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Protective Effect of *Solanum nigrum*, Vitamin C or Melatonin on the Toxic Effect of Acrylamide on Rats

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Abstract: *Acrylamide, used in many fields, from industrial manufacturing to laboratory work, is also formed during the heating process through the interactions of amino acids with carbohydrate. Therefore, acrylamide poses a significant risk for both human and animal health. This study aimed to investigate if Solanum nigrum (leaves+fruits), vitamin C or melatonin treatment could modulate toxic effect of acrylamide and to evaluate their antioxidant and immuno-stimulant effects. One hundred Sprage-Dawley rats weighing 220-260 gm (M: 336) were divided into 5 groups: 1-control (C), 2-acrylamide treated, 3-solanum nigrum + acrylamide treated group; 4-Vitamin C+acrylamide treated group; 5-melatonin+acrylamide treated group. After 4 weeks, the rats were sacrificed. In the acrylamide treated group, most of the studied biochemical parameter were significantly changed as decreased total protein, Albumin, globulin, IgG, IgM and cholinesterase on comparing with the normal control group. Also urea, creatinine, aspartate aminotransferease (AST), alanine aminotransferase (AST), Alkaline phosphatase (Alk Ph) were significantly increased ($P < 0.001$). In the co-treatment of the acrylamide infected rats with Solanum nigrum (leaves+fruits), vitamin C, or melatonin the increased levels tend to decrease and vise versa. Therefore, supplementation with Solanum nigrum (leaves+fruits), vitamin C or melatonin can be useful when there is a risk of acrylamide toxicity.*

Key words: *Acrylamide, Oxidative stress, Immunoglobulin.*

Aim of the study: Because exposure of humans to acrylamide can come from many sources especially the diet, a need exists to understand its role in human health and to find a way to minimize its toxic effect through using *Solanum nigrum* (leaves+fruits), vitamin C or melatonin.

I. Introduction

Acrylamide (ACR, CH_2=CH-$CONH_2$) is water soluble, α, β- unsaturated vinyl monomer of poly acrylamide (conjugated) reacted molecule with a significantly high chemical activity **[1,2].** It is used in water purification, making of cosmetics, glues and paper **[3]**. It is a contaminant in certain potatoes and grain-based foods cooked at high temperature. Acrylamide is listed by the World Health Organization (WHO) as probable human carcinogen **[4].**

Dietary ACR is largely derived from heat-induced reactions (Maillard reaction) between the predominantly amino group of the free amino acid precursor asparagine and carbonyl groups of reducing sugar as glucose and fructose during heat processing of plant-derived foods such as potato fries and cereals. Orally consumed ACR is absorbed into the circulation then distributed to various organs, and reacts with DNA, neurons, hemoglobin, and essential enzymes **[5[,** causing several toxic effects as human neurotoxicant and suspected carcinogen **[6,7].** Acrylamide (ACR) is one of the most important contaminant in the environment, which was shown to be a neurotoxicant, reproductive toxicant, hepatotoxic and carcinogen in animals **[8].** Acrylamide is quickly absorbed in the digestive tract. The excretion via the urine is fast and half of the acrylamide is cleared from the body in a few hours **[9]**. Presence of acrylamide in a variety of commonly consumed human foods has raised public health concerns **[10].**

ACR causes lipid peroxidation **[11]**. The toxicity of ACR is at least in part related to free radicals and free radical-mediated oxidative stress so we tried to study the protective effect of melatonin, vitamin C and *Solanum nigrum* (leaves+fruits) as antioxidant against the toxic effect of acrylamide.

Melatonin, the chief indolamine produced by the pineal gland. Melatonin was initially studied in terms of its role in endocrine physiology. However, evidence has been accumulated showing that melatonin influences the function of a variety of tissues not related to the endocrine system **[12)** and that it has been played an important role in antioxidant and free radical scavenger **[13-15]**.

Numerous medicinal plants and their formulation are used for liver disorders in ethnomedical practice. Herbs play a vital role in the management of various liver disorders. *Solanum nigrum* (Family: Solanceae) commonly called Black nightshade or deadly nightshade, known as Enab Eldib here in Egypt and is traditionally used for inflammatory, diuretic and liver disorders. *Solanum nigrum* (leaves+fruits) are effective in the treatment of cirrhosis of the liver. Freshly prepared extract of the plant is effective in the treatment of cirrhosis

of the liver and also serves as an antidote to opium poisoning. The leaves are used as poultice for rheumatic and gouty joints, skin diseases, used in the treatment of anti tuberculosis and are said to produce Diaphoresis **[16-18]**. The leaves are reported to contain several constituents e.g. flavonols like Quercetin, Hyperoside, Steroids and alkaloids like Sitosterol, Solamargine, Stigmastrol, Campesterol, Cholesterol, Solasodine, Sapogenin like Tigogenin, phenolic and flavonoid contents [**19,20**].

II. Material and Methods

ACR compound purity is 99% and purchased from Sigma Chemical Company.
The LD_{50} of acrylamide in rats was 150 mg/ kg BWT **[21]**.

Animals and Dosing

This study was carried out on one hundred adult male Sprague Dawley rats with an average body weight ranging from 220-260 g. Animals were kept in stainless steel wired meshed cages during the whole experimental period under hygienic conditions, fed on standard control diet and provided with water *add libtum*, through the experiment. Rats were fed on control diet for 10 days before starting the experiment (adaptation period). The standard rat chow diet (AIN-93 M diet formulated for adult rodents) was prepared according to the **National Research Council (NRC) [22] and [23].** Rats were divided into five equal groups (20 rats/group); the first group (G1: Control) fed on standard control diet; the second (2^{nd}) group (G 2) fed on normal control diet and intoxicated with acrylamide through interaperitoneal injection (40 mg/kg BW/day), the 3^{rd} group (G 3) as G 2 i.e. intoxicated with acrylamide and fed with normal diet supplemented with dried *Solanum nigrum* (leaves & fruits, 1:1, 2g/kg diet), the 4^{th} group (G4) as G 2 and fed with diet supplemented with vitamin C (1000 mg/kg diet), and the 5^{th} group (g 5) as G 2 and was administered melatonin at 10 mg/kg BW/day i.p. The experiment lasted for 30 days. Clinical signs and mortality percentage (%) were recorded along the experimental period.

Samples

At the end of the experimental period, rats were fasted over night, anesthetized under diethyl ether, and then the rats from each group were sacrificed. The blood was collected and kept in tubes with and without anticoagulant for serum and plasma separation and for whole blood. Serum and plasma samples were kept at -70 °C till analysis. Liver was removed, washed with saline, dried and then weighed. Relative liver weights (RLW) were calculated as follow: RLW= [absolute liver weight (LW)/Final Body Weight (FBW)] X 100.

Biochemical Analysis

The following parameters were monitored: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea, creatinine, total protein, and some immunoglobulin biomarkers as IgG, IgM). The protein, albumin, globulin, A/G, urea and creatinine contents were estimated by the method of **[24-27]** respectively. ALP activities were measured by the methods of **[28]**. ALT and AST activities were measured spectrophotometrically by the methods of **[29]**. Plasma LDH were determined using kinetic endpoint kits (SGM Italia, Rome, Italy) according to the method of **[30].** Cholinesterase was determined according to **[31].** Immunoglobulins biomarkers were estimated using Rat ELISA Kits supplied by Kamiya Biomedical Company (12779 Gateway Drive, Seattle, WA 98168) as Rat IgM ELISA for the quantitative determination of IgM in rat biological samples, Cat. No. KT-419; Rat IgG ELISA for the quantitative determination of IgG in rat biological samples, Cat. No. KT-418.

III. Statistical Analysis

The results are expressed as Mean ±SE. Analysis of data was performed by one-way analysis of variance (ANOVA) followed by Post hock Duncan test using the SPSS v 11 (statistical package for social sciences) software for windows. P value less than 0.05 was considered statistically significant.

IV. Results

Clinical Signs

Administration of ACR to male rats result in marked alterations in behaviour, revealing nervous manifestations (abnormal neurobehavioral) in the rats treated with ACR as ataxia, increased landing of the limbs and weakness of the muscles. The severity of the clinical signs appeared on the 22^{nd} days of ACR treated group.

Mortality and Post-Mortem Picture

Mortalities started at 22^{nd} day of administration of ACR in acrylamide treated groups. It reached 10% by the end of the experiment (In group 2, 2 rats out of 20 were dead (10%); in group 3 and 4, 1 rats out of 20

was dead (5%). In groups 2-5, both dead and sacrificed rats revealed generalized enlargement and paleness of body organs with different degrees.

Body Weight

Results of our study reveal reduction of body weight in group treated with acrylamide. Results shown in table (1) reveal that this group had the lowest amount of food intake/day (14.39+0.22 gm/d). The results presented in table (1) also reveal lower body weight gain (BWG: -36.55%) and higher RLW in G 2 (4.05 vs 2.21, acrylamide treated group vs normal control group respectively). Supplementation of the diets with *Solanum nigrum* (leaves+fruits) or vitamin C or melatonin improves body weight gain and liver weight in comparison with acrylamide treated group ($P < 0.001$, Table 1). The higher BWG was noticed in G 3, acrylamide infected rats treated with *Solanum nigrum* compared with the other two treated groups.

Biochemical Analysis

Acrylamide treated group revealed a highly significant decrease of total protein, albumin and globulin levels when compared with normal control group (Table 2). Also the reduction may be due to increased necrosis in the liver. The levels of total protein, albumin and globulin of infected rats begins to improve when treated with *Solanum nigrum* (leaves+fruits), vitamin C or melatonin but still significantly lower than normal control group.

Table 2 revealed that, the administration of acrylamide resulted in significant decrease ($P<0.05$) in IgG and IgM. The data in Table 2 postulated that, the administration of *Solanum nigrum* (leaves+fruits), vitamin C or melatonin with acrylamide (G 3: G 5) resulted in significant ($P< 0.05$) increase in the IgG and IgM as compared to control group.

Urea and creatinine concentrations were studied to assess the renal functions. As shown in Table 2, urea and creatinine levels were tended to increase significantly ($P < 0.001$) by ACR treatment (G 2). This high level tends to decrease after treatment with *Solanum nigrum*, vitamin C or melatonine (Table 3).

As an indication of hepatic injury, the ALT, AST and Alkaline phosphatase levels were significantly higher in the acrylamide-treated group when compared with the control group (the increase reach 90.67%, 248.77% and 64.33% respectively, $P < 0.001$). *Solanum nigrum* (leaves+fruits) or vitamin C or melatonine treatment decreased both the ALT and AST levels significantly ($P <0.001$) compared with the acrylamide-treated group (Table 3).

Serum lactate dehydrogenase (LDH) activity, as an indicator of generalized tissue damage, showed a significant increase in the acrylamide-treated group ($P < 0.001$) and this effect was reversed significantly by *Solanum nigrum* (leaves+fruits), vitamin C or melatonine treatment ($P < 0.001$, Table 3).

The results presented in Table 3 reveal that rats treated with acrylamide significantly decreases cholinesterase activity (-33%, $P < 0.001$) compared to normal group. The value of cholinesterase begins to increase but still significantly lower than normal control group. Acrylamide infected rats treated with *Solanum nigrum* or vitamin C shows better result than melatonine treatment.

V. Discussion

Mechanism of Acrylamide Toxicity

Several mechanisms have been suggested to explain how acrylamide induce its toxic effect. Acrylamide contains α, β-unsaturated amide system that reacts with nucleophilic compounds via a Michael addition. The major site of reaction is sulfhydryl groups contained on proteins and amino acids. Once absorbed, acrylamide may be conjugated by glutathione-S-transferase (GST) to N-acetyl-S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P 450 (CYP450) to produce glycidamide, which is more reactive toward DNA and proteins than the parent compound, acrylamide **[32]**, or forming a DNA reactive epoxide **[33-36].** Oxidative stress is considered as one of the important mechanisms of toxic effects of acrylamide. ACR causes oxidative damage through inducing the generation of reactive oxygen species (ROS) which enhanced the production of lipid peroxidase reducing the antioxidant defence systems **[37].** Liver, kidney, brain and erythrocyte GST have significant binding capacity with acrylamide, being higher with the liver **[38]**.

Toxicokinetics of acrylamide

Toxicokinetic studies in **humans** have shown a terminal elimination half-life of 2.4–7.0 h for acrylamide **[39]**. Over 60% of acrylamide taken up by the body can be recovered from the urine **[39]**; of which 86% are GSH conjugates **[40]**. Both acrylamide and glycidamide have a significant affinity for binding to plasma proteins, in particular, to Hb **[40]** leading to glycidamide–Hb adduct. Acrylamide can be found in human placenta and breast milk **[41]**, thus easily transferable to fetus or newborn infants. Toxicokinetic studies of acrylamide in **rodents** have shown that rodents metabolize acrylamide to glycidamide more efficiently than

humans **[42]**. The estimated plasma half-life for acrylamide and glycidamide in male Sprague Dawley rats was approximately 2 hours **[43].**

Clinical Signs

The marked alterations in behaviour in rats treated with ACR agree with the previously mentioned by **[44,45].**

Total protein, albumin and globulin

Results shown in table (2) revealed that treatment of the infected acrylamide rats with *Solanum nigrum*, vitamin C or melatonine shows better result. Biochemical findings (change) may be attributed to protein degradation which manifested by the low level of total protein, albumin and globulin in our result. The obtained results were found to be supported by the results of **[1]** who reported that there were steady decreases in hepatic protein level with higher doses of ACR which can be resulted from retarded protein synthesis, or to change in protein metabolism or to the leaking out of protein reserves from hepatocytes. ACR molecule has two reactive sites, viz, the conjugated double bond and the amide group which can conjugate with the -SH group of a sulfur containing amino acids and α-NH_2 group of a free amino acid. The above statments can explain the unavailability of few amino acids for protein synthesis. Further being an electrophilic compound, ACR can bind with proteins which can make them undetectable.

IgG, IgM

Results shown in table (2) reveal the effect of administration of *Solanum nigrum*, vitamin C or melatonine and acrylamide on immune functions (IgG and IgM), it was clear that, *Solanum nigrum* or vitamin C or melatonine elicited significant increase of IgG and IgM in groups 3-5 when compared with acrylamide intoxicated rats, while acrylamide significantly decrease them in group 2 in comparison with control group. On the other hand, administration of *Solanum nigrum* (leaves+fruits) *or vit*amin C or melatonine in group 3: 5 respectively elevates the level of IgG near to the control level. This could be attributed to the antioxidant activity of *Solanum nigrum* (leaves+fruits) or vitamin C or melatonine. These results agree with **[46]** 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**.** 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 **[47].** 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 **[48]**.

Urea and Creatinine

Our result for the effect of acrylamide upon urea and creatinine disagree with **[49]** where he mentioned that acrylamide in permissible limits 0.5 mg/kg BWT produces a non significant decrease in creatinine and significant decrease of urea values in rats. **Susan *et al.* (2003) [50]** mentioned that 40% to 70% of the acrylamide dose which reach blood stream was extracted in the urine in 24 hours in male albino rats, which may indicate that kidney can perform its function to some extent during acrylamide toxicity.

Alanine and Asprtate Transaminase (ALT and AST)

There was a significant increase in serum AST and ALT activities in acrylamide treated groups. These results are similar to those recorded by **[51,11]** in serum and plasma of mice and rats respectively post ACR intoxication. These results confirmed by the hypothesis that recorded by **[51]** who attributed the significant increase in serum AST, ALT levels to the bipolar nature of ACR, where the CH_2=CH part may undergo hydrophobic interactions while the $CONH_2$ part can form hydrogen bonds with the cell components. This property may enhance its ability to alter the cell membrane structure and make the parenchymal cell membrane of liver more permeable, thereby causing the active retention of enzymes and making them appear first in the extracellular space and then in the blood. The previous changes were confirmed by histopathological findings. In addition, acrylamide induced cellular transformation and increase in the degree of membrane permeability of liver **[52]**. The disruption of these membranes may cause the translocation of liver enzyme ALT and AST into the blood, as observed in this study.

On the contrary to our result **[53]** reported that the serum level of AST and ALT in Wister rat was not much increased after continuous intake of trace of acrylamide in frying oils for 12 weeks.

ALP activity

The main source of this enzyme is the liver and bone, the enzyme induction is the source of the raised activity and the billiary obstruction or bone diseases simulate this production **[54]**. These results agree with **[55]** and disagree with **[49]**.

Lactate Dehyrogenase (LDH)

LDH is present in almost all cells of the body and predominate in higher levels in erythrocytes, heart, liver, skeletal muscles and kidneys and increase in myocardial affections, hepatic and muscular diseases. The increase of the activities of some cellular enzymes as lactate dehydrogenase (LDH) in acrylamide treated group may be due to cell membrane affection which will lead to leakage of cellular enzymes [**56]**. Also, LDH is recognized as a potential marker for assessing the toxicity of a chemical **[57],** so elevations in its activity may be associated with diseases affecting organs where LDH is present as myocardial infarction, renal infarction and haemolysis. The present increase in LDH activity in rats intoxicated with arylamide indicated damage to any of the organs producing this enzyme.

Cholinesterase

Acetylcholine **e**sterase (AChE) is an enzyme secreted by the liver in an active form into plasma; its production is often depressed in liver disease **[58]**. AChE hydrolyzes the neurotransmitter acetylcholine, in synapses of the brain, automatic and neuromuscular junction of voluntary nervous system, into choline and acetic acid. This reaction is necessary to allow cholinergic neuron to return into its resting state after activation. Acetylcholine is a compound synthesized at the nerve endings, which acts in transmitting impulses from nerve to muscles fibber **[58]**. Acrylamide is well-known neurotoxic compound that produces central and peripheral distal axonopally.AChE inhibition leads to accumulation of acetylcholine at cholinergic sites causing disturbances in normal nervous system function **[59]**. In our study, there is a significant decrease in serum cholinesterase concentrations in group 2 (acrylamide intoxicated) as compared with the control group. The decrease begins to increase in acrylamide intoxicated rats treated with *Solanum nigrium* (leaves+fruits), vitamin C or melatonin (Table 4). Our results agree with **[60]** who suggested that this decrease may be due to inhibition of "anterograde" transport of acetylcholine esterase. **LoPachin 2002; LoPachin *et al.*, 2002** and **Sickles *et al.*, 2002 [61-63]** reported that nerve terminals were the primary site of acrylamide action and that synaptic dysfunction and subsequent degeneration. Moreover, it interferes with kinesin, the motor protein for anterograde axonal transport in the nerve cell result in inhibition of anterograde axonal transport **[64]**. Cholinesterase levels inhibition (decrease) is an indicator for possible neurotoxic effect of organic compound (acrylamide) **[65].** Also **Richard and LoPachin, (2004) [66]** reported that ACR inhibits the corresponding membrane-fusion processes, impairs neurotransmitter release, promotes downstream degeneration and due to electrophilic nature of ACR, ACR contributes to adduct formation with sulfhydryl groups on certain proteins that are critically involved in membrane fusion.

Our results reveals that treatment with *Solanum nigrum* (leaves+fruits), vitamin C give better result even better than melatonine although all are considered as antioxidants. The leaves of *Solanum nigrum* were found to be richer in polyphenols than stem and fruit. *Solanum nigrum* leaves contained the highest concentration of gentisic acid, luteolin, apigenin, kaempferol, and m-coumaric acid. However, the anthocyanidin existed only in the purple fruits **[67]**. The major active components are glycoalkaloids, glycoproteins, and polysaccharides. It also contains polyphenolic compounds such as gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin **[68]**.

VI. Conclusion

Acrylamide caused many adverse effects in the tissues reflected in significant increase in liver enzymes. The administration of *Solanum nigrum* (leaves+fruits) vitamin C or melatonine in combination with acrylamide significantly improves liver enzymes as well as improves immune status reflected in increased Ig G and Ig M.

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Table (1): Initial, final body weight (IBW, FBW), body weight gain (BWG), amount of diet eaten/ day, liver weight (LW) and relative liver weight. of intoxicated and treated groups.

	IBW	FBW	BWG	diet/day	Liver weight	RLW
	gm					
G1	238.90±2.09	309.80±2.42	70.90±1.05	19.54±0.16	6.86±0.08	2.21±0.03
G2	235.65±1.34	199.10±1.67 [a]	-36.55±2.37 [a]	14.39±0.22 [a]	8.04±0.09 [a]	4.05±0.07 [a]
G3	235.95±2.73	291.05±1.85 [a,b]	55.10±1.75 [a,b]	16.75±0.12 [a,b]	7.17±0.07 [a,b]	2.46±0.03 [a,b]
G4	234.15±1.15	281.70±1.17 [a,b,c]	46.55±1.49 [a,b,c]	16.62±0.25 [a,b]	7.09±0.13 [a,b]	2.51±0.04 [a,b]
G5	235.85±1.14	281.50±1.24 [a,b,c]	45.65±1.66 [a,b,c]	16.62±0.12 [a,b]	7.09±0.05 [a,b]	2.52±0.02 [a,b]

G1 (a): Control, G2: acrylamide treated, G3: *Solanum nigrum* + acrylamide treated group; G4: Vitamin C + acrylamide treated group; G5: melatonin+acrylamide treated group. a: G1, b: G2, c: G3, d: G4, e: G5; Significance of P < 0.01.

Tabke (2): level of Total protein, Albumin, Globulin, A/G ratio and immunoglogulins IgG and IgM of intoxicated and treated groups.

	T. Protein	Albumin	Glob	A/g	IgG	IgM
	gm/dl				mg/dl	
G1	6.62±0.07	4.21±0.02	2.41±0.08	1.77±0.05	717.32±7.22	180.07±1.76
G2	5.31±0.02 [a]	3.34±0.02 [a]	1.97±0.03 [a]	1.71±0.04	579.50±9.31 [a]	140.39±1.17 [a]
G3	6.18±0.02 [a,b]	3.73±0.03 [a,b]	2.44±0.03 [b]	1.54±0.03 [a,b]	654.78±5.51 [a,b]	166.15±1.72 [a,b]
G4	6.13±0.07 [a,b]	3.71±0.09 [a,b]	2.42±0.10 [b]	1.61±0.11	651.51±6.31 [a,b]	165.97±2.10 [a,b]
G5	5.99±0.06 [a,b,c]	3.56±0.05 [a,b,c]	2.43±0.03 [b]	1.46±0.03 [a,b]	615.05±6.43 [a,b,c,d]	165.18±2.13 [a,b]

G1 (a): Control, G2: acrylamide treated, G3: *Solanum nigrum* + acrylamide treated group; G4: Vitamin C+ acrylamide treated group; G5: melatonin+acrylamide treated group. a: G1, b: G2, c: G3, d: G4, e: G5; Significance of P < 0.01.

Table (3): Level of Urea, Creatinine, aspatate aminotransferease, Alanine amino transferase, Alkaline phosphatise, lactate dehydrogenase and cholinesterase of intoxicated and treated groups.

	Urea	Creatinine	AST	ALT	Alk Phos	LDH	Cholinesterase
	mg/dl		IU/L				
G1	34.11±0.49	0.97±0.02	39.46±1.03	38.40±0.64	64.18±1.23	765.51±18.59	736.64±7.22
G2	65.59±0.51 [a]	3.13±0.02 [a]	137.61±0.99 [a]	73.21±0.85 [a]	105.55±0.65 [a]	1182.79±10.92[a]	492.83±4.55 [a]
G3	41.77±0.94 [a,b]	1.50±0.03 [a,b]	65.07±0.72 [a,b]	44.81±0.84 [a,b]	70.97±1.11 [a,b]	965.06±9.30 [a,b]	656.69±4.48 [a,b]
G4	41.37±0.69 [a,b]	1.51±0.03 [a,b]	66.14±0.84 [a,b]	47.36±0.16 [a,b,c]	80.23±0.50 [a,b,c]	965.06±9.30 [a,b]	646.82±3.05 [a,b]
G5	46.73±0.66 [a,b,c,d]	1.59±0.03 [a,b]	70.25±0.57[a,b,c,d]	49.59±0.17 [a,b,c,d]	80.19±3.00 [a,b,c]	991.97±3.61 [a,b,c,d]	604.74±3.02 [a,b,c,d]

G1 (a): Control, G2: acrylamide treated, G3: *Solanum nigrum* + acrylamide treated group; G4: Vitamin C + acrylamide treated group; G5: melatonin+acrylamide treated group. a: G1, b: G2, c: G3, d: G4, e: G5; Significance of P < 0.01.