# Antioxidant and Immunostimulant Effect of Carica Papaya Aqueous Extract in Acrylamide Intoxicated Rats

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**Abstract:** The present study was conducted to evaluate the antioxidant and immunostimulant effects of The Carica papaya fruit aqueous extract (CPF, Caricaceae) against acrylamide induced oxidative stress and improvement of Immune functions which affected by free radicals liberating acrylamide in rats. Sixty male wistar albino rats (195 – 230 g) were assigned to four groups, (fifteen/group). The first group used as control group and received normal physiological saline orally daily. The second group was supplemented with acrylamide 0.05% in drinking water. The third group was gastro-gavaged with 250 mg/kg of papaya fruit extract orally on daily basis. The fourth group was supplemented with acrylamide 0.05% in drinking water and gastro-gavaged with 250 mg/kg of papaya fruit extract orally on daily basis. The chosen dose of papaya fruit extract was based on the active pharmacological dose range obtained from the orientation study earlier conducted. The experimental period was extended to forty day. At the expiration of the experimental period and night fasting, blood samples were collected from the orbital venous sinus. The sera were separated and used for determining of IgG and IgM and the stomach, liver and kidney homogenates for estimation of MDA, GSH level, SOD and CAT activity as a biomarker of lipid peroxidation and antioxidative stress. The obtained results revealed that, acrylamide caused significant increases in MDA and decrease of GSH level, SOD and CAT activity due to the oxidative stress induced by acrylamide on membrane polyunsaturated fatty acids in rat's stomach, liver and kidney while administration of CPF aqueous extract, was significantly ameliorated the increased levels of MDA and decline of GSH, SOD and CAT activity in the stomach, liver and kidney tissues caused by acrylamide toxicity. Meanwhile, CPF aqueous extract significantly increased immune functions (IgG and IgM) while acrylamide significantly decrease it specially IgG. Thus, this study suggests that acrylamide-induced oxidative stress in rats can be ameliorated by administration of CPF aqueous extract.
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## 1. Introduction

Damage induced to cellular constituents by oxygen-derived free radicals have been accepted to play a crucial role in the pathogenesis of a wide range of chronic and degenerative disorders (aging, atherosclerosis, neurodegeneration cancer, cataract), as well as in acute clinical conditions (Halliwell and Gatteridge, 1989). Millions of people in various traditional systems have resorted to the use of medicinal plants to treat their ailments; this could be as a result of the high cost of orthodox health care, or lack of faith in it, or may be as a result of the global shift towards the use of natural, rather than synthetic products. Therefore, substances with antioxidant properties have recently been given unprecedented attention as possible therapeutic and preventative agents (Emerit et al., 1990). Carica papaya Linn. (family: Caricaceae) popularly known as pawpaw and more commonly known as the papaya (Watson, 1997) is native to Southern Mexico, Central

America and Northern South America, the papaya is now cultivated in most countries with tropical climate like Malaysia, the West Indies and throughout Africa (Sofowa, 1996). It is an interesting tree in that the male and female parts exist in different trees. The fruits, leaves, seeds and latex are used medicinally. Pawpaw fruits has a juicy taste rich in antioxidant nutrients like carotene, vitamin C, vitamin B, flavonoids, folate, panthotenic acids and minerals such as potassium and magnesium, the fruit is also a good source of fibre all these are reported to promote the functions of cardiovascular system and provide protection against colon cancer (Fischer, 1998; Franco *et al*., 1993). Sofowa, (1996) revealed that, the biologically active constituents of papaya include chymopapain and papain, are used in the treatment of arthritis and digestive disorders, also extracts of the ripe fruits are used for a variety of medicinal purposes ranging from treatment of ringworm, malaria and hypertension. While extracts of unripe fruit have been used in treatment of diabetes (Oke, 1998).). Traditionally, the leaf extract was used as a tonic for the heart, analgesia and treatment for stomach ache (Giove Nakazawa, 1996). The extract is also known to have antioxidant properties (Nobuya et al., 1995 and Rahmat et al., 2004). Despite the wide and historical use of Carica papaya in the traditional management of many diseases, the scientific validation of its use as

antioxidant is lacking. In view of this, the current preliminary study was designed to evaluate the antioxidant potentials of the fruit extract in rats. Acrylamide (ACR) is a water soluble vinyl monomer used extensively in the production of polyacrylamide with several uses (Gold and Schaumburg, 2000). It is used in water purification, making of cosmetics, glues and paper, as a soil stabilizer and for the production of polyacrylamide gel electrophoresis (Tareke et al., 2002). It is a contaminant in certain potatoes and grain-based foods cooked at high temperature. Its presence in these foods occurs through maillard reaction between amino acids especially asparagine and certain reducing sugar either glucose and/ or fructose (Stadler et al., 2002). In rodent models, ACR had significant carcinogenic effect and damage to the nervous system (LoPachin et al., 2003). The acrylamide toxicity is considered to be hepatotoxic, genotoxic and causes lipid peroxidation (Abou Donia et al. 1993; Mukul Das et al., 1982; Cihak and Vontorkova, 1998). The aim of present study is to determine the possible antioxidant and immunostimulant effects of Carica papaya L aqueous extract in acrylamide intoxicated rats.

## 2. Material and methods

### 2.1. Chemicals

Acrylamide and all reagents used for the determination of oxidative indices were purchased from Sigma chemicals (St Louis, Mo, USA). Other reagents of analytical grade were obtained from normal commercial sources.

### 2.2. Plant authentication and extract preparation

Matured fresh unripe *C. papaya* fruit was obtained in a local garden and was authenticated in Department of nutrition, Faculty of agriculture, Damanhur University. The fruit was peeled and the cream coloured seeds inside discarded, 100 g of the fruit was soaked in 100ml of distilled water and incubated at room temperature for 72 h. The extract was sieved into a clean container and kept in the refrigerator until use. (Oduola et al., 2007).

### 2.3. Animals

Sixty male wistar albino rats (195 – 230 g) obtained from were used for the study. They were kept in rat cages in well ventilated house, temperature of 27 – 30 $^{o}$C, 12 h natural light and 12 h darkness, with free access to tap water and dry rat pellet. They were allowed to acclimatize for 15 days prior to the experiment. All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, 1985). Treatment of the animals was in accordance with the Principles of Laboratory Animal Care. Rats were divided into four equal groups of 15 rats each. The first group used as control group and this received 250 µl of physiological saline orally daily. The second group was supplemented with acrylamide 0.05% in drinking water. The third group was gastro-gavaged with 250 mg/kg of papaya fruit extract orally on daily basis. The fourth group was supplemented with acrylamide 0.05% in drinking water and gastro-gavaged with 250 mg/kg of papaya fruit extract orally on daily basis. The chosen dose of papaya fruit extract was based on the active pharmacological dose range obtained from the orientation study earlier conducted. The experimental period was extended to forty day.

### 2.4. Samples.

At the expiration of the experimental period, fasted control and other thee groups were anesthetized under diethyl ether, the blood was collected from orbital venous sinus and kept without anticoagulant at room temperature for one hour, then centrifuged at 3000 rpm/30 min. The non-hemolysed serum was obtained in clean sterilized rubber stoppered glass vials and stored at -20 °C until used for determination of IgG and IgM according to (Ojala et al., 1981). Then the rats from each group were sacrified by cervical dislocation. After that, the stomach, liver and kidneys were eviscerated and perused with ice-cold 0.05 M tris- HCl buffer pH7.4 containing 0.25 M sucrose. The stomach, liver and kidneys were blotted, dried, weighted and the homogenized in the ice-cold buffer with twelve stroks in a tight-fitting potter Elvehagen homogenizer. Lipid peroxides as malondialdehyde (MDA) were measured spectrophotometrically after the reaction with thiobarbituric acid (Placer et al., 1966). Reduced glutathione (GSH) was assayed by Spectrophotometric technique; the method is based on reductive cleavage of 5.5. dithiobis 2-nitrobezoic acid (DTNB) by sulphydryl group to yield yellow colour with maximum absorbance at 412 nm (Sedlack and Lindsay, 1968). Catalase activity was determined according to the method of (Sinha, 1971). Superoxide dismutase activity was determined according to the method of (Misra and Fridovich, 1972).

### Statistical analysis

The results are expressed as Mean ± SE. Analysis of data was performed by one-way analysis of variance (ANOVA). P value less than 0.05 was considered statistically significant.

## 3. Results

The result postulated in Table1 revealed that, the administration of acrylamide alone in drinking water significantly ($P< 0.05$) increased lipid peroxidation as expressed by increased MDA level in stomach, liver and kidneys. In the contrary papaya fruit extract (PFE)

when supplemented in combination with acrylamide (4th group) reveals a significant (P< 0.05) decrease in the level of MDA in stomach, liver and kidneys as compared to second group.

Table 2 show that, the administration of acrylamide resulted in significant decrease (P< 0.05) in the level of GSH in stomach, liver and kidneys. The data in Table 2 represented the extension of study to determine the effect of PFE in combination with acrylamide (4th group) on the level of glutathione. The present findings revealed that, the supplementation of PFE resulted in significant (P< 0.05) increase in the content of GSH in stomach, liver and kidneys as compared to second group.

Table 3 show that, the administration of acrylamide resulted in significant decrease (P< 0.05) in the activity of CAT in stomach, liver and kidneys. The data in Table 3 represented the extension of study to determine the effect of PFE in combination with acrylamide (4th group) on the activity of CAT. The supplementation of PFE resulted in significant (P< 0.05) increase in the activity of CAT in stomach, liver and kidneys as compared to second group.

Table 4 show that the administration of acrylamide resulted in significant decrease (P< 0.05) in activity of SOD in stomach, liver and kidneys. The data in Table 4 showed that, the administration of PFE in combination with acrylamide (4th group) resulted in significant (P< 0.05) increase in the activity of SOD in stomach, liver and kidneys as compared to second group.

Table 5 revealed that, the administration of acrylamide resulted in significant decrease (P< 0.05) in IgG and IgM. The data in Table 5 postulated that, the administration of PFE in combination with acrylamide (4th group) resulted in significant (P< 0.05) increase in the IgG as compared to second group.

**Table (1):** Effect of acrylamide (0.05% in water) and CPF aqueous extract (250mg/kg) and their combination on lipid peroxidation (nmol MDA/gm) of stomach, liver and kidneys of rats.

| Groups | LP. (nmol MDA/g wet tissue) | | |
|---|---|---|---|
| | Stomach | Liver | Kidney |
| **Group I (Control)** | 100.06 ± 1.61[c] | 112.1 ± 1.3[c] | 115.93 ± 1.75[c] |
| **Group II (Acrylamide)** | 176.8 ± 1.72[a] | 165.44 ± 1.89[a] | 186.66 ± 1.35[a] |
| **Group III (CPF)** | 80.66 ± 1.51[d] | 99.3 ± 1.2[d] | 114.3 ± 1.75[c] |
| **Group IV (Acrylamide+ CPF)** | 152.96 ± 1.42[b] | 133.5 ± 1.69[b] | 148.92 ± 1.35[b] |

Means within the same column carrying different letters are significantly different (P< 0.05).

**Table (2)**: Effect of acrylamide (0.05% in water) and CPF aqueous extract (250mg/kg) and their combination on G-SH (µmol/gm) of Stomach, liver and kidneys of rats.

| Groups | GSH (µmol/g wet tissue) | | |
|---|---|---|---|
| | Stomach | Liver | Kidney |
| **Group I (Control)** | 32.49 ± 0.60[a] | 78.61 ± 1.31[b] | 61.95 ± 1.12[b] |
| **Group II (Acrylamide)** | 15.79 ± 0.57[c] | 39.39 ± 1.32[d] | 31.03 ± 1.41[c] |
| **Group III (CPF)** | 33.46 ± 0.30[a] | 98.17 ± 1.35[a] | 79.39 ± 1.37[a] |
| **Group IV (Acrylamide+ CPF)** | 24.97 ± 0.77[b] | 59.02 ± 1.33[c] | 58.11 ± 1.61[b] |

Means within the same column carrying different letters are significantly different (P< 0.05).

**Table (3)**: Effect of acrylamide (0.05% in water) and CPF aqueous extract (250mg/kg) and their combination on catalase activity (CAT) (K/Sec/mg protein) of Stomach, liver and kidneys of rats.

| Groups | CAT (K/Sec/mg protein) | | |
|---|---|---|---|
| | Stomach | Liver | Kidney |
| **Group I (Control)** | 66.87±54b | 104.50±14a | 88.23±23b |
| **Group II (Acrylamide)** | 43.74±35c | 73.20±18c | 50.53±43c |
| **Group III (CPF)** | 79.95±57a | 108.76±13a | 103.78±23a |
| **Group IV (Acrylamide+ CPF)** | 63.76±64b | 91.46±15b | 83.67±46b |

Means within the same column carrying different letters are significantly different (P< 0.05).

**Table (4):** Effect of acrylamide (0.05% in water) and CPF aqueous extract (250mg/kg) and their combination on superoxide dismutase activity (SOD) (U/mg protein) of Stomach, liver and kidneys of rats.

| Groups | SOD (U/ mg protein) | | |
|---|---|---|---|
| | Stomach | Liver | Kidney |
| **Group I (Control)** | 105.6 ± 3.18a | 270.73±26a | 93.56±15b |
| **Group II (Acrylamide)** | 68.65 ± 5.19b | 187.85±38c | 80.32±16c |
| **Group III (CPF)** | 107.6 ± 1.49a | 274.58±27a | 111.43±18a |
| **Group IV (Acrylamide+ CPF)** | 104.8 ± 2.27a | 233.98±24b | 90.76±15b |

Means within the same column carrying different letters are significantly different (P< 0.05).

**Table (5)**: Effect of acrylamide (0.05% in water) and CPF aqueous extract (250mg/kg) and their combination on IgG and IgM of rats.

| Groups | Serum Immunoglobulin (mg/dl) | |
|---|---|---|
| | IgM | IgG |
| **Group I (Control)** | 16.06±0.58a | 123.00±4.20b |
| **Group II (Acrylamide)** | 12.01±1.34b | 89.17±5.55d |
| **Group III (CPF)** | 17.04±1.26a | 138.60±6.14a |
| **Group IV (Acrylamide+ CPF)** | 13.15±0.68b | 107.70±4.61c |

Means within the same column carrying different letters are significantly different (P< 0.05).

**Discussion**

The present study evaluated the antioxidant and immunostimulant activities of Carica papaya L aqueous extract against acrylamide toxicity. Cells posses a variety of primary and secondary defenses against lipid peroxidation and other deleterious effect of oxidative damage. Primary defenses were mainly preventative which depend on scavenging/ inactivation of Reactive Oxygen Species (ROS) or redox metal ions before lipid peroxidation take place. They include superoxid dismutase (SOD), glutathione peroxidase (GPx) which scavenge superoxide anion radical and hydrogen peroxide at low concentration, catalase which scavenge hydrogen peroxide at high concentration where as secondary defenses had protective role which involve excision/ repair of any lesion that do develop (Girotti, 1990). Glutathione is an abundant and important antioxidant tripeptide and essential biofactor synthesized in all living cells. It function mainly as an effective intracellular reductant. It protect cells from free radicals mediated damage caused by drugs and ionizing radiation. It forms an important substance for GPx, GST and several other enzymes which were involved in free radical scavenging (Rahman and Macnee, 1999).

The result presented in Table1 revealed that the administration of acrylamide in drinking water significantly increased lipid peroxidation as expressed by an increase in MDA levels in tissues. Acrylamide is able to increase lipid peroxidation by inducing oxidative stress with generation of free radicals (Jiazhong et al., 1998). These results are in agreement with other reports that showed an increase in lipid peroxidation in brain and liver upon administration of acrylamide (Srivastava et al., 1983). A recent study on human erythrocytes also shows acrylamide induced MDA formation and a decrease in glutathione peroxidase activity in the erythrocyte (Catalgol et al., 2009). However, in this study, administration of PFE counteracted the effects of acrylamide. These results come in accordance with others who reported the effects of Carica papaya leaf (CPL) aqueous extract on alcohol induced acute gastric damage and the immediate blood oxidative stress level in rats, the results showed that gastric ulcer index was significantly reduced in rats pretreated with CPL extract as compared with alcohol treated controls (Indran et al., 2008). The same authors revealed that, biochemical analysis indicated that, the acute alcohol induced damage is reflected in the alterations of blood oxidative indices and CPL extract offered some protection with reduction in plasma lipid peroxidation level and increased erythrocyte glutathione peroxidase activity, therefore, Carica papaya leaf may potentially serve as a good therapeutic agent for protection against gastric ulcer and oxidative stress. In vivo and In vitro evaluation of the antioxidant effects of dried papaya juice in rats showed that, the blood total antioxidant power was increased significantly while blood lipid peroxidation levels decreased significantly (Mehdipour et al., 2006). A novel electron spin resonance (ESR) technique for evaluating oxidative stress and location of its damage in the brain of spontaneously hypertensive rats (SHR) has been described to evaluate the ability of fermented papaya preparation (FPP, a product of yeast fermentation of Carica papaya Linn.) to modulate oxidative stress of SHR brain results revealed that, FPP have up-regulated the redox defense activity in the SHR brain (Fumihiko et al., 2009).

The enzymatic antioxidant defense systems are the natural protectors against lipid peroxidation. Table 2, 3 and 4 demonstrates significant reduction in the level of GSH and activity of CAT and SOD respectively in the tissues by acrylamide. This suggests an increased utilization of this antioxidant enzyme with subsequent depletion to counter the increased level of free radicals induced by acrylamide in these tissues. These results concur with previous studies which reported a decrease in the activity of GST and other antioxidant enzymes in rat brain on subsequent exposure to acrylamide (EL-Ballal and EL-Manankhly, 1998). This is concurs also with other reports that showed significant decrease in the level of glutathione (GSH) in brain and liver of rats upon acrylamide administration (Srivastava et al., 1983). Acrylamide are electrophilic compounds, which property facilitates them to react with vital cellular nucleophiles possessing SH, $NH_2$ and OH groups. GSH is a cellular non-protein sulfhydryl molecule, which on administration of acrylamide is accompanied by its significant depletion in cells by reacting with SH group of glutathione. This results in formation of glutathione S-conjugates which is the initial step in the biotransformation of electrophiles (like acrylamide)

into mercapturic acid with subsequent excretion in the urine. Hence, the body uses glutathione for detoxification and excretion of acrylamide in the body (Edward, 1975). This study also, agree with other reports which reported significant decrease in GSH content and GST activity in corpus striatum of rat brain and liver intoxicated with ACR (Shukla-Pradeep et al., 2002). In the contrast, Awad et al., (1998) reported an increased activity of antioxidant enzymes after incubation of acrylamide with liver slices.

Administration of PFE in combination with acrylamide restored the activity of this enzyme in all tissues. This was in agreement with previous studies which reported that, Carica papaya contains antioxidant phytochemicals, such as vitamin C, beta-carotene, lycopene and vitamin E all of which acts as antioxidant and subsequently decrease the consumption of these antioxidant enzymes to combat oxidative stress (Aruoma et al., 2006; Amer et al., 2008; Looze et al., 2009; Azarkan et al., 2004; Gouado., et al., 2007 and Anuar et al., 2008). In a small double-blind, placebo controlled study, a fermented extract of Carica papaya was administered to elderly patients without major diseases, the fermented Carica papaya preparation supplemented group showed a significant enhancement of the individual's antioxidant defense system (Marotta et al., 2006 and Osata et al., 1995).

The Carica papaya extract (CPE) have hepatoprotective effect against carbon tetrachloride intoxicated rats and it may be mediating its protective effects either by decreasing the metabolic activation of carbon tetrachloride, or by acting as a chain-breaking antioxidant for scavenging free radicals or by a combination of these effects. In an earlier study, the presence of alkaloids, flavonoids, saponin, tannin, anthraquinones, and anthacyanosides in CPE was reported (Adeneye et al., 2009). Also, previous independent studies have reported that the protective actions of hepatoprotective medicinal plants are mediated by their flavonoids or alkaloids components or by their combination via antioxidant and free radicals scavenging activities (Adeneye et al., 2008). The presence of these active biological principles may thus be accounting for the biological effect of CPE and could be via antioxidant and/or free radicals scavenging activities. However, further studies will still be required to substantiate this. Oral administration of fermented Papaya Preparation (F.P.P.), a health food prepared from a yeast fermented mixture of medicinal plants, has been reported to decreased lipid peroxidation and increase the superoxide dismutase activity in rat (Nobuya et al., 1995). The same authors reported that, the antioxidant action of papaya could be exerted through different mechanisms, i.e. by metal-cheletion, by scavenging of hydroxyl radical, by an effect on antioxidant enzymes, or by reaction with peroxyl radicals.

Concerning the effect of the oral administration of CPF aqueous extract and acrylamide on immune f unctions (IgG and IgM) (Table 5) it was cleared that, CPF aqueous extract elicited significant increase the IgG and IgM in group III while acrylamide significantly decrease it in group II in comparison with control group. On the other hand, administration of CPF aqueous extract in group IV elevate the level of IgG near to the control level. This could be attributed to the potent antioxidant activity of CPF aqueous extract (Fischer, 1998; Franco *et al*., 1993). These results were nearly correlated with that of other authers who stated that there is a lot of information about the role of free radicals in the immune defense mechanism where the involvement of Free radicals leading to weakness of immunity (Ivanov, 2008). Also, these findings were coincided with others who concluded that the supplementation with the antioxidant protected immune responses in individuals exposed to certain environmental sources of free radicals (Bendich, 1993). Many of the protective functions of immune cells depend on the fluidity of the membranes of the cell. As the concentration of polyunsaturated fatty acids in the membranes is increased, the potential for membrane lipid peroxidation mediated by free radicals also is increased. Lipid peroxidation decreases membrane fluidity, which adversely affects immune responses. Mice fed oxidized lipids show marked atrophy of the thymus and T-cell dysfunction. Loss of membrane fluidity has been related directly to the decreased ability of lymphocytes to respond to challenges to the immune system (Bendich, 1990).

Although our bodies can synthesis antioxidant enzymes, we also need additional intake of dietary antioxidants to enhance our immunity and protect us from the harmful effects of free radicals and oxidative stress.

**Conclusion**

Acrylamide caused many adverse effects in the tissues reflected in significant increase in lipid peroxidation, decrease in glutathione levels and decreased activities of catalase and superoxide dismutase. The administration of papaya fruit aqueous extract alone or in combination with acrylamide significantly lowered lipid peroxidation, and enhanced glutathione levels, activity of catalase and superoxide dismutase as well as improves immune status reflected in increased Ig G and Ig M.

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