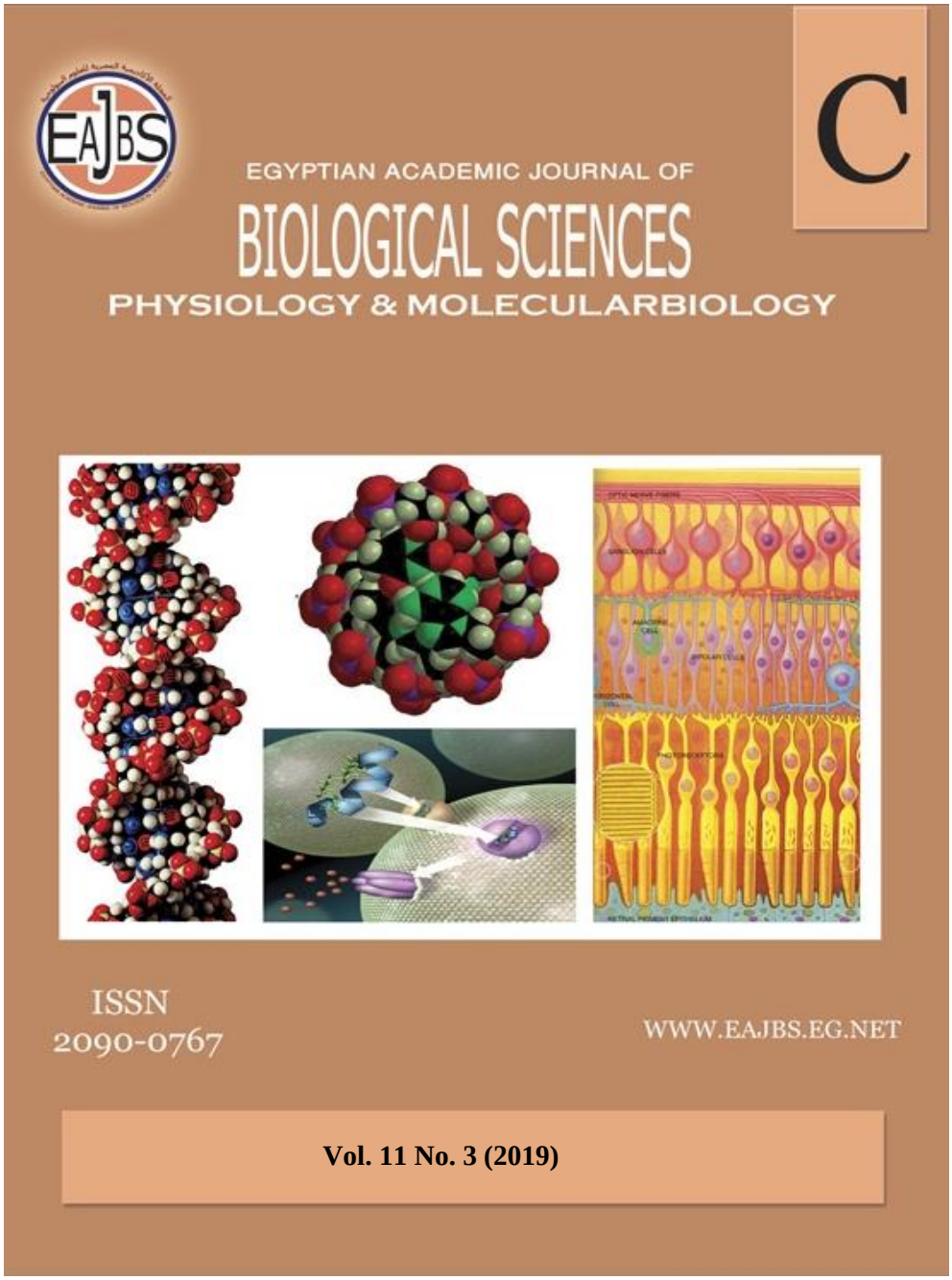

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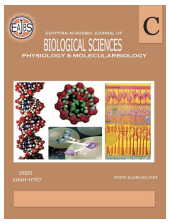

Ameliorative Effects of *Annona muricata* (Graviola) and Fullerene C_{60} Against Toxicity Induced by Carboplatin in Male Albino Rats

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Carboplatin, Oxidative stress, *Annona muricata*, Fullerene C_{60}.

ABSTRACT

This study was designed to demonstrate the ameliorating effects *Annona muricata* (Graviola) plant extract and fullerene C_{60} on hematological and biochemical disorder alteration induced by intraperitoneal (i.p.) injection of carboplatin (CRP) on male rats. Five groups of adult male rats we established (n=10). Group1: animals were administered normal saline i.p. (10 ml/kg b.wt) for 40 days and served as a normal group. Group 2: rats were injected i.p. with a single dose of CRP (80 mg/kg b.wt), then received a saline solution for the remaining 40 days and served as a control group. Group 3: animals were oral administration of *Annona muricata* leaves extract at a dose of 200 mg/kg/day for 40 consecutive days after 24 hours of i.p. injected with a single dose of CRP (80 mg/kg b.wt.). Group 4: animals were treated orally by fullerene C_{60} (4 mg/ kg b.wt.) daily for 40 days after 24 hours of i.p. injected a single dose of CRP (80 mg/kg b.wt.). Group 5: each rat in this group was daily oral administration of *Annona muricata* leaves extract at a dose of (200 mg/kg b.wt.**)** + fullerene C_{60} (4 mg/ kg b. wt.) for 40 days after 24 hours of injected a single dose of CRP. Results obtained revealed that CRP administration in the control group (gp. 2) significantly reduced the levels of red blood cells (RBCs), haemoglobin (Hb), blood Platelets (PLTs) and PCV value, with a marked reduction in white blood cells (WBCs). Additionally, there is an elevation in serum ALT, AST, ALP, total bilirubin, creatinine and urea associated with a reduction in albumin, total protein, and uric acid. In addition, hepatic and renal thiobarbituric acid reactive substances (TBARS) levels were significantly increased by CRP administration while levels of endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were reduced. This study confirmed the risk of increased oxidative stress, hepatotoxicity and nephrotoxicity due to CRP administration. In addition, *Annona muricata* leaves extract and fullerene C_{60}, clarified modulatory role against the cellular damage produced by free radical-induced by CRP.

INTRODUCTION

CRP is one of platinum-set chemotherapeutic (Simon *et al*., 2014). CRP is a block of platinum that characterizes a class of an active anticancer agent generally used to treat several types of cancer such as ovarian, head-neck, small cell lung cancer, thyroid, gastric, breast, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, and sarcomas. CRP is chosen from the other platinum (cisplatin, etc.) groups due to its reduced adverse effects and enhanced resistance (Rajeswarana *et al*., 2008 and Chen *et al*., 2017). Since CRP has a less toxic adverse result shape than cisplatin, it is existence tried as a replacement for cisplatin in many cancer therapy treatments (Kazim *et al.,* 2002). On the other hand, its therapeutic application is limited by its harmful side effects, the greatest significance of which is its renal and liver toxicity, which can lead to kidney failure and liver enzymes dysfunctions. The current reviews showed that the cytotoxic action of CRP is one of the most vital mechanisms involved in CRP induced toxicity results from its oxidative stress, which may result from either overproduction of free radicals or from deficiency of some antioxidant defense systems. which plays a significant role in CRP induced toxicity of kidney and liver.

Medicinal plants are a good source of natural antioxidants believed to exert their effect by reducing the formation of the final active metabolite of the drug-induced systems or by scavenging the reactive molecular species to prevent their reaching a target site. *Annona muricata* is a genus of tropical fruit trees belonging to the family Annonaceae, of which there are about 119 species (Neela and Alexander, 2010). This plant contains chemical compounds that show antitumor, pesticidal, antiviral and antimicrobial effects, thus signifying several potentially beneficial applications. Ripe *Annona muricata* pulp extract contains three prominent acetogenins: asimicin, bullatacin, and bullatalicin. Usually, the leaves are used for headaches, insomnia, cystitis, liver problems, diabetes, hypertension and as an inflammatory, antispasmodic and antidysenteric. *Annona muricata* has therapeutic uses dropping elevated blood pressure (Sushmita *et al.,* 2012).

Nanotechnology by the handling of features of materials is able to provide superior drug distribution systems for better regulatory and treatment of diseases. In other words, the nanostructures working as drug delivery systems have several advantages which make them the greatest of classical delivery systems. Fullerene C_{60} is well-known to be proficient in inactivating hydroxyl radicals by attaching to double bonds (Andrievsky *et al*., 2009). Though, this mechanism cannot clarify enough increases in the lifespan of rats. Such kind of antioxidative activity is also credited to natural phenolic antioxidants that do not have high senescence retarding activity (Banks *et al*., 2010). Fullerene C_{60} has both antioxidant and antitumor potential and may be beneficial in controlling cell responses to an anti-cancer drug. The liver and kidney tissues have high oxidative metabolism and very sensitive to free radicals. The greatest severe side effect of the anti-cancer drug is a free radical-mediated injury to those cells. In this study, the beneficial effect of fullerene C_{60} on liver and kidney antioxidant protection against anticancer drug treatment (Prylutska *et al*., 2014).

The current work was suggested to study the significance of CRP on specific biochemical changes in the blood, liver, and kidney of rats as a consequence of lipid peroxidation which was induced by free radical after CRP- induced liver and renal

toxicity and the therapeutic effect of *Annona muricata* plant extract, fullerene C_{60}, and their administration together on this experimental model.

MATERIALS AND METHODS

Experimental Animals:

Fifty adults of male Albino rats of approximate age and weight (6-8 weeks) (weighing 200 ± 50 g). Animals were kept in clean cages and given a normal diet and clean water ad libitum. Cages were placed in an air-conditioned room (23 ± 3ºC) with 12:12 hour light: dark cycle. Animals were kept for two weeks before starting the experiment for acclimatization, during which they subjected to clinical and laboratory examinations. The experimental protocol was approved by the experimental animal ethics committee, Faculty of Science, South Valley University, Egypt.

Drugs and Chemicals:

- **Carboplatin**

It is an anticancer drug and was purchased from Ebewe Pharma co. Austria in vial form. CRP injection (80 mg/kg) according to Zuleyha *et al*. (2019).

- ***Annona muricate:***

The leaves of *Annona muricata* were obtained from a farmer, Qena, Egypt January 2017. They were taxonomically identified by the Department of Botany, Faculty of Science, South Valley University of Qena, Egypt.

Preparation of *Annona muricata* Leaves Ethanolic Extract:

Annona muricata fresh leaves were air-dried at room temperature. the air-dried leaves of the plant were crushed into fine powder in a Waring commercial blender. 150g of the powdered leaves were extracted with 500ml ethanol for three days (with occasional shaking). The extract was concentrated in a rotary evaporator at a reduced pressure to produce a crude ethanolic extract, the crude extract thus obtained was frozen at 4ºC (Gavamukulya *et al.,* 2015); and then used in this study. Portions of the crude plant extract residue were weighed and dissolved in distilled water for use on each day of our experiment. 200mg of this extract/kg b.wt. was dissolved in distilled water and administered to the animals.

- **Fullerene C_{60}:** Fullerene C_{60} (purity 99.9%) was obtained from Lydow Group Limited Research Corporation (China) and used without further purification.
- **Virgin Olive Oil:** Virgin olive oil is obtained from a Colavita Extra Virgin Olive Oil Company, which extracted from Olives harvested and pressed in Italy.

Fullerene C_{60}-Olive Oil Solution Preparation:

After sourcing the high purity C_{60}, we prepared C_{60}-olive oil solution according to Batti *et al.* (2012), we took 50 mg (milligrams) of high purity C_{60} (fullerene) and added it to 10 ml of olive oil. This solution was then stirred for two weeks non-stop in the laboratory without probable heat and mechanical failure. Once the two weeks had passed, we then used a centrifuge for one hour on the C_{60} Olive Oil solution. The centrifuge typically spins a solution at a high rate of speed to allow undissolved particles to collect at the bottom of the container, once it was prepared, it would be used in the experiment. After the centrifuge was completed supernatant solution was then finally filtered. The filtrate (C_{60} Olive Oil) used in the biochemistry Millipore filter paper that had 0.25 micro-meter porosity.

Experimental Design:

Male rats (200-250) gm. was used for the present study and they were classified into 5 groups, 10 animals of each group.

The First Group*:* animals were intraperitoneal (i.p.) Injected with only saline solution Nacl 0.9% (10 ml/kg b.wt) for 40 days, this group served as a normal group.

The Second Group: animals were i.p. injected a single dose with CRP (80 mg/kg), and then after 24 hours received a saline solution for the remaining 40 days, this group served as a control group.

The Third Group: animals were i.p. injected single dose with CRP (80 mg/kg), and then after one day treated orally by *Annona muricata* leaves extract at a dose of 200 mg/kg/day for 40 consecutive days.

The Fourth Group: animals were i.p. injected single dose with CRP (80 mg/kg), and then after one day were received orally fullerene C_{60} (4 mg/ kg b. wt.) daily for 40 consecutive days.

The Fifth Group: animals were i.p. injected single dose with CRP (80 mg/kg), and then after one day were treated orally by *Annona muricata* leaves extract (200 mg/kg/day) and received also fullerene C_{60} (4 mg/ kg b. wt.) orally for 40 consecutive days.

All animals were sacrificed at the end of the experiment.

Blood Collection:

At the end of the experiment, the blood of all animals was divided into two portions one portion was taken in EDTA containing tubes from every animal. This blood was used for the examination of the complete blood picture. The other portion of blood was left in clean tubes at room temperature to clot, then after an hour; serum was separated by centrifugation for 30 minutes at 3000 rpm. The sera were collected in labeled epindorff's tubes and stored at - 20 °C until used for biochemical analysis. A part of the right lobe of the liver and a part of the kidney were dissected and washed with physiological saline solution, dried, weighed and homogenized in phosphate buffer (pH 7.4) and kept frozen until used for biochemical assays. The obtained results as follow:

Hematological Analysis:

The hematological evaluation consisted of erythrocytes (RBCs), white blood cells (WBCs), platelets (PLT) counts and Hb content, the determination by Automated Hematology Analyzer (Diff3) Mek6410/Mek-6420.

Biochemical Analysis:

- **Serum Indices of Hepatotoxicity and Renal Toxicity:**

Determination of alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein and albumin, creatinine, urea, and uric acid was performed according to the manufacturer's protocol of reagent kits purchased from Sigma Chemical Company (St. Louis, MO, USA).

- **Liver and Kidney Homogenate:**

Reduced glutathione (GSH), catalase assay (CAT), superoxide dismutase (SOD) and malondialdehyde MDA were performed according to the manufacturer's protocol of reagent kits purchased from spectrum Diagnostics, Egypt.

Statistical Analysis:

The variability degree of results was expressed as Means ± S.D. The data were statistically analyzed by one-way ANOVA analysis of variance (prism computer program) and the least significant difference (L.S.D) was used to test the difference between treatments.

RESULTS

Hematological Indices:

Rats treated with CRP resulted in a highly significant decrease in RBCs count, WBCs count, PLTs count, Hb content and PCV value at ($p<0.01$). These results were recorded in table (1), when compared with the normal group. On the other hand, Animals treated with *Annona muricata* leaves extract, fullerene C_{60} and (*Annona muricata* + fullerene C_{60}) recorded a graduated improvement as a highly significantly increased at ($p<0.01$) in the count of RBCs, WBCs, PLTs, Hb content, and PCV value when compared with CRP (gp 2) but it did not reach to normal animals (gp 1).

Table 1: Effect of *Annona muricata*, fullerene C_{60}, and *Annona muricata* + fullerene C_{60} for 40 days of treatment after a single dose of CRP injection on hematological parameters (RBCs, WBCs, Platelets count, Hb content and PCV value) in males of Albino rats.

Groups	RBCs count ($x\ 10^6/mm^3$)	WBCs count ($x\ 10^3\ mm^3$)	Hb content (g/dL)	Platelets count ($x\ 10^3\ mm^3$)	PCV (%)
	Mean ±S.D.	Mean ± S.D.	Mean±S.D.	Mean ± S.D.	Mean±S.D.
Normal	6.9 ± 0.58	8.1 ± 0.57	12.66 ± 0.8	503.6 ± 42.22	37.43 ± 2.2
Carboplatin (CRP)	3.66 ± 0.46^{--a}	4.97 ± 0.73^{--a}	8.15 ± 1.04^{--a}	296.8 ±36.81^{--a}	22.43 ± 1.6^{--a}
CRP + *Annona muricata*	6.04 ± 0.36 $^{--a\ ++b}$	6.38 ± 0.78 $^{--a\ ++b}$	10.76±0.79$^{-a\ ++b}$	387.1 ± 48.37$^{--a\ ++b}$	30.98 ± 1.6$^{--a\ ++b}$
CRP +Fullerene C_{60}	5.8 ± 0.35 $^{--a\ ++b}$	7.05 ± 0.86 $^{-a\ ++b}$	10.73 ±0.63 $^{--a\ +^{+b}}$	490.3 ±52.08 $^{++b}$	31.85±2.28 $^{--a\ +^{+b}}$
CRP+ *Annona muricata* +Fullerene C_{60}	6.2 ± 0.49 $^{-a\ ++b}$	7.86 ± 0.89 $^{+^{+b}}$	11.51 ±0.69 $^{-a++b}$	497.7 ±36.98 $^{++b}$	33.81 ± 1.78^{--a++b}

Results are expressed as mean ± S.D. of 10 animals for each group.
-a = significantly decreased compared with normal at p<0.05
--a = highly significant decrease compared with normal at p<0.01.
++b = highly significant increased compared with control at p<0.01.

Liver Function:

In the present study, as indicated in table (2) serum ALT, AST, ALP and total bilirubin activities of rats treated with CRP showed that there was a highly significant increase at (p<0.01), accompanied with highly significantly decrease at (p<0.01) in the activities of albumin and total protein as compared with normal animals. While treatment with *Annona muricata* leaves extract, fullerene C_{60} and *Annona muricata* leaves + fullerene C_{60} of CRP-treated rats showed a highly significant decrease in the activities of ALT, AST, ALP and total bilirubin in comparison with rats intoxicated with CRP in control group. On the other hand, the activities of albumin and total protein recorded highly significantly increase at (p<0.01) when compared with a control group, but when compared with the normal rats the results indicated that the activities of albumin and total protein were improved but not approached to the normal level.

Table 2: Effect of *Annona muricata*, fullerene C_{60}, and *Annona muricata* + fullerene C_{60} for 40 days of treatment after a single dose of CRP injection on liver function parameters (serum ALT, AST, ALP, total bilirubin, albumin, and total protein) in males of Albino rats.

Groups	ALT (Units/ml)	AST (Units/ml)	ALP (IU/L)	Total Bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
	Mean ± S.D.	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**
Normal	74.28 ± 6.19	63.68± 3.65	46.7 ± 4.61	1.3±0.26	7.07±0.4	3.62±0.28
Carboplatin (CRP)	133 ± 9.65 $^{++a}$	114.1± 4.22^{++a}	109.5 ± 5.21^{++a}	2.9±0.66^{++a}	4.13±0.5^{--a}	1.83±0.44^{--a}
CRP + *Annona muricata*	93.2 ± 5.11$^{++a\ --b}$	85.51± 3.15$^{++a\ --b}$	78.61 ± 4.06$^{++a--\ b}$	1.98±0.3$^{++a\ --\ b}$	6.03±0.58$^{--a\ ++b}$	2.7±0.49$^{--a\ ++b}$
CRP +Fullerene C_{60}	82.49 ± 4.21$^{+a\ --\ b}$	78.45 ± 5.24$^{++a\ --\ b}$	63.1 ± 9.95$^{++a--\ b}$	1.67±0.18 $^{--\ b}$	6.11±0.59$^{--a\ ++b}$	3.13±0.34 $^{++b}$
CRP + *Annona muricata* +Fullerene C_{60}	80.15 ± 4.11$^{+a\ -\ -\ b}$	76.88 ± 7.76$^{++a\ --\ b}$	57.56 ± 5.3$^{++a--\ b}$	1.43±0.28 $^{--\ b}$	6.49±0.59 $^{-a\ ++b}$	3.34±0.43 $^{++b}$

Results are expressed as mean ± S.D. of 10 animals for each group.
+a = significantly increased compared with the normal at p<0.05.
++a = highly significantly increased compared with the normal at p<0.01.
-a = significantly decreased compared with normal at p<0.05.
--a = highly significant decrease compared with normal at p<0.01.
++b = highly significant increased compared with control at p<0.01.
--b = highly significant decreased compared with control at p<0.01.

Kidney Function:

As shown in table (3) creatinine and urea in the serum of rats intoxicated with CRP (gp 2) resulted in a highly significant increase at (p<0.01) associated with a highly significantly decreased in uric acid compared with the normal rats. On other hand, levels of serum creatinine and urea showed a highly significant decrease at (p<0.01) with a highly significant increase in uric acid in treated rats intoxicated with CRP (gp 3 and 4) comparing with the control group but also it still not reaches to normal values. As well as, levels of

serum creatinine and urea of rats intoxicated with CRP decreased a highly significantly at ($p<0.01$) while uric acid increased a highly significantly when treated with *Annona muricata* leaves extract and fullerene C_{60} together in comparison with rats intoxicated with CRP in group 2. These results were approached to the normal level.

Table 3: Effect of *Annona muricata*, fullerene C_{60}, and *Annona muricata* + fullerene C_{60} for 40 days of treatment after a single dose of CRP injection on kidney function parameters (serum creatinine, urea, and uric acid) in males of Albino rats.

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Normal	0.44 ± 0.07	22.5 ± 2.8	4.13 ± 0.49
Carboplatin (CRP)	1.04 ± 0.17^{++a}	63.2 ± 4.9^{++a}	1.38 ± 0.37^{--a}
CRP + *Annona muricata*	0.74 ± 0.1$^{++a\ --b}$	31.1 ± 2.6$^{++a\ --b}$	2.68 ± 0.69$^{--a\ ++b}$
CRP +Fullerene C_{60}	0.55 ± 0.07^{--b}	26.5 ± 2.02$^{+a\ --b}$	3.42 ± 0.48$^{-a\ ++b}$
CRP + *Annona muricata* +Fullerene C_{60}	0.48 ⊥ 0.05^{--b}	23.5 ⊥ 2.16^{--b}	3.82 ⊥ 0.61^{++b}

Results are expressed as mean ± S.D. of 10 animals for each group
+a = significantly increased compared with the normal at $p<0.05$.
++a = highly significantly increased compared with the normal at $p<0.01$.
-a = significantly decreased compared with normal at $p<0.05$.
--a = highly significantly decreased compared with normal at $p<0.01$.
++b = highly significantly increased compared with control at $p<0.01$.
--b = highly significantly decreased compared with control at $p<0.01$.

Liver and Kidney Tissues Homogenate:

As indicated in table (4), rats intoxicated with CRP recorded a highly significant increase at ($p<0.01$) in Malondialdehyde (MDA) level, with a marked reduction in GSH, CAT and SOD activities comparing with normal rats. On the other hand, intoxicated rats treated with *Annona muricata* leaves extract, fullerene C_{60} and *Annona muricata* leaves extract + fullerene C_{60} induced a highly significant decrease at ($p<0.01$) in MDA, associated with a highly significant increase at ($p<0.01$) in GSH, CAT and SOD activities comparing with intoxicated rats in group 2. These results recorded a remarkable improvement compared with normal animals.

Table 4: Effect of *Annona muricata*, fullerene C_{60}, and *Annona muricata* + fullerene C_{60} for 40 days of treatment after a single dose of CRP injection on reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and Malondialdehyde (MDA) activities in liver and kidney tissues of male Albino rats.

Groups	GSH (mmol/g. tissue)		CAT (U/g)		SOD (U/g. tissue)		MDA (n mol/g. tissue)	
	Mean ± S.D.		Mean ± S.D.		Mean ± S.D.		Mean ± S.D.	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal	1.89±0.21	1.91±0.27	1.88±0.11	2 ±0.34	253.5±10.42	280.2±12.72	53.81±4.19	49.28±4.2
Carboplatin (CRP)	0.69±0.12^{--a}	0.62±0.18^{--a}	0.77±0.16^{--a}	0.85±0.15^{--a}	213.9±8.64^{--a}	215.6±9^{--a}	112.1±6.78^{++a}	92.26±4.43^{++a}
CRP + *Annona muricata*	1.45± 0.17$^{--a\ ++b}$	1.39± 0.13$^{--a\ ++b}$	1.41± 0.25$^{--a\ ++b}$	1.4± 0.41$^{--a\ ++b}$	242.8± 6.59$^{--a\ ++b}$	249.7± 10.07$^{--a\ ++b}$	70.27± 8.69$^{++a\ --b}$	79.13± 5.88$^{++a\ --b}$
CRP +Fullerene C_{60}	1.68±0.16$^{-a\ ++b}$	1.58±0.1$^{--a\ ++b}$	1.66±0.21$^{-a\ ++b}$	1.73±0.29^{++b}	252.7±9.38^{++b}	264.2±6.61$^{--a\ ++b}$	60.21±5.68^{--b}	60.57±5.24$^{++a\ --b}$
CRP + *Annona muricata* +Fullerene C_{60}	1.72± 0.13$^{-a\ ++b}$	1.71± 0.18$^{-a\ ++b}$	1.77± 0.16^{++b}	1.85± 0.4^{++b}	255.1± 9.1^{++b}	274.1± 8.09^{++b}	58.14± 4.38^{--b}	56.9± 4.97$^{++a\ --b}$

Results are expressed as mean ± S.D. of 10 animals for each group.
++a = highly significantly increased compared with the normal at $p<0.01$.
-a = significantly decreased compared with normal at $p<0.05$.
--a = highly significant decrease compared with normal at $p<0.01$.
++b = highly significantly increased compared with control at $p<0.01$.
--b = highly significantly decreased compared with control at $p<0.01$.

DISCUSSION

Effect of *Annona muricata* Leaves Extract and Fullerene C_{60} on Some Hematological Parameters of Rats Intoxicated with CRP:

In the current study, it was detected that due to single dose of CRP treating rats, the number of RBC, WBC, PLT, Hb content, and mean values of PCV were significantly decreased, Equivalent results were stated by several authors (Hassan and Chibber, 2010 and Ashry and Elkady, 2014). The results indicate that after CRP treated rats, the number of WBC was significantly decreased. This might be due to infection and inflammation during CRP treatment. The main molecular mechanism of CRP is myelotoxicity is due to its ability to bind with cellular DNA and reduce the cell unable to replication (Waykar *et al*., 2018). Adjacent to this, an alternative mechanism of CRP is its ability to induce oxidative stress (Malarczyk *et al*., 2003).

Reactive oxygen species are toxic to bone marrow cells and maybe can activate apoptosis and affect cell cycle, producing anemia and a decrease in leukocyte count (Hoagland, 1982). Myelosuppression subsequent in leukopenia and thrombocytopenia is a common and a major difficulty of cancer chemotherapy (Mahadev, 1998). Many authors (Bhavaraju *et al*., 2004 and Atrooz *et al*., 2008) detected that the number of WBC was decreased after exposure to CRP administration.

On the other hand, the platelet count was highly significantly decreased compared to a normal group. This might be due to CRP preventing bone marrow activity or could be due to decreased production or increased ingesting of platelets or due to the increased platelet aggregation (Olas *et al*., 2005). CRP causes oxidative stress in human platelets and lymphocytes, which might reflect on their life expectancy, the initiation of apoptosis, and thereby eventually reduced the number of these cells in the blood of experimental animals (Khynriam and Prasad, 2001). CRP reduced the platelet count in rats under experiment, and depleted the platelet number and caused cumulative anemia (Markovic *et al.*, 2011).

After CRP injection, the total number of RBC and Hb content were highly significantly decreased as compared to normal animals. Similar results were reported by Sirage (2009). The previous results recommended that there was an etiological association between anemia and CRP action. This relation could be pronounced through different mechanisms, including the damage of bone marrow cells or increased osmotic breakability of RBC. Thus, CRP healing might lead to anemia as a result of either destruction of the activity of hematopoietic tissues, reduced erythropoesis, and enhanced RBCs destruction because of the altered RBCs membrane permeability, increased RBCs mechanical fragility, and defective iron metabolism (Markovic *et al*., 2011). The reduction of RBC and Hb content attributed to the hemorrhage, hemolysis or because of reduced blood formation in bone marrow due to CRP toxicity and that led to imbalance between production and loss, inhibition of DNA synthesis in bone marrow precursor cells, leaving both RNA and protein synthesis intact and inhibition of many steps of heme biosynthesis in rats, as result of CRP action (Waykar *et al*., 2018). The reduction in the Hb content is related to the suppression of erythropoiesis and iron supply to erythroblasts (Cazzola, 2000).

It was also found that CRP therapy inhibited the production of renal erythropoietin, which resulted in a lower RBC production. The nephrotoxic effect of CRP showed a negative effect on erythropoiesis that resulted in the low production of RBCs

(Hassan *et al*., 2010). CRP causes anemia by interfering in iron metabolism (Gao et al., 2013). Due to CRP treatment, the mean values of PCV value was significantly decreased as compared to the normal group. Similar results were reported by a number of authors (Nasr, 2014; Maheswari and Manohari, 2015 and Geyikog *et al*., 2016).

It is stated that reactive oxygen species (ROS) increases hemoglobin glycation and erythrocyte fragility and bone marrow can be impaired by direct oxidation (Ghosh *et al*., 2005 and Niforou, 2014). More specifically haemoglobin derived iron might contribute to the pathogenesis of CRP by inducing oxidative stress (Baliga *et al*., 1998).

In the current study it was observed that after *Annona muricata* leaves extract and fullerene C_{60} treatment, the total number of RBC, WBC, PLT, Hb content and the mean values of PCV of experimental rats were significantly increased as compared to control group. Antioxidants can prevent cell damage caused by the action of reactive oxygen species (ROS) and free radicals (Cherubini *et al*., 2005). The antioxidant activities are related to a number of different mechanisms, such as free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl (Robards *et al*.,1999).

Recently, medicinal plants have received particular attention as a highly efficient antioxidant and have the free radical scavenging capacity. Therefore, the use of plants and herbs as antioxidants in processed food is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants (Sacchetti *et al.*, 2005), where plants usually contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, and more (Agati *et al*., 2012 and Chiang *et al*., 2013). *Annona muricata* is a natural antioxidant, which may contain flavonoids, ascorbic acid, tocopherols, catalase, and phenolic compounds all of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals (Adewole and Ojewole, 2009).

It is obvious that the fullerene C_{60} administrated to rats intoxicated with CRP, caused hematological changes in the blood of rats, which characterized by a pronounced improvement of the total number of RBC, WBCs and PLT, remarkable improvement in Hb contents and significant increase in PCV value. This improvement in RBC may be attributed to the fullerene C_{60} reacting with both; the drug and the membrane proteins of the erythrocytes. Therefore, it may diminish the complication of the side effects of CRP (Grebowski *et al.,* 2013). It is worth to mention that, the mechanical properties of the human RBC membrane can be changed due to the presence of C_{60} nanoparticles (Zhang *et al.,* 2013).

Also, fullerene C_{60} improves platelet count is attended to increase in RBC count. This is in harmony with Radomski *et al.* (2005) who revealed that the exposure to carbon nanoparticles led to a significant increase in platelet count in the blood. On the other hand, there is a deficiency of studies on the effects of fullerene C_{60} on blood cells. For example; Fullerene C_{60} induced negligible changes in various biochemical and hematologic parameters (Yamashita *et al*., 2013). Therefore, the present study detected that an improvement in the WBCs count may be attributed to the anti-inflammatory activity of fullerene C_{60} (Mamontova *et al*., 2013). In addition, an enhancement in PCV in rats treated with C_{60}. It is well known that the hematocrit measures the volume of red blood cells compared to the total blood volume, on this

foundation, any change in the RBC number effects on PCV and Hb.

In the present study it was observed that after the combined treatment of CRP along with *Annona muricata* leaves extract and fullerene C_{60}, the total number of WBC, RBC, PLT, Hb content and mean values of PCV of experimental rats were highly significantly increased as compared to animal treated with CRP. *Annona muricata* and fullerene C_{60} have a protective role against many drugs.

Effect of *Annona muricata* Leaves Extract and Fullerene C_{60} on Some Liver Biochemical Parameters of Rats Intoxicated With CRP:

It was found that rats induced by CRP showed a highly significant increase in the activities of the serum AST, ALT, ALP enzymes and total bilirubin value. The current results are in agreement with other studies (Maheshwari *et al*., 2019). In addition, results clearly indicated a highly significant decrease in albumin and total protein in rats intoxicated with CRP. The results documented are in agreement with the results of previous detectives (Ashry, 2014 and Mir *et al*., 2015).

The reduction in the total protein and albumin was recognized due to oxidative stress produced by CRP. CRP are inducers of reactive oxygen species (ROS) (Mansour *et al*., 2006). In the presence of reactive oxygen species (ROS), proteins can be injured by direct oxidation of their amino acid residues and cofactors or by secondary attack via lipid peroxidation (Ahmida, 2012). Liver injury leads to a decrease in the synthetic ability of hepatocytes, producing a reduction in serum total protein and albumin levels (Bass, 2003 and Ganong, 2006). Also, the reduction in the serum albumin and total protein may similarly refer to the renal damage result in excretion in urine (Vosilinko and Grebenev, 1990).

It worthily indication that, necrosis or membrane impairment releases the enzyme into circulation and therefore it can be measured in the serum. increase levels of AST indicate liver damage that happens in many diseases such as viral hepatitis as well as cardiac infarction (heart attack) and muscle damage. AST catalyzes the change of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver, and consequently is an enhanced parameter for noticing the liver damage. Raised levels of serum enzymes indicate cellular leakage and functional integrity loss of cell membranes in the liver (Ashraf, 2013).

Commonly, ALP concentration is related to the functioning of hepatocytes, a high level of ALP in the blood serum is related to the increased synthesis of it by cells lining bile frequently in response to cholestasis and increased biliary pressure (Gopalakrishnan and Dhanapal, 2014). Similarly, ALP is membrane-bound and its variation is likely to affect the membrane permeability and create an imbalance in the transport of metabolites (Mehana *et al*., 2012 and Soliman, 2016).

The obtained results show that CRP administration produced severe liver damage characterized by severe activation of Kupffer cells, degenerated hepatocytes and moderate enlargement of sinusoids (Maheshwari *et al*., 2019). Almost every drug has been associated with hepatotoxicity due to the vital role of the liver in drug metabolism (Tolman, 1998). Additionally, the liver functions were damaged in CRP treated group as reproduced by elevation in the activities of ALP and AST in the plasma when compared with the control rats, this finding is parallel to previous studies that reported significant increase in the levels of serum ALT, AST and ALP in rats after long term usage of cisplatin (Maheshwari *et al*., 2019).

Furthermore, the current results showed pronounced improvement in the activities of serum AST, ALT, ALP and total bilirubin with a marked increase in total protein and albumin in rats treated with *Annona muricata* extract, this finding indicates that plant extract tended to prevent the impairment and suppressed the leakage of enzymes through cellular membranes. These results are in agreement with those obtained by Nwogu *et al*. (2010) and Owolabi (2013) who stated that treatment of rats with *Annona muricata* leaves extract significantly reduced the elevated levels of ALT, AST, ALP, and total bilirubin near to the individual normal values indicating stabilization of plasma membrane as well as repair of hepatic tissue damage induced by CRP. Treatment of *Annona muricata* removed the CRP intoxication reduced compared to CRP alone in the control group. The defense conferred could be due to the occurrence of bioactive compounds such as acetogenins, flavonoids, alkaloids, saponins, tannins and vitamin C in *Annona muricata* leaves (Usunobun and Okolie, 2015 and Usunobun *et al*., 2015).

In the present results fullerene, C_{60} treated rats revealed a marked decrease in the activities of serum AST, ALT, ALP and total bilirubin associated with a pronounced increase in total protein and albumin compared to rats intoxicated with CRP, this indicated that fullerene C_{60} tended to prevent the damage and suppressed the leakage of enzymes through cellular membranes. This result is in agreement with Batti *et al*. (2012) who stated that fullerene C_{60} improved the altered activities of liver enzymes which are intoxicated by CCl_4 intoxication. Fullerene C_{60} provided antioxidant activity (Liu *et al.,* 2014). Fullerene C_{60} produced a stabilization of plasma membrane as well as repair of hepatic tissue damage that can be considered as an expression of the functional enhancement of the hepatocytes (Azevedo Costa *et al*., 2012). Similarly, Bisaglia *et al.* (2000) stated that fullerene C_{60} can suppress the cellular apoptosis of the liver by inhibition of oxidative stress. It is well recognized that fullerene C_{60} is able to scavenge a large number of radicals per molecule (Krusic *et al.,* 1991) which is attributed to its free radical sponge properties due to delocalized π double bond system in fullerene C_{60} structure, So that it can increase the antioxidative defense within the liver (Halliwell *et al.,* 1995) and the liver enzymes and protein synthase as a consequent.

Effect of *Annona muricata* Leaves Extract and Fullerene C_{60} on Some Kidney Biochemical Parameters of Rats Intoxicated with CRP:

The present data indicate that a single dose of CRP caused renal dysfunction in rats as showed by elevation of plasma creatinine and blood urea levels with a remarkable decrease in uric acid level. This statement is in support of previous studies (Boisdron-Celle *et al*., 2001) and it is an indication of renal toxicity which causes a reduction in glomerular filtration rate leading to the accumulation of creatinine and urea in the blood. CRP significantly increased platinum accumulation in the kidney as compared to the normal group. It has been recommended that the covalent binding of platinum to renal proteins and platinum accumulation in the kidney may have a role in the nephrotoxicity induced by this platinum-containing anticancer agent (Kazim *et al*., 2002).

The renal platinum accumulation and nephrotoxicity subsequent CRP are related to the variances in the pharmacokinetics and pharmacodynamics in rats (Boisdron-Celle *et al*., 2001). Metabolites of drugs are excreted in the kidneys and some may cause cellular impairment which could lead to kidney dysfunction (Singhal *et al*., 1998).

Studies demonstrate that nephrotoxicity induced by chemical agents is one of the significances of the accumulation of certain metabolites in kidneys (Sener *et al.* 2003).

CRP induced nephrotoxicity may similarly be due to reduced antioxidant enzyme activities and the destruction of antioxidant enzyme protein expressions in the kidney of the rat. The antioxidant enzymes are the first line of protection against free radicals/reactive oxygen species (ROS) induced oxidative renal damage (Kazim *et al*., 2002). This study statements the changes in renal function and its association with endogenous oxidants/antioxidant systems and platinum content in kidney tissues of rats in response to CRP at different time points. Previous studies had shown that CRP is toxic at advanced clinical therapeutic doses in cancer patients (Maldoon *et al*., 2000).

However, *Annona muricata* treatment prior to CRP administration significantly lowered the serum urea and creatinine levels, thus improving renal function. In respect to *Annona muricata* leaves extract dealing animals, when compared with rats intoxicated with CRP the improvement takes place. The preventive of leaves extract of *Annona muricata* effects on rats intoxicated with CRP, side effects have been attributed to the aptitude of plant preventing the failure of the renal antioxidant status or due to having antioxidant and radical scavenging action of leaves extract of *Annona muricata* plant, which has useful effects of mediators of large vessel damage so it has ability to the prevention nephropathy**.** Leaves extract of *Annona muricata* plant displayed improved renal function where it induced a reduction in the creatinine and urea (Usunobun and Okolie, 2016).

The standard results when paralleling the levels of urea, creatinine and uric acid in the groups treated with fullerene C_{60} with rats intoxicated with CRP, the enhancement will be clear. In fact, there are not enough studies about the effect of fullerene C_{60} on kidney function parameters, but Mori *et al.* (2007) recommended that the administration of fullerene C_{60} prohibited radical formation, liver and kidney injury, and death. Moreover, fullerene C_{60} has the ability to aggregate in serum and it is mainly distributed to the liver and kidney (Nielsen *et al.*, 2008) and they act as the free radical scavenger and protect the cells from damage which lead to reappearance kidney function parameter to the normal value. There is a study supports the anti-apoptotic effects of fullerene derived in kidney cells exposed to oxidative stress (Chien *et al.*, 2001). It is worth to indicate that, the injured kidneys were returned to the normal level because of nanoparticle treatments (wang *et al.*, 2006).

Effect of *Annona muricata* Leaves Extract and Fullerene C_{60} on Antioxidant Enzymes of Liver and Kidney Tissues Homogenate of Rats Intoxicated with CRP:

The results of existing study revealed that a single dose of CRP produced a patent oxidative influence as showed by a highly significant increase in the level of MDA in liver and kidney tissues homogenate as one of the main end products of lipid peroxidation, associated with a highly significant decrease in the activities of SOD, CAT and reduced GSH. These are in agreement with those documented by (Kazim *et al*., 2002 and Kazim *et al*., 2004).

This effect may be a minor occurrence following the CRP induced increase in free radical generation and a decrease in lipid peroxidation protecting enzymes. CRP can cause the generation of oxygen free radicals, for instance, hydrogen peroxide, superoxide anions, and hydroxyl radicals. The hydroxyl radical is proficient in removing a hydrogen

atom from polyunsaturated fatty acids in membrane lipids to recruit lipid peroxidation. These radicals can evoke widespread tissue damage, reacting with macromolecules, for instance, membrane lipids, proteins, and nucleic acids. Furthermore, the reduction of glutathione may contribute to CRP induced lipid peroxidation. (Kim *et al*., 2004)

CRP intoxication produced a sense reduction of GSH. The reduced form of GSH converts oxidized to GSSG on interacting with free radicals. (Gupta and Kumar, 2003). Excessive production of free radicals caused oxidative stress, which leads to damage to biomolecules and can induce lipid peroxidation. (Ip *et al*., 1996 and Maheshwari *et al*., 2015). In the present study, the decreased level of GSH was associated with enhanced lipid peroxidation in CRP intoxicated rats. Therefore decrease in the activity of these enzymes may consequence in a number of harmful effects due to the accumulation of superoxide radicals and hydrogen peroxide (Maheshwari *et al*., 2015).

The reduction of glutathione in tissues is a vital rate, which can lead to damage of the cellular resistance against reactive oxygen species and may result in peroxidative injury (Deleve and Kaplowitz 1990). Additionally, it has been revealed that the reduction of glutathione reduced by buthionine sulfoximine can consequence in potentiation of CRP toxicity (Hu *et al.* 1999).

CRP induced liver and renal toxicity may be due to an increase of reactive oxygen species produced due to metal-catalyzed reactions and increased lipid peroxidation, as a result of damage of liver and renal antioxidant enzyme activities and protein expressions as well as decrease of glutathione. CRP caused a dose-dependent decrease in the activities of the antioxidant enzymes superoxide dismutase and catalase in the liver and kidney. This damage to the antioxidant enzymes can result in the additional generation of superoxide anions and organic peroxides in the liver and renal tissues, leading to oxidative damage. Supplementary, the reduction of these antioxidant enzymes in the liver and kidney can lead to improved reactive oxygen species induced lipid peroxidation. The inhibition of antioxidant enzyme activities in liver and kidney after administration of single dose of CRP maybe because of (1) direct binding of CRP to vital sulfhydryl groups of these enzymes; (2) reduction of copper and zinc which are important for superoxide dismutase action (DeWoskin and Riviere, 1992); and (3) increased reactive oxygen species and organic peroxides which inactivate antioxidant enzymes (Pigeolet *et al.* 1990).

Administration of *Annona muricata* and fullerene C_{60} restored the activities of GSH, SOD, and CAT in CRP intoxicated rats. Results revealed that *Annona muricata* and fullerene C_{60} prohibited excessive free radicals accumulation and protected the liver and kidney from CRP induced hepatotoxicity and renal toxicity, thus *Annona muricata* and fullerene C_{60} may act by inducing the clearing enzymes and these enzymes might purify the free radicals produced following CRP intoxication. Treatment with *Annona muricata* and fullerene C_{60} also decrease in MDA levels which is responsible for the formation of lipid peroxidation. Therefore, *Annona muricata* and fullerene C_{60} reduced the levels of MDA indicating a protective antioxidant effect on the animals treated with CRP.

Similarly, fullerene C_{60} has antioxidant properties, the current study records that clearly. There are four potential mechanisms in the C_{60} liver and kidney protector were suggested, the first one is C_{60} can scavenge great numbers of free radicals (Gharbi *et al.*, 2005 and Andrievsky *et al.*, 2009). The reaction detected for C_{60} free radical scavenger

is a Dielsen Alder like reaction with retinol and retinyl esters inside the liver cells (Moussa *et al.,* 1998), the second mechanism is that can act as a breakdown catalyst for $O_2^{\bullet}/H_2O_2$, as it has been assumed for its tris-malonic acid derivatives (Ali *et al.,* 2004). The third mechanism is that fullerene C_{60} activities as a cytochrome P450 inhibitor as it has been stated for some fullerene products (Gharbi *et al.,* 2005), and the fourth mechanism is it can deactivate Kupffer cells through the buildup and congestion with a large number of fullerene C_{60} collections (Tykhomyrov *et al.,* 2008).

In the current study, the reduction of MDA rate shows that the aptitude of fullerene C_{60} to scavenge the free radicals as displayed in the first and second mechanisms which stated. Remarkably, the C_{60} has an aptitude to get a constructive charge by absorbing inside some protons and this compound could pass into mitochondria. Such a process permits for the slight separation of respiration and phosphorylation. This, in order, indicates the diminution in ROS production (Chistyakov *et al.,* 2013). Additionally, in this study the recorded data it has shown a highly significantly increase in SOD activity. Fullerene C_{60} could act as a SOD derivative, this fact was sustained before by previous study. Ali et al. (2004) indicated that fullerene C_{60} could act as superoxide/catalase imitative. In this study the best protection effect was obtained when treated with fullerene C_{60} after intoxicated with CRP and this results in agreement with previous study, which displayed the effects of fullerene C_{60} oil solutions on toxicity (Gharbi *et al.,* 2005).

The results of the existing work displayed that group of rats administered *Annona muricata* leaves extract + fullerene C_{60} revealed a highly significant increase of GSH, SOD, and CAT with a highly significant decrease of MDA in liver and kidney tissues, this group represent the greatest an improvement due to the presence of antioxidant production which reducing of free radicals.

It could be concluded that carboplatin, led to impairment of liver and kidney functions. Moreover, either *Annona muricata* leaves extract, or fullerene C_{60} alone or combined as a powerful antioxidant against results in suppression to the adverse effect caused by carboplatin, furthermore, *Annona muricata* + fullerene C_{60} together causing a marked regeneration than each of them alone noticed in most of the hematological and biochemical analysis.

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ARABIC SUMMERY

الاثار التحسينية لـ *Annona muricata* (القشطة) والفوليرين C_{60} ضد السمية المستحدثة بواسطة الكاربوبلاتين فى ذكور الفئران البيضاء

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صممت هذه الدراسة لإظهار الآثار التحسينية لمستخلص نبات *Annona muricata* (القشطة) والجسيمات النانوكربونية فوليرين C_{60} على الأضرار الناجمة على المكونات الخلوية للدم والقياسات البيوكيميائة المستحدثة بواسطة الحقن فى الغشاء البريتونى على ذكور الفئران. قد تم تصميم الدراسة على خمس مجموعات من ذكورالفئران البالغة (١٠ فئران بكل مجموعة). المجموعة ١: تم حقنها فى الغشاء البريتونى بمحلول ملحي من كلوريد الصوديوم (١٠ مل /كجم من وزن الجسم) واعتبرت كمجموعة طبيعية. المجموعة ٢: حقنت بجرعة واحدة فى الغشاء البريتونى بالكاربوبلاتين (٨٠ ملجم /كجم من وزن الجسم) ، ثم حقنت فى الغشاء البريتونى بمحلول ملحى من كلوريد الصوديو يوميا لمدة أربعون يوما وإعتبرت المجموعة الضابطة. المجموعة ٣: تم إعطاء الحيوانات مستخلص أوراق *Annona muricata* من خلال الفم بجرعة (٢٠٠ ملجم /كجم يوميا) لمدة ٤٠ يوما متتالين بعد ٢٤ ساعة من الحقن بجرعة واحدة فى الغشاء البريتونى من الكاربوبلاتين (٨٠ ملجم /كجم من وزن الجسم). المجموعة ٤: تم علاج الحيوانات عن طريق الفم بالفوليرين C_{60} من خلال الفم بجرعة (٤مجم/كجم) من وزن الجسم يوميا لمدة ٤٠ يوما بعد ٢٤ ساعة من حقنها بجرعة واحدة فى الغشاء البريتونى من الكاربوبلاتين (٨٠ ملجم /كجم من وزن الجسم). المجموعة ٥: كل فأر في هذه المجموعة تم علاجه عن طريق الفم يوميا بمستخلص أوراق *Annona muricata* بجرعة (٢٠٠ ملجم /كجم يوميا) + الفوليرين C_{60} بجرعة (٤مجم/كجم) لمدة ٤٠ يوما بعد ٢٤ ساعة من حقنها بجرعة واحدة من الكاربوبلاتين.

كشفت النتائج المتحصل عليها أن الحيوانات التى تم إعطاؤها الكاربوبلاتين فى المجموعة الضابطة سجلت نقص ملحوظ فى عدد كريات الدم الحمراء، كريات الدم البيضاء، الصفائح الدموية، تركيز الهيموجلوبين ونسبة حجم خلايا الدم الحمراء المتجمعة (PCV). بالإضافة إلى ذلك، هناك ارتفاع فى أنشطة كلا من ALT, AST, ALP و مستوى البيليروبين الكلي، الكرياتينين واليوريا مع إنخفاض ملحوظ فى مستوى الألبيومين, البروتين الكلى وحمض اليوريك. إضافة إلى ذلك، أظهرت النتائج أن الكاربويلاتين سجل زيادة فى مستويات المواد التفاعلية حمض الثيوباربيتوريك (TBARS) فى أنسجة كلا من الكبد والكلي في حين كان هناك نقص معنوي ملحوظ في نشاط السوبر أوكسيد ديسميوتيز(SOD) , نشاط إنزيم الكتاليز (CAT) وكمية الجلوتاثيون (GSH). أكدت هذه الدراسة خطر زيادة الإجهاد التأكسدي، والسمية الكبدية والكلوية بسبب إعطاء الكاربوبلاتين. بالإضافة إلى ذلك، أظهرت النتائج دورمستخلص أوراق القشطة و الفوليرين C_{60} ضد الضرر الخلوي نتيجة الجذور الحرة الناجمة عن الكاربوبلاتين.