by incubation at 83°C for 20 min. Subsequently, to each sample 160 ml of DPA solution [150 mg DPA in 10 ml glacial acetic acid, 150 ml of sulfuric acid and 50 ml acetaldehyde (16 mg:ml)] wereadded and incubated at room temperature for 24h [13]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm wavelength using the formula:

$$\%\text{Fragmented DNA} = \frac{OD(S)}{OD(S) + OD(P)} \times 100$$

Micronucleus test by acridine orange fluorescent staining: Acridine orange staining of erythrocytes was performed according to Ueda et al. [14]. To assess this assay, ten rats from each location were sacrificed after exposure period. The bone marrow cells were collected from all animals and re-suspended in a small volume of fetal calf serum (FBS; Sigma) on a 0.003% acridine orange-coated glass slide. The slide was then covered with a cover glass to prepare bone marrow specimens. Slides were dried overnight and fixed with methanol for 10 min. bone marrow specimens were examined in a blinded manner using fluorescence microscopy at 600X or higher magnification with a blue excitation wavelength (e.g. 488 nm) and yellow to orange barrier filter (e.g., 515 nm long pass). Two slides per one animal were labeled to get blinded micronuclei scoring. To avoid obtaining unbiased results, the slides were observed once by one observer who has sufficient experience of micronucleus test. The number of micronucleated polychromatic erythrocytes (%MnPCEs) were measured at a rate of 3000 polychromatic erythrocytes (PCEs) per one animal.

Gene expression analysis

1. Isolation of total RNA: TRIzol® Reagent (cat#15596-026, Invitrogen, Germany) was used to extract total RNA from liver tissues of male rats according to the manufacturer's instructions with minor modifications.

Briefly, tissue samples (50 mg) were homogenized in 1 ml of TRIzol® Reagent. Afterwards, the homogenized samples were incubated for 15 min at room temperature. A volume of 0.2 ml of chloroform per 1 ml of TRIzol® Reagent was added. Then, the samples were vortexed vigorously for 15 seconds and incubated at room temperature for 3 min. The samples were centrifuged for no more than 12 000 g for 15 min at 4 °C. Following centrifugation, the mixture was be separated into lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA was remained exclusively in the aqueous phase. Therefore, the upper aqueous phase was carefully transferred without disturbing the interphase into a fresh tube. The RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol. A volume of 0.5 ml of isopropyl alcohol was added per 1 ml of TRIzol® Reagent used for the initial homogenization. Afterwards, the samples were incubated at 15 to 30 °C for 10 min and centrifuged at not more than 12,000 x g for 10 min at 4 °C. The RNA was precipitated which was often invisible before centrifugation, formed a gel-like pellet on the side and bottom of the tube. The supernatant was removed completely. The RNA

pellet was washed once with 1 ml of 75% ethanol. The samples were mixed by vortexing and centrifuged at no more than 7 500 g for 5 min at 4 °C. The supernatant was removed and RNA pellet was air-dried for 10 min. RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water by passing solution a few times through a pipette tip.

Purity of total RNA was assessed by the 260/280 nm ratio which was between 1.8 and 2.1. Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for reverse transcription (RT), otherwise they were stored at -80°C.

2. Reverse transcription (RT) reaction: The complete Poly(A)$^+$ RNA isolated from male rats from all groups tissues were reverse transcribed into cDNA in a total volume of 20 µl using RevertAid^{TM} First Strand cDNA Synthesis Kit (Fermentas, Germany). An amount of total RNA (5µg) was used with a master mix (MM). The MM was consisted of 50 mM $MgCl_2$, 10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, 50 µM oligo-dT primer, 20 IU ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 IU MuLV reverse transcriptase. The mixture of each sample was centrifuged for 30 sec at 1000 g and transferred to the thermocycler. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with a denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for cDNA amplification through Real Time polymerase chain reaction (RT-PCR).

3. Quantitative Real Time- PCR (qRT-PCR): An iQ5-BIO-RAD Cycler (Cepheid. USA) was used to determine the male rats cDNA copy number. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction conditions consisted of denaturation at 95.0 °C for 3min, 30 cycles of denaturation at 95.0 °C for 15 sec, annealing at 60.0 °C for 30 sec and extension at 72.0 °C for 30 sec and then final step consisted of several cycles at 60.0°C for 10 sec with an increase of 0.5°C until 95°C. Each experiment included a distilled water control. The sequences of specific primer of the genes used [15, 16] and product sizes are listed in (Table 1). At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers.

4. Calculation of gene expression: First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae [17]:

$Ef = 10^{-1/slope}$

Efficiency (%) = (Ef – 1) x 100

The relative quantification of the target to the reference was determined by using the

ΔC_T method if E for the target (Hsp70a, MT and CYP450) and the reference primers (β-Actin) are the same [17]:

$Ratio_{(reference/\ target\ gene)} = Ef^{C_T(reference) - C_T(target)}$

Biochemical analysis: Oxidation parameters for stress were estimated in serum as follow:

Determination of enzyme activity: Determination of glutathione peroxidase activity: glutathione-S-transferase activity measurements were carried out according to Miranda et al. [18]. The reaction mixture was consisted of 8 mM H2O2, 40mM guaiacol, 50 mM sodium acetate buffer, pH 5.5 and a suitable amount of the enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed at 30 s intervals. One unit of glutathione peroxidase activity was defined as the amount of enzyme which increases the O.D. 1.0/min under standard assay conditions.

Statistical analysis: All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System followed by Scheffé-test to assess significant differences between groups. The software was used is SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). The values were expressed as mean±SEM. All statements of significant were based on probability of $P < 0.05$.

RESULTS

Effects of different concentrations of DJKM on DNA fragmentation induced by benzene: Assessment of DNA fragmentation in liver tissues of male rats' genome following benzene treatment as a surrogate for oxidative stress induced damage is summarized in Figure 1.

The results showed that the DNA fragmentation following DJKM25, DJKM50 and DJKM75 treatments were relatively similar to that of the control group. However, the DNA fragmentation increased to 337.5% following benzene treatment in comparison to that of the control group (Fig 1).

In contrary, the DNA fragmentation decreased significantly following DJKM25+benzene, DJKM50+benzene and DJKM75+benzene treatments compared with benzene alone (Fig. 1). Moreover, the lowest DNA fragmentation induced by benzene was observed by DJKM75+benzene treatment.

Effects of DJKM on micronucleus formation induced by benzene: Effect of different concentrations of DJKM on micronucleated polychromatic erythrocytes (MnPCEs) formation in the bone marrow cells of male rats is summarized in Table (2). The results showed that low, medium and high doses of DJKM did not increase significantly the incidence of MnPCEs in comparison to control group (Table 2). However, exposure of male rats with benzene treatment increased significantly the incidence of MnPCEs (23.4± 1.3) compared with control group (7.6± 0.4, Table 2).

On the other hand, treatment of male rats with low, medium and high doses of DJKM combined with benzene decreased significantly the MnPCEs formation in the bone marrow cells of male rats (Table 2). Whereas, the higher decrease of MnPCEs formation was showed with the treatment of the high dose of DJKM combined with benzene (14.9± 0.4) in comparison with control benzene alone (23.4± 1.3, Table 2).

Effects of DJKM on the gene expression alterations induced by benzene: Hsp70a, MT and CYP450 mRNA expression were quantified by real-time RT-PCR (Figures 2-4). In DJKM treatment groups (DJKM25, DJKM50 and DJKM75) expression of stress related genes (Hsp70a, MT and CYP450) elevated similar expression values to control group (Figure 2-4). However, benzene exposure increased significantly the mRNA expression values of Hsp70a, MT and CYP450 by 303, 338 and 194% of control group, respectively (Figs 2-4).

In contrast, comparing with benzene treatment alone, expression values of Hsp70a, MT and CYP450 genes decreased significantly in DJKM25+benzene, DJKM50+benzene and DJKM75+benzene groups, respectively (Figs 2-4). Moreover, the lowest expression of Hsp70a, MT and CYP450 genes was observed by DJKM75+benzene treatment (Figs 2-4).

Determination of glutathione peroxidase activity: The present study showed that male rats treated with DJKM25, DJKM50 and DJKM75 represented relatively similar levels of the glutathione peroxidase activity compared to control group (Table 3). However, male rats exposed to benzene showed significantly low levels of the glutathione peroxidase activity compared to control group (Table 3). In contrary, these low levels of glutathione peroxidase activity induced by benzene alone were increased significantly with DJKM25+benzene, DJKM50+benzene and DJKM75+benzene treatments, in which the higher level was showed with DJKM75+benzene (Table 3).

DISCUSSION

The current study was aimed to investigate the efficacy of detoxified *Jatropha curcas* seed meal on petrochemicals such as benzene induced genetic toxicity. The obtained results revealed that DNA fragmentation and incidence of MnPCEs increased significantly following benzene treatment in comparison to those in control rats. In agreement with our finding cell transforming and genotoxic effects of benzene and its metabolites using cultured mammalian cells have been reported [19]. Furthermore, 70% DNA breakage was observed with p-benzoquinone or 1,2,4-benzenetriol exposure , respectively. Benzene metabolites, hydroquinone and benzoquinone are highly potent to induce DNA strand breakage in Chinese hamster ovary cells [20]. The enhanced production of oxygen-derived free radicals (ODFR) by redox-cycling of phenoxy radicals has been implicated in the oxidative damage of DNA [21] Rao [22] suggested glutationyl hydroquinone to be a possible toxic metabolite of benzene with pro-oxidant property for the degradation of DNA. The involvement of ODFR in benzoquinone-induced DNA damage has been observed using the comet assay of peripheral blood mononuclear cells [23]. Additional studies have shown that modulation of topoisomerase II enzyme by benzene's metabolites play a key role in benzeneinduced DNA damage [24, 25].

The current study also observed that alterations in the expression of stress related genes (Hsp70a, MT and CYP450) were found following benzene treatment in comparison to those in control rats. In agreement with our results alterations in gene expression in Swiss mouse spleen lymphocytes exposed to benzene or its metabolites hydroquinone and p-benzoquinone has been reported [26]. Hydroquinone and p-benzoquinone caused 50% inhibition in RNA synthesis. The inhibition of T-cell proliferation and reduced production of interleukin- 2 by p-benzoquinone was suggested to be a possible cause of benzene-induced aplastic anemia [26].
Bioactivation of benzene causes increased generation of ODFR, which in turn activates the oncogene c-

myb gene leading to the formation of leukemic cells [27]. Studies on C57BL/6 normal and p53 knockout mice have shown that benzene suppresses the cell cycle by p53 mediated overexpression of p21, a cyclindependent kinase inhibitor, resulting in dynamic change of haemopoiesis during and after benzene exposure [28]. Gene expression profiles in bone marrow cells from C57BL/6 p53 +/+ and isogenic p53 +/− mice chronically exposed for 15 weeks to 100 ppm of inhaled benzene revealed that p53 homozygous mice expressed significantly higher levels of a majority of key genes involved in p53-regulated DNA damage response pathway compared with p53 heterozygous bone marrow cells [29]. The mice lacking expression of NQO1 and NQO2 protein showed myelogenous hyperplasia of the bone marrow and increased granulocytes in the peripheral blood accompanied by decreased apoptosis [30]. Short-term exposure of NQO1−/− mice to benzene demonstrated substantially greater benzene-induced toxicity as compared with wild type mice [30]. Nitric oxide has also been suggested to be an important mediator of benzene-induced bone marrow suppression and haematopoietic impairment in mice [31].

Additionally, our results revealed that male rats exposed to benzene showed significantly low levels of the glutathione peroxidase activity compared to control group. Glutathione has been suggested to play an important role in protecting against benzene induced chromosome aberrations and sister-chromatid exchange formation by conjugating with toxic metabolites [32]. Tice et al. [33] reported a dose-dependent increase in chromosome aberrations and sister-chromatid exchange following benzene exposure which decreased the levels of antioxidant glutathione levels. Antioxidants are closely related to their biofunctionalities, such as the reduction of cellular abnormalities like DNA damage, mutagenesis, carcinogenesis and which is also associated with free radical propagation in biological systems [34]. One of the main objectives of the present work was to evaluate the potential toxicological effects of the treatment with different doses of *Jatropha curcas*. In addition, the efficacy of detoxified *Jatropha curcas* seed meal on petrochemicals such as benzene induced genetic toxicity was determined. The results of the present work found that DNA fragmentation, incidence of MnPCEs and the expression of stress related genes following DJKM25, DJKM50 and DJKM75 treatments were relatively similar to that of the control group.

In disagreement with our results Oskoueian et al. [35] reported that several extracts of jatropha meal including phorbol esters (PEs) and Phorbol 12-myristate 13-acetate (PMA) induced nucleosome-sized DNA fragmentation. They found that DNA fragmentation was a natural phenomenon that takes place in cells undergoing apoptosis. The presence of DNA cleavage bands in cells treated with PEs indicated the similar cytotoxic effect of PEs to that of PMA. However, our results did not find higher DNA fragmentation attributed to DJKM because this meal used in the present work does not contain phorbol esters. We prepared the meal to be free of the phorbol esters. Additionally the meal was autoclaved (121 ºC) to remove heat-labile anti-nutrients, trypsin inhibitor and lectin.

In addition, the current results revealed that DNA fragmentation, incidence of MnPCEs, and alterations in the expression of stress related genes induced by benzene treatment were decreased significantly following DJKM+benzene treatments. In agreement with our observations, Sundari et al. [36] reported that Jatropha curcus was able to protect human peripheral blood lymphocytes (HPBL) from the DNA damage induced by ultraviolet radiation-B. These results suggested that J. curcas exhibited strong antioxidant property and its extracts protected UVB-radiation-induced DNA damage in HPBL.

The free radical scavenging activity of the *J. curcas* fractions might be due to the presence of steroids and terpenoids which are known to occur in *J. curcas* plant [37]. Previous report showed that high concentrations of the methanol extract have been reported to be more effective in quenching free radicals in the system [38]. Recent reports show that 1,1-diphenyl-2- picrylhydrazyl (DPPH) is a stable free radical that has been used extensively to determine the free radical scavenging ability of various compounds, as a measure of their antioxidant potential [39]. The present study suggests that extracted meal of J. curcas possess a quantitative examination and showed that there was a general trend of significantly higher antioxidant activity.

It has been well established that plant extracts and their active compounds enhances DNA repair mechanism and inhibits DNA strand breaks in radiation exposed cells [40]. Antioxidant potential of J. curcas has been proposed for its anti-genotoxic potential. Because DNA damage induced by benzene is mainly mediated by ROS, acompound with antioxidant potential has capable to intercept the ROS before it attack DNA. Several investigators have demonstrated that natural antioxidants scavenge ROS and protect cellular DNA against agents-induced oxidative damage [41]. Hence, it can be postulated that J. curcas, by virtue of its free radical scavenging

capacity and DNA repairing capacity restore DNA damage induced by benzene. The DNA damage induced by benzene is reduced by J. curcas, meal as measured by the decrease DNA fragmentation, micronucleus formation and gene expression. The antioxidant activity of the J. curcas, meal is attributed to the hydrogen donating ability of phenolic compounds present in the extracts [42].

CONCLUSION

In conclusion, the present study has clearly shown that J. curcas, meal against in vivo free radicals cavenging activity and benzene –induced DNA fragmentation, micronucleus formation and gene expression changes. Further, it offers protection to cellular DNA damage in vivo cells. The antioxidant property of this extract might be the reason for its DNA repair capacity during benzene exposure.

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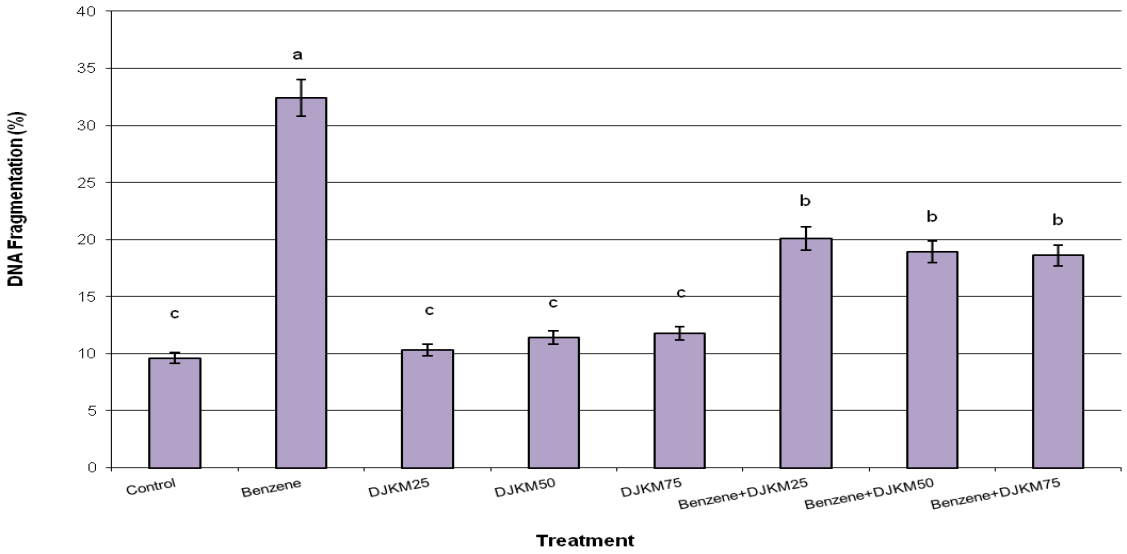

Figure 1: Rate of DNA fragmentation in liver tissues after benzene and/or DJKM treatments. Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (P<0.05, Scheffé-Test). While, [b,ab] Mean values within tissue with similar superscript letters were not significant differences (P>0.05).

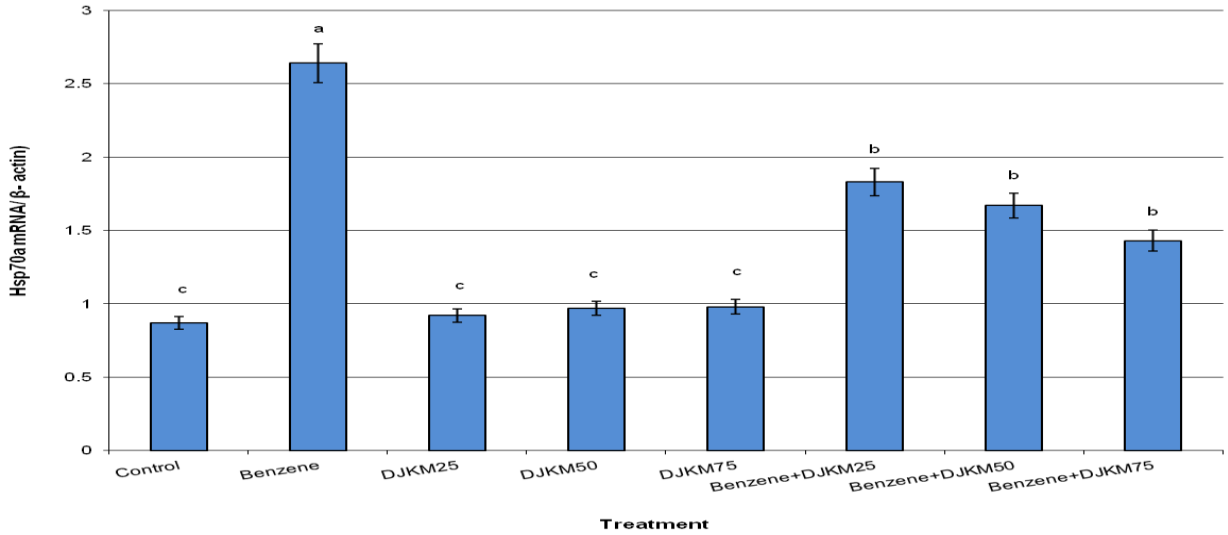

Figure 2: The alterations of Hsp70a mRNA in liver tissues of male rats after benzene and/or DJKM treatments. Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (P<0.05, Scheffé-Test). While, [a,b,c] Mean values within tissue with similar superscript letters were not significant differences (P>0.05).

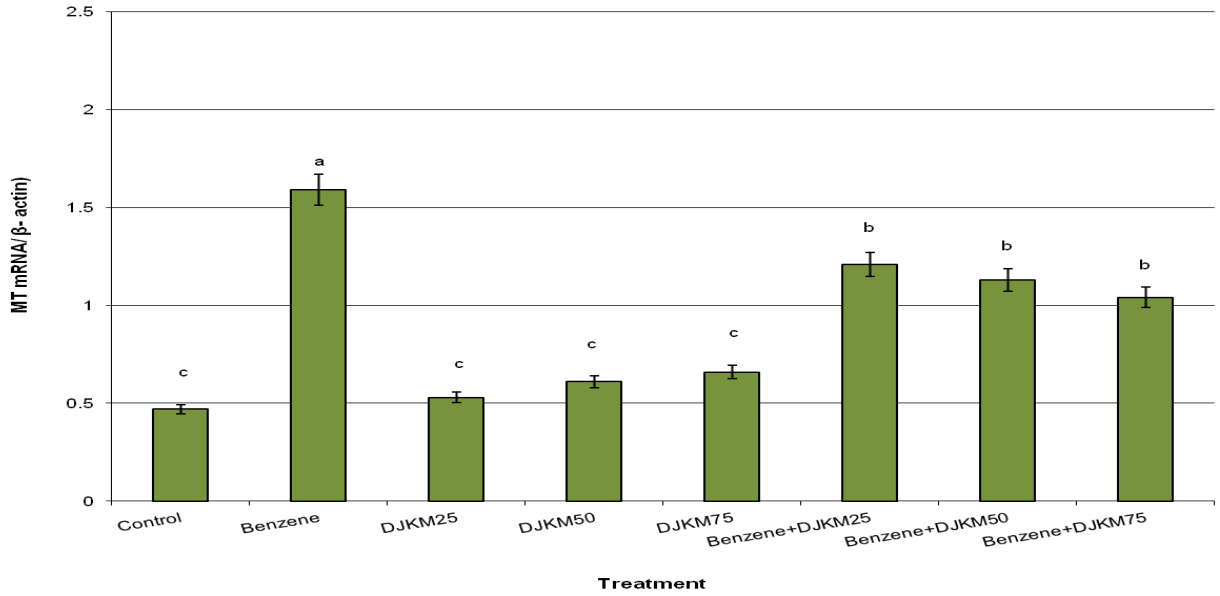

Figure 3: The alterations of MT mRNA in liver tissues of male rats after benzene and/or DJKM treatments. Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (P<0.05, Scheffé-Test). While, [a,b,c]Mean values within tissue with similar superscript letters were not significant differences (P>0.05).

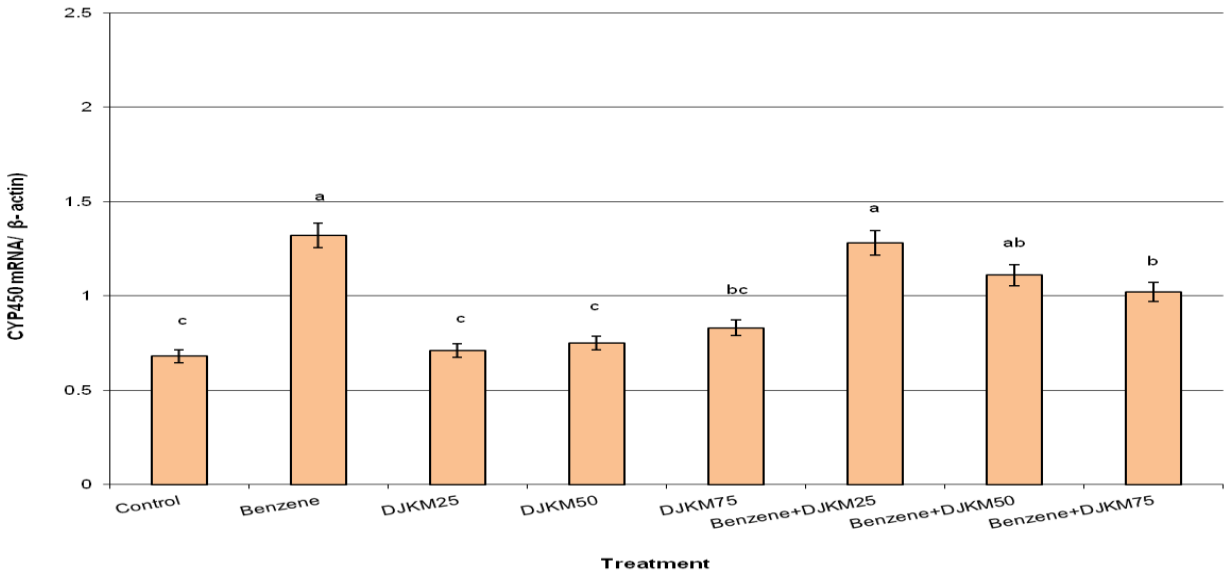

Figure 4: The alterations of CYP450 mRNA in liver tissues of male rats after benzene and/or DJKM treatments. Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (P<0.05, Scheffé-Test). While, [a,b,c]Mean values within tissue with similar superscript letters were not significant differences (P>0.05).

Table 1. Primers sequences used for RT-PCR

Gene	Primer sequence (5′–3′)[a]	Annealing Tm (°C)
Hsp70a	F: CGG GAG TTG TAG CGA TGA GA R: CTT CCT AAA TAG CAC TGA GCC ATA A	60
MT	F: TTT TGT TTT CAA GGT GGA ACC R: AGA GGT TGG TGA ACT TTG TGG	55
CYP450	F: ATC AAG CAA GGG GAC GAG TT R: GCT CGC TGA CAA TCT TTT GC	57
β-Actin	F: TGG GGC AGT ATG GCT TGT ATG R: CTC TGG CAC CCT AAT CAC CTC T	55

[a] F: forward primer; R: reverse primer. Tm: temperature.

Table 2. Micronucleated polychromatic erythrocytes (MnPCEs) of male rats exposed to benzene and /or fed on detoxified jatropha kern meal (mean ± SEM)

Treatment	MnPCEs / 3000 PCEs
Control	7.6 ± 0.4^{c}
Benzene	23.4 ± 1.3^{a}
DJKM25	8.1 ± 0.2^{c}
DJKM50	8.5 ± 0.3^{c}
DJKM75	9.1 ± 0.4^{b}
Benzene+DJKM25	16.2 ± 0.3^{b}
Benzene+DJKM50	15.8 ± 0.5^{b}
Benzene+DJKM75	14.9 ± 0.4^{b}

DJKM: detoxified jatropha kern meal, ^{a,b,c} values with different superscripts within columns represent significant statistical differences ($P< 0.05$, Scheffé-Test). ^{a,b,c} values with similar superscripts within columns represent no significant statistical differences ($P < 0.05$, Scheffé-Test).

Table 3. The amount of glutathione peroxidase activity in male rats exposed to benzene and /or fed on detoxified jatropha kern meal (mean ± SEM)

Treatment*	Glutathione peroxidase activity (U/mg tissues/min)
Control	6.2 ± 0.06^{a}
Benzene	1.9 ± 0.08^{d}
DJKM25	5.4 ± 0.11^{a}
DJKM50	5.8 ± 0.10^{a}
DJKM75	5.1 ± 0.07^{ab}
Benzene+DJKM25	3.2 ± 0.08^{c}
Benzene+DJKM50	3.6 ± 0.10^{bc}
Benzene+DJKM75	4.2 ± 0.12^{b}

DJKM: detoxified jatropha kern meal, ^{a,b,c,d} values with different superscripts within columns represent significant statistical differences ($P< 0.05$, Scheffé-Test). ^{a,b,c,d} values with similar superscripts within columns represent no significant statistical differences ($P < 0.05$, Scheffé-Test).

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