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Ability of aqueous extract of *Phoenix dactylifera* to effectively alleviate paracetamol-induced hepatotoxicity in experimental Wister albino rats

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ABSTRACT

Objective: To investigate the preventive, protective and ameliorative activity of the aqueous extract of *Phoenix dactylifera* L. (*P. dactylifera*) against paracetamol-induced hepatotoxicity.
Methods: A total of 50 male albino rats were used for the study and 2 g/kg body weight of paracetamol and 400 mg/kg body weight of aqueous extract of *P. dactylifera* were administered orally for the study. They were divided into 5 groups, namely group A (vehicle control), group B (paracetamol control), group C (preventive), group D (ameliorative) and group E (protective), with 10 rats in each group. Group B was administered with paracetamol for 7 days; group C was administered with the extract for 7 days before administering with paracetamol for 7 days; group D was administered with paracetamol for 7 days, then the extract for 7 days; while group E was administered with paracetamol and the extract simultaneously for 7days.
Results: The study revealed that the extracts of date palms contained active chemical compounds such as anthocyanins, phenolics, sterols, carotenoids and flavonoids. The levels of antioxidant enzymes activity such as superoxide dismutase, catalase, peroxidase were found to be reduced while malondialdehyde level was significantly increased in the paracetamol-treated group. This trend was reversed in groups where the extract was administered, as the antioxidant enzymes level in the liver was raised.
Conclusions: This study has shown that the aqueous extract of *P. dactylifera* can mitigate the hepatotoxicity effect of paracetamol with a better ameliorating effect than protective or preventive.

1. Introduction

Date palm, also called *Phoenix dactylifera* (*P. dactylifera*) tree, is regarded as a vegetable with health benefits and has been used traditionally to remedy a number of disease conditions[1,2]. The fruits of date palms are commonly eaten in many parts of the world and are used as a vital component of the diet and a staple food in most Arabian countries[3,4]. It contains 70%–80% carbohydrates in the form of monosaccharide (fructose and glucose) which are absorbed easily by human bodies[5]. It also contains salt and minerals, dietary fibre, fatty acids, amino acid, protein, mineral salts and vitamins[6]. Its pulp is very rich in phytochemicals like anthocyamins, phenolic (such as sinapic acids, p-coumaric, ferulic, vanillic acid and caffeic acid), procyanidins, carotenoids and flavonoids[7,8]. The presence of all these important biomolecules makes it an important fruit for promoting good health and for pathological conditions in most regions of the world. The antioxidant property of this fruit can be traced to the wide range of phenolic compounds and vitamin C in it[9]. Researchers also found that date might have an effect on the glycaemic and lipid level and control of diabetic patients[10,11].

Paracetamol (acetaminophen) is a widely used analgesic and antipyretic drug that is sold over the counter but causes liver problem if an over dose of it is consumed. It is metabolised in different phases by the liver to excretable sulphate or glucuronide conjugate[12]. The liver toxicity of paracetamol, however, is attributed to the formation of toxic metabolite, due to the activation of a portion of the paracetamol by cytoP450 to highly reactive metabolite known as N-acetyl-p-benxoquinone imine (NAPQI)[13,14] which is initially conjugated with reduced glutathione (GSH) to form

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mercapturic acid[15]. Although if the rate of the formation of NAPQI exceeds its detoxification by GSH, NAPQI would therefore oxidize tissue macromolecules and alter the homeostasis of calcium after depletion of GSH[16]. This research was to investigate the preventive, protective and ameliorative effect of the aqueous extract of date palm against oxidative stress from paracetamol-induced hepatotoxicity.

2. Materials and methods

Acetaminophen was bought from Sigma-Aldrich Co., France. Date palm (*P. dactylifera*) was obtained from Nigerian Institute for Oil Palm Research, Benin City. They were further verified in the Department of Botany of University of Benin. The fruits were sun-dried to a constant weight, the seeds were removed and the fruits were ground to fine powder. Fifty grams of the pulverized fruit were then placed in 1 L of distilled water for 72 h with constant stir to avoid fermentation. A 15 g solution of the extract was prepared and stored in a refrigerator. This solution was used as a stock crude extract.

Proximate analysis was then carried out on the pulverised sample to determine the moisture content, total ash, total lipid, crude fibre and crude protein.

The extract was tested for the presence of bioactive compounds such as carbohydrates, phenol, tannins, flavonoid, saponins, glycosides, steroid, terpenoids and alkaloids using standard methods described by Sofowara *et al.*[17], Trease and Evans[18] and Harborne *et al*.[19].

2.1. Experimental animal and design

A total of 50 male Wister albinos rats were used for this study. They were obtained from the Anatomy Department of University of Benin, Benin City. The rats were grouped with 10 rats per group and placed in five metallic cages. The rats were allowed to access to food and water unlimitedly to acclimatize for 2 weeks.

The grouped rats were named as group A (vehicle control), group B (paracetamol control), group C (preventive), group D (ameliorative) and group E (protective). Rats in groups B, C, D and E were induced orally with paracetamol (2 g/kg body weight) for 7 days by gavage.

The plant extract served as the stock crude drug. It was administered orally by the means of gavage at a dose of 400 mg/kg body weight for rats in groups B, C, D and E. Rats in group C were administered with the extracts for 7 days prior to the induction of paractemol this served as the preventive group, rats in group D were given the extract at 400 mg/kg body weight after being induced with paracetamol for 7 days. This served as the ameliorative group, while rats in group E were administered with the extract alongside paracetamol. This served as the preventive group.

2.2. Animal sacrifice

At the end of 14 days of treatment, the rats were anesthetized with chloroform in a closed chamber. The animals were dissected and their livers were put in labelled containers and transferred into ice-cold containers and then stored in a refrigerator in preparation for assay. One gram of the liver harvested from the experimental animal after 14 days of the experiment was homogenised in 10 mL of normal saline solution and the homogenate was centrifuged at 20 000 r/min for 15 min. The supernatant was collected and used for the antioxidant analysis.

2.3. Antioxidant assay

2.3.1. Estimation of malondialdehyde (MDA) level

This was based on the method of Guttridge and Wilkings[20], a modification of the procedure used by Hunter *et al.*[21]. The principle that underlay this assay was that MDA, a product of lipid peroxidation, when heated with thiobabituric acid, in the presence of an acid formed a pink or redish complex that was measured spectrophotometrically at 535 nm.

2.3.2. Superoxide dismutase (SOD) activity

This was determined according to the method of Misra and Fridovich[22]. Adrenaline auto-oxidises rapidly in aqueous solution to adrenochrome, whose concentration can be determined at 420 nm using a spectrophotometer. The auto-oxidation of adrenaline depended on the presence of superoxide anions. The enzyme SOD inhibited the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anions. The degree of inhibition was thus a reflection of the activity of the SOD and it was determined at one unit of the enzyme activity.

2.3.3. Peroxidase assay

Peroxidases were oxidoreductases which used H_2O_2 as an electron acceptor for catalysing different oxidative reactions.

The Chance and Maehly method utilized pyrogallol as shown below:

H_2O_2 + Pyrogallol → $2H_2O$ + Purpurogallin (donor) (oxidized donor)

The formation of purpurogallin was measured by measuring its absorbance at 420 nm spectrophotometrically.

2.3.4. Estimation of catalase assay

Catalase was present in nearly all animal cells, plants and bacteria and acted to prevent the accumulation of noxious H_2O_2 which was converted to O_2 and water, *i.e.* $H_2O_2 \rightarrow 2H_2O + O_2$.

The release of O_2 caused a discolouration of the $KMnO_4$ which was measured spectrophotometrically. The rate of discolouration was proportional to the catalase activity. Catalases which were less widely distributed catalyzed the above reaction.

2.4. Data analysis

Data for each parameter were analyzed separately using One-way ANOVA followed by Duncan's multiple comparisons test. $P < 0.05$ was considered statistically significant.

3. Result

Animals treated with 2 g/kg body weight paracetamol showed

significant decrease in SOD, catalase and peroxidase activity as compared to the ameliorative, preventative, protective groups and the vehicle control group.

Animals treated with 2 g/kg body weight paracetamol showed significant decrease in the total protein level and an increase in MDA level as compared to the ameliorative, preventative, protective groups and the vehicle control group (Tables 1–4).

Table 1

Phytochemical analysis of aqueous extract of *P. dactylifera.*

S/N	Phytochemical	Tests performed	Aqueous extract of *P. dactyliferia*
1	Carbohydrate	Molisch test	+
2	Saponins	Foam test	+
3	Alkaloids	Dragendorff test	+
4	Reducing sugars	Benedict test	++
5	Tannins	Lead acetate test	+
6	Flavonoids	Aluminum chloride method	+
7	Steroids	Ring test	+
8	Cardiac glycoside	Ring test	+

P. dactylifera tested positive for all the phytochemicals listed above.

Table 2

Proximate analysis of *P. dactylifera.*

Crude protein	Cude fiber	Lipids	Moisture content	Ash	Non-protein energy
14.42	3.50	10.00	15.00	5.00	52.08

Table 3

Effect of the aqueous extract of *P. dactylifera* on SOD, catalase and peroxidase activity levels (unit/mg protein) in paracetamol-induced hepatotoxicity in experimental rats.

Groups	SOD activity	Catalase activity	Peroxidase activity
Group A	0.225 ± 0.015^{a}	3.286 ± 4.250^{a}	4.905 ± 4.350^{a}
Group B	0.130 ± 0.029^{b}	1.027 ± 5.300^{a}	2.930 ± 5.890^{b}
Group C	0.171 ± 0.005^{b}	1.247 ± 2.150^{a}	6.038 ± 6.220^{a}
Group D	0.252 ± 0.014^{b}	5.999 ± 3.500^{b}	10.416 ± 8.080^{ab}
Group E	0.187 ± 0.019^{b}	3.476 ± 8.300^{a}	7.370 ± 4.680^{ab}

Each value is the mean of 6 replicates. Values in column followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's multiple range tests.

Table 4

Effect of the aqueous extract of *P. dactylifera* on total protein and MDA level in paracetamol-induced hepatotoxicity in experimental rats.

Group	Total protein level (mg/dL)	MDA level (µg/mg protein)
Group A	2.706 ± 4.870^{a}	2.150 ± 0.480^{a}
Group B	0.647 ± 1.120^{b}	6.603 ± 1.770^{b}
Group C	3.650 ± 6.320^{a}	1.540 ± 0.190^{a}
Group D	5.504 ± 9.530^{b}	0.388 ± 0.030^{a}
Group E	1.862 ± 3.220^{a}	2.123 ± 0.130^{a}

Each value is the mean of 6 replicates. Values in column followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's multiple range tests.

4. Discussion

The phytochemical screening of chemical constituents of the aqueous extract of date palm showed a wide range of phenolic compounds including alkaloids, saponins, tannins, steroid, flavonoids and procyanidins as shown in Table 1 above. Reducing sugars, carbohydrate and cardiac glycoside were also found. They were known to have medicinal values as well as physiological activities[17].

Proximate analysis of aqueous extract of date palm in Table 2 showed the presence of crude protein, crude fibre, ash, non-protein energy, lipids and moisture. They were therefore rich in certain nutrients and provided a good source for rapid energy (70%–80%) [23].

In Table 3, the activity of SOD was significantly decreased ($P < 0.05$) in paracetamol-controlled rats. The role of SOD depletion in the pathogenesis and intoxication has been reported by various studies performed using different experimental models[24,25]. A decrease in SOD production could be attributed to an enhanced superoxide generation and utilization of this enzyme during reactive metabolites detoxification. SOD is well known to be the primary defense mechanism against oxidative stress in tissue. Catalase and peroxidase are also antioxidant enzymes which help in intracellular detoxification of hydrogen peroxide. The activities of the antioxidant enzymes were inhibited ($P < 0.05$) due to the high level of toxic metabolite emanating from the detoxification process.

In Table 4, the total protein was significantly reduced in the paracetamol control group ($P < 0.05$) as compared to the vehicle control. The MDA level of the paracetamol control group was significantly increased ($P < 0.05$) as compared to the vehicle control.

Acetaminophen is a well-known antipyretic and analgesic drug that is harmless in therapeutic doses, but it has been shown to result in fatal liver cell death in experimental animals as well as humans when an overdose is used[26], and is now a well-established hepatotoxic agent for pre-clinical research. The access to paracetamol in pharmacies without prescription has led to it being kept in many homes and, therefore, it is not surprising that it is often involved in accidental and deliberate self-poisoning. The drug is considered safe with an excellent safety record with respect to unwanted side effects. However, intentional or accidental use of paracetamol overdose could cause life-threatening damage to the liver and other diseases[27].

The beneficial effect of aqueous extract of date palm was observed to have increased the activity of SOD, catalase and peroxidase in the preventive, ameliorative and protective groups, since the effect of paracetamol toxicity overwhelms the natural antioxidant enzyme in the hepatocytes. This effect of date palm in preventing the progress of liver damage from paracetamol toxicity is as a result of the presence of flavonoids and other phytochemicals[5], which enhances the activity of antioxidant enzymes. The ameliorative group was found to have the highest value for peroxidase activity. The total protein was increased in aqueous extract of date palm treated group with the ameliorative group having the highest value for total protein.

MDA, one of the end-products of polyunsaturated fatty acid peroxidation, is a good indicator of the degree of lipid peroxidation[28], which is related to paracetamol-induced hepatotoixicity. The significant increase in MDA level observed in the liver homogenate of paracetamol-induced hepatotoxicity in rats was reduced by the treatment of aqueous extract of date palm, indicating its ability to break the chain reaction of lipid peroxidation. This protective effect of *P. dactylifera* on acetaminophen-induced hepatotoxicity in rats appears to be related to the inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action. Based on these results,

we may suggest that the therapeutic potential of aqueous extract of date palm is dependent on antioxidant mechanism.

This study has shown that *P. dactylifera* has a therapeutic action by inhibiting stress due to oxidative action from paracetamol hepatotoxicity. However, the study revealed that aqueous extract of *P. dactylifera* has a better ameliorative effect than protective or ameliorative effects and thus can be used in ameliorating liver damage from the use of paracetamol.

Conflict of interest statement

We declare that we have no conflict of interest.

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