Amelioration Effect of *Carica papaya* Fruit Extracts on Doxorubicin – induced Cardiotoxicity in Rats

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**ABSTRACT**

Papaya (*Carica papaya* Linn) belongs to the family Caricaceae. Papaya is commonly known for its food and nutritional values throughout the world. Papaya possess excellent medicinal properties for treatment of different ailments. This article focuses on the antioxidant, free radical scavenging activity and antitoxin activity of papaya. The antioxidant activity of *Carica papaya* Fruits, have the highest of reducing power which were 184.15, 151.19 and 1

**Key words:** *Carica papaya* fruits; Antioxidant and Antitoxin.

**INTRODUCTION**

The doxorubicin (DOX) is an anthracycline glycoside antibiotic and an anticancer drug that cause DNA intercalation as well as inhibit the DNA replication. DOX has a wide spectrum of antineoplastic activity such as breast cancer, leukemia, and sarcoma. One of the most common adverse effects of DOX is cardiotoxicity which is clinically manifested as congestive heart failure (Ali et al., 2002; Haybar et al., 2019).

Free radical damage is one of the most prominent reasons of disturbing diseases that are accountable for killing millions of people in the world and this can manifest as heart attacks and cancers. Free radicals naturally occur in the body as a result of chemical reactions during normal cellular processes such as adaptation of food into energy in the body. Antioxidants are powerful free radical scavengers in the human body. Several researches on antioxidants in biological systems have confirmed their neutralizing effects on oxidative stress that predispose the human body to lethal diseases and thus, making keen interest in valuation of antioxidant potentials of consumable food compounds antioxidants comprise a number of chemical compounds (Ahiakpa et al., 2010).

There is an increasing evidence for the enhancing effect of free radicals involved in the primary pathogenic mechanism of doxorubicin-induced cardiotoxicity (Li et al., 2000; Mahmoud and Ali, 2012) in rats. These highly toxic reactive oxygen species react with cellular molecules including nucleic acids, proteins and lipids, thereby causing cell damage. Several studies have reported that adriamycin administration inhibit the activity of the antioxidant enzymes and resulted in imbalance between the generation of free radicals and the antioxidant defense resulted in adriamycin-induced tissue toxicity (Gnanapragasam et al., 2004).

*Carica papaya* Linnaeus, (pawpaw), belongs to the family of *Caricaceae*. *Papaya* is not a tree but herbaceous succulent plants that possess self-supporting stems. In traditional medicine, different parts of *C. papaya* including its leaves, barks, roots, latex, fruit, flowers, and seeds have a wide range of reputed medicinal application (Tiwari et al., 2011).

Due to the great importance of doxorubicin in chemotherapy for the treatment of many types of cancer, researchers have expended great efforts trying to prevent or attenuate its side effects. In this sense this work aim to find the best solvent for the optimal extraction of active ingredients from different parts of papaya including peel, seeds, pulp and fruit, then we will evaluate the their antioxidant and free radicals scavenging activities in vitro, the best extract will use to ameliorate the toxic effect of doxorubicin on heart of rats.

MATERIALS AND METHODS

Plant materials
Fruits samples of *Carica papaya* were kindly obtained from Agricultural Research Center, Giza, Egypt. Samples were dried in oven at 55°C and ground into a fine powder. The powder was divided into methanolic, ethanolic and aqueous extract.

Powdered air-dried fruit (100 gm) of dried samples were extracted with distilled water by boiling at temperature from 80 to 100°C in reflux for 3h to achieve an initial extract. The extract was filtered after cooling to room temperature. Finally, the extract was lyophilized and preserved at −20°C until further use (Kim et al., 2011). Fruits powdered (1Kg) of the plant was extracted by soaking at room temperature for six times with methanol (10 L), then the successive extraction was carried out by using methanol. Tow extracts were obtained and then concentrated to dryness under vacuum and reduced pressure using the rotary evaporator at 45°C.

The yields of samples were 25.09, 23.41 and 22.00%, of methanolic, ethanolic and aqueous extracts respectively. All tests were conducted in Sciences Academy of Experimental Researches, Mansoura, Egypt.

Determination of reducing power, (FRAP) radical scavenging activity
Reducing power of *Carica papaya* extracts, were determined according to the method of Oyaiu, (1986). Extract (0–100mg) from each sample in 0.2mol phosphate buffer, pH 6.6 (2.5ml) was added to 2.5ml potassium ferricyanide (10mg/ml), mixture was incubated at 50°C for 20min. Trichloroacetic acid (TCA) (2.5ml, 100mg/ml), was added to the mixture then centrifuged at 650g for 10 minutes. The supernatant (2.5ml) was mixed with distilled water (2.5ml) and 0.5ml ferric chloride solution (1mg/ml) was added and the absorbance of the resultant color was measured using a Spekol 11 (Carl Zeiss Jena) spectrophotometer at 700nm. Higher absorbance of the reaction mixture indicated greater reducing power. The free radical scavenging activity (% antiradical activity) was calculated using the following equation:

\[
\text{Increase in reducing power} \% = \left( \frac{A_{\text{Test}} - A_{\text{Blank}}}{A_{\text{Blank}}} \right) \times 100
\]

Determination of (DPPH) radical scavenging activity
The DPPH free radical scavenging activity of *Carica papaya* extracts at different concentrations were measured from bleaching of the purple color of (2,2 Diphenyl -1-picryl hydrazyl) was based on the method of Pratap et al., (2013). Exactly 0.1 ml solution of different concentration of the extract was added to 1.4 ml of DPPH and kept in dark for 30 min. The absorbance was measured at 517 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The percentage inhibition was calculated by using the following equation:

\[
\text{Percentage inhibition} \% = \left( \frac{A_{\text{Blank}} - A_{\text{Test}}}{A_{\text{Blank}}} \right) \times 100
\]

Experimental animals
A number of 48 male rats (200-220 g) were obtained from the animal house of Sciences Academy of Experimental Researches, Egypt. The rats were kept for adaptation under normal laboratory conditions for 7 days before the beginning of the experiment. All rats were fed on balanced basal diet and allowed free access of water.

Experimental design
In the experimental design the rats were assigned into eight groups of six animals each as described by Komolafe et al., (2013); El-Sayed et al., (2011).

- **Group 1:** Normal group was given saline (1 ml/kg body weight).
- **Group 2:** Control group was given saline (1 ml/kg) + DOX (15 mg/kg body weight).
- **Group 3:** Aqueous extract of *C. papaya* (100 mg/kg bw).
- **Group 4:** Methanolic extract of *C. papaya* (100 mg/kg bw).
- **Group 5:** Ethanolic extract of *C. papaya* (100 mg/kg bw).
- **Group 6:** Aqueous extract of *C. papaya* (100 mg/kg bw) + DOX (15 mg/kg bw).
- **Group 7:** Methanolic extract of *C. papaya* (100 mg/kg bw) + DOX (15 mg/kg bw).
- **Group 8:** Ethanolic extract of *C. papaya* (100 mg/kg bw) + DOX (15 mg/kg bw).

*C. papaya* extracts (100 mg/kg bw aqueous, methanolic and ethanolic) was administered orally to healthy experimental rats once daily for 9 consecutive days and thereafter, the rats, were challenged with single intraperitoneal dose of doxorubicin (15 mg/kg bw) on the 7th day according to El-Sayed et al., (2011). Animals were sacrificed 48 h after doxorubicin administration to harvest serum and heart tissues which were used for various biochemical analyses.

Blood samples were collected from the tail canthus by heparinized tubes. Then, each blood sample was centrifuged (10000 rpm) to obtain clear serum where serum glucose levels for fasting animals were determined immediately. Serum blood samples were kept at refrigerator under freezing conditions for the determination of the other parameters.

Determination of plasma biochemical parameters
The activities of Lactate dehydrogenase (LDH); Creatine phosphokinase (CK); Aspartate aminotransferase (AST) were assayed according to Lum and Gambino, (1974); Tsung et al., (1983); Reitman and Frankel, (1957), respectively.

Determination of antioxidant parameters
The levels of Malondialdehyde (MDA) and reduced glutathione (GSH) were determined as described by Ohikawa et al., (1979); and Moron et al., (1979), respectively. The activities of superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) according to Kakkar et al., (1984); Haque et al., (2003); Mohadas et al., (1984), respectively.

Statistical analysis
Statistical analyses of all experimental data were done using the statistical software package CoStat, (2005). All comparisons were first subjected to one way analysis of variance (ANOVA) and significant differences between treatment means were determined using Duncan’s multiple range test at P<0.05 as the level of the significance.
RESULTS AND DISCUSSION

Reducing power of plant leaves extracts

Efficiency of methanolic and aqueous leave extracts to reduce Fe+++ to Fe++ was determined according to the method described by Sroka and Cisowski, (2003). Optical density of reaction mixture was measured at wave length 700nm using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer.

The obtained data are presented in Table (1), the absorbance showed the reducing power for different concentrations of crude aqueous, methanolic and ethanolic extracts of Carica papayas fruits. Data expressed as absorbance at 700nm for producing color as a result for using three concentrations (5, 10, 20 and 40 mg/ml) for each sample.

Carica papayas fruits have the highest percentage of reducing power which was ranged from 69.28% to 184.15%, for methanolic extract at the concentrations of 5 and 40mg/ml, respectively. While, ethanolic extract of Carica papayas fruits have the average percentage of reducing power which was ranged from 54.76% to 151.19%, at concentrations of 5 and 40mg/ml, respectively. Followed by aqueous extract of the same plant, which was ranged from 24.76% to 139.52%, at concentrations of 5 and 40mg/ml, respectively. High levels of reducing power specified the presence of some compounds which could be considered electron donors and could react with free radicals to convert them into more stable products Arabshahi and Urooj, (2007).

The results were in the same trend with those reported by Sheneni et al., (2018), who revealed that, the percentage antioxidant activity (AA%), which was (62.4%), of C. papaya ethanolic extract. While, the antioxidant activity of the same plants determined by reducing potential (RP) was (1.2) at 700nm. by Addai et al., (2013), who found that, the scavenging effect of papaya fruits extract using FRAP radical ranged from 19.93mg GAE/100g to 180.28 mg GAE/100g.

Our results agree with Lydia et al. (2016), they found that acetone has the highest radical scavenging with 1.388 g / M FeSO4 equivalents, followed by ethanol (0.5772 g / M FeSO4 equivalents) and aqueous extract (0.1168 g / M FeSO4 equivalent). Dada et al. (2016), results revealed that the extract of unripe pawpaw peel had higher ferric reducing antioxidant property, which was (112.35 mg AAE/100g), compared to the unripe papaya seed extract, which was (102.78mg AAE/100g).

Determination of antioxidant activity using the 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity of both methanolic and aqueous extracts ready from the two studied plant species are reported in Table (2). The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC50) is a parameter widely adapted to measure the antioxidant activity Sanchez et al., (1998). The lower EC50 pointed to the higher antioxidant activity.

From Table (2), it is clear that, the scavenging effect (IC50) of methanolic extracts of Carica papayas fruits have the most effective of inhibition percentage (0.809), at a concentration 40mg/ml, followed by ethanolic and aqueous extract of the same plant, which were (0.832 and 0.896), at the same concentrate. While, the scavenging effect (IC50) of Carica papayas fruits have the lowest effective of inhibition percentage (1.082, 1.071 and 1.065), of aqueous, ethanolic and methanolic extracts, at a concentration 5mg/ml respectively.

Sheneni et al., (2018), who revealed that, the antioxidant scavenged using the (DPPH)+ radical, was (IC50 100μg/mL), of C. papaya ethanolic extract. Wong and Khor, (2014), Results showed that both ethyl acetate fractions from the fruits and seeds of Carica papaya are high in their antioxidant activities (IC50 values of 30.61μg/ml and 25.97μg/ml respectively.

Our results agree with Zahra et al., (2017), who found that, the DPPH scavenging activity of Carica papaya fruit, were 68.36% and 3.06%, of ethanolic and water extract, respectively. Lydia et al., (2016), who found that, In the current study, acetone has more scavenging activity (86.5%) followed by ethanol (82.02%) and aqueous extract (31.68%).

The results were in the same line with those reported by Addai et al., (2013), who found that, the scavenging effect of papaya fruits extract using DPPH radical ranged from 10.48% to 72.19%. El-Nekety et al., (2017), who found that, the DPPH radical scavenging activity of the water and ethanolic extract of papaya fruits, were 32.73% and 20.75%, respectively.

Effect of C. papaya L extracts on DOX induced changes in heart weight, heart weight to body weight percentage and mortality rate percentage

Data in Table (3) revealed that, the effect of C. papaya extracts on body weight of rat, it was normal and ranged from 229.3 to 230.7 gm, respectively. when compared with effect of DOX, which was 223.3gm. While the effect was negative on heart weight and mortality, which was 0.250 gm and 50%, of (DOX 15 mg/kg), after two days of treatment, compared with normal rat which was 0.348 and 0%, of heart weight and mortality, respectively. While, the effect of aqueous, methanolic and ethanolic C. papaya extracts at treatment of (100 mg/kg), on heart weight, were 0.332, 0.341 and 0.336 gm, after 9 days, respectively. Likewise, the effect of the same extracts on mortality, were 0%. Moreover, when the treatment by DOX 15 mg/kg with aqueous, methanolic and ethanolic C. papaya extracts at treatment of (100 mg/kg), were 0.334, 0.338 and 0.332 gm of Heart weight/ body weight. While, the effect of the same treatment on mortality, were 16.6, 0.00 and 16.6%, respectively.
The obtained data were agreed with those by Nweahujo et al., (2014), who studied the Acute toxicity of C. papaya methanol root extract, which was safe in rats at the tested oral doses (500–2000 mg/kg), also there was no mortality within the study period.

Imosene et al., (2018), who found that an increased body weight was observed in the pups of the treated groups (200mg/kg Carica papaya and Carica papaya + 2.5Gy gamma irradiation) on days 28, which were 45.14g and 42.56g, respectively. compared with the control and irradiated groups at days 28, were 37.52g and 37.74g, respectively. Moreover, the mean brain weight of the treated groups (200mg/kg Carica papaya and Carica papaya + 2.5Gy gamma irradiation) on days 28, which were 1.36g and 1.52g, respectively.

**Effect of C. papaya extracts on DOX-induced changes in various antioxidant biomarkers**

From Table (4), it could be noticed that the antioxidants (GSH), (GST), (GPx), (GR) and (SOD) decreased with the injection of (DOX 15mg/kg), which were (11.87μ mole/g), (110.24n M /mg), (70.26n M /mg), (103.98n M /mg) and (4.27U/mg), respectively. While the increased (MDA) at injection of (DOX 15mg/kg), which was (21.88U/mg). Compared with normal rat, which was, (11.87μ mole/g), (110.24n M /mg), (70.26n M /mg), (103.98n M /mg) and (4.27U/mg), of the same antioxidant biomarkers, respectively.

Data in Table (4), showed that, the methanolic extract have the highest effective (100mg/kg), an increase (GSH), (GST), (GPx), (GR) and (SOD) decreased with the treatment of (DOX 15mg/kg), which were (19.33μ mole/g), (140.93n M /mg), (114.21n M /mg), (149.08n M /mg) and (8.59U/mg), respectively. While, the decreased (MDA) at treatment with the same extract, which was (19.69U/mg). Compared with the injection of (DOX 15mg/kg) of control group.

Data in Table (6), revealed that, the ethanolic extract has an average effect (100mg/kg), an increase (GSH), (GST), (GPx), (GR) and (SOD) decreased with the injection of (DOX 15mg/kg), which were (17.40μ mole/g), (136.07n M /mg), (114.21n M /mg), (149.08n M /mg) and (7.94U/mg), respectively. While, the decreased (MDA) at treatment with the same extract, which was (20.15U/mg). Compared with the injection of (DOX 15mg/kg) of control group.
From the same Table, it was clear that the effect of *C. papaya* extracts, on antioxidants without injecting DOX, within normal limits at compared with normal group.

Obtained data were agreed with those by Ojo et al., (2018), who found that the effect of the administration of *Carica papaya* aqueous root extract (100mg/kg), on SOD, GPx, CAT and MDA levels of arsenic induced rat, which were (8.54μg/mg protein), (9.28μg/mg protein), (11.48μg/mg protein) and (3.72x10⁻⁸nmol/ml), respectively. While, the effects of the administration of *Carica papaya* aqueous root extract (150mg/kg) on the same antioxidant parameter, which were (9.28μg/mg protein), (96.98ng/mg protein), (14.98μg/mg protein) and (3.72x10⁻⁸nmol/ml), respectively. Compared with Arsenic control, which were (2.21μg/mg protein), (22.28μm/mg protein), (3.87μm/mg protein) and (9.87x10⁻⁸nmol/ml), respectively. When the normal control rats were, (9.22μg/mg protein), (96.21nm/mg protein), (14.64μm/mg protein) and (3.70x10⁻⁸nmol/ml), respectively.

El-Nekeety et al., (2017), they said that, the effect of water extract of papaya fruits on GSH-Px, SOD, TAC and MDA, in the kidney of rats fed OTA-contaminated diet, were (230.67unit/mg protein), (225.76unit/mg protein), (27.49μm/g) and (32.05μm/g), respectively. While, the effect of ethanolic extract of papaya fruits on the same parameter, were (233.78unit/mg protein), (228.74unit/mg protein), (27.37μm/g) and (40.09μm/g), respectively. Compared with ochratoxin (OTA) control rats, which (122.73unit/mg protein), (101.76unit/mg protein), (17.14μm/g) and (91.74μm/g), respectively. When the normal control rats were, (250.53unit/mg protein), (237.93unit/mg protein), (27.19μm/g) and (53.99μm/g), respectively.

Imosemi et al., (2018), they said that the effect of (*Carica papaya* + 2.5γg gamma irradiation), on levels of lipid peroxidation (LPO), glutathione peroxidase (GPx), reduced glutathione (GSH) and hydrogen peroxide (H2O2) in the brain, were (17.10 μM/mg), (391.90 μg/mg), (62.50 μg/ml/mg) and (8.30 μM), respectively. While, the effect of *Carica papaya* only on the same parameters, were (9.68 μM/mg), (388.04 μg/mg), (62.25μg/ml/mg) and (8.30μM), respectively. Compared with gamma irradiation group, which were (23.96 μM/mg), (356.69 μg/mg), (63.15 μg/ml/mg) and (9.95 μM), respectively. When the control group, were (10.88 μM/mg), (342.46 μg/mg), (61.55 μg/ml/mg) and (8.25 μM), respectively.

**Effect of *C. papaya* extracts on toxic substances (DOX-induced) of blood biochemical in rats**

Data in Table (5), it could be noticed that the blood biochemical (LDH), (CK) and (AST) increased with the injection of (DOX 15mg/kg), which were (534.84IU/L), (635.55IU/L) and (219.15IU/L), respectively. Compared with normal rat, which was, (393.83IU/L), (357.20IU/L) and (131.26IU/L), of the same blood biochemical, respectively.

From the same Table, it was clear that that, the methanolic extract has an average effect (100mg/kg), an decrease blood biochemical (LDH), (CK) and (AST) increased with the injection of (DOX 15mg/kg), which were (409.13IU/L), (376.29IU/L) and (149.04IU/L), respectively. Followed by the ethanolic extract has an average effect (100mg/kg), an decrease blood biochemical (LDH), (CK) and (AST) increased with the injection of (DOX 15mg/kg), which were (417.31IU/L), (391.88IU/L) and (158.89IU/L), respectively. While, the aqueous extract (100mg/kg), a decrease (LDH), (CK) and (AST) increased with the injection of (DOX 15mg/kg), which were (427.45IU/L), (409.00IU/L) and (191.61IU/L), respectively. Compared with the injection of (DOX 15mg/kg) of control group.

From the same Table, it was clear that the effect of *C. papaya* extracts, on antioxidants without injecting DOX, within normal limits at compared with normal rat.

This finding was in the same line with Nwaehujor et al., (2014), who described that effect of *Carica papaya* methanol root extract (75mg/kg), on serum biochemical parameters in rats, (Total bilirubin, ALP, AST ALT, gamma glutamyl transferase (GGT), blood urea nitrogen and triglycerides), were (0.30mg/dl), (114.52IU/l), (21.73IU/l), (23.77IU/l), (97.91IU/l), (53.44mg/dl) and (58.11mg/dl), respectively. Compared with of normal control rats, which were (0.43mg/dl), (112.68IU/l), (10.13IU/l), (26.71IU/l), (93.17IU/l), (46.33mg/dl) and (62.31mg/dl), of the same parameters, respectively.

Achieved data were arranged with those described by Oduola et al., (2007), who found that the effect of intake of extract of unripe *Carica papaya* (100mg/kg), on some liver function tests, (Total bilirubin, Conjugated bilirubin, Total protein, Albumin, ALT, AST and ALP), which were (13.03mmol/l), (3.65mmol/l), (68.55g/l), (36.28g/l), (23.55IUl), (10.12IU/l) and (112.63IU/l), respectively. Compared with of normal control rats, which were (12.23mmol/l), (2.88mmol/l), (69.86g/l), (36.11g/l), (23.37IU/l), (9.96IU/l) and (113.36IU/l), of the same parameters, respectively.

Sadeque and Begum, (2010), who established that the effect of *Carica papaya* aqueous extract on mean serum bilirubin, ALT, AST and ALP levels in CCI4 treated rats, were (0.6mg/dl), (194.3u/L), (265u/L) and (425u/L), respectively. Compared with CCI4 treated group, which (0.7mg/dl), (525u/L), (265u/L) and (425u/L), respectively. When the control group, were (0.5mg/dl), (40.3u/L), (54u/L) and (276u/L), respectively.

**Conclusions**

The effect of natural extracts as antioxidant when tested using (FRAB and DPDPH radical) showed high ability of these plant to scavenging the free radicals in laboratory. Additionally, the methanolic extract of *Carica papaya* fruit showed the active effect as antioxidant when tested on rats.

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