

DAILY USE OF *CAMELLIA SINENSIS* EXTRACT CAN PROTECT ACRYLAMIDE INDUCED ORGAN PATHOLOGIES IN MICE

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ABSTRACT

Human beings are vulnerable to a variety of toxins. Nowadays concern has aroused to opt defensive mechanisms in the form of foodborne phytochemicals against these toxicants. Current research investigated the protective potential of green tea aqueous extract against Acrylamide (AA) induced organ toxicity. Eighty albino mice weighing (26±2g) divided equally into eight groups following CRD experimental mode. Three groups were orally exposed to various acrylamide concentrations (20µg/g, 40µg/g, 80µg/g B.W of mice), other three were treated with respective doses of AA along with green tea extract for thirty days regularly. Control (without treatment) and GE (only green tea extract) groups were also maintained during current experiment. Blood and organs (liver, kidneys and testes) were extracted on euthanasia of the animals after 24hrs of last treatment, for serological and histopathological studies, respectively. Aqueous extract of *Camellia sinensis* was also assessed for total phenolic contents and antioxidant activity. The observations indicated that AA intoxication reduced testes weight, size, testosterone and ALP level while increased kidneys and liver size as well as ALT, bilirubin, creatinine and urea levels significantly ($p \leq 0.05$) as compared to control group. Histopathological anomalies in testicular, hepatic and renal tissues were also obvious in AA group. However, pathologies were much suppressed when green tea is co-administrated with acrylamide except for the highest concentration of acrylamide. It is deduced by above mentioned results that AA exposure is noxious in mice and green tea extract showed protective potential against its toxicity.

Keywords: Acrylamide, Toxicity, Green Tea, Biochemistry, Histopathology.

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INTRODUCTION

Acrylamide has been used in industries since 1970 for production of polyacrylamide. (Hashimoto and Aldridge., 1970). Polyacrylamides have various applications such as, paper strengthening, waste water treatment, production of biomedical and scientific enquiry materials like electrophoresis gels and personal care products such as lotions, cosmetics and deodorizers, hence a major source of occupational poisoning (Arihan *et al.*, 2011). Acrylamide is also formed in foods that are rich in carbohydrates when they are cooked above 120°C especially by the reaction of reducing sugars and amino acids like asparagine (Mottram *et al.*, 2002; Stadler *et al.*, 2002). Acrylamide levels were reported as high as 2300g/kg in potato chips and fries (Pedreschi *et al.*, 2004). Intake of potato, bread or coffee are the major dietary sources by which humans are exposed to acrylamide (NIH, 2004). These findings raised global interest towards acrylamide. However, better knowledge about its production and presence in food and its impact on human health is of marked importance.

Workers exposed to AA during mining, tobacco smoking and flocculation processes of acrylamide

containing products in industries and also during tunnel construction. Exposure of human to acrylamide takes place through various potential means including ingestion, inhalation and dermal contact, respectively (Smith *et al.*, 2000; Tareke *et al.*, 2002; Schettgen *et al.*, 2003). Many chemicals bio-transform to highly reactive metabolites, which induce cellular toxicity and acrylamide is the one of them.

According to EPA, EU and National Toxicology Program (EPA, 1990; EU, 2000; NTP, 2004) studies on mice and rats proved acrylamide and glycidamide as possible carcinogens. Additionally, acrylamide induces genotoxicities comprising micronuclei formation (Schrieve-Schwemmer *et al.*, 1997). Acrylamide was extensively studied for its reproductive toxicity along with germ cell mutagenicity (Favor and Shelby, 2005). Many scientific researches were conducted to gauge the reproductive toxicities of acrylamide (Sakamoto and Hashimoto, 1986), testicular damages (Tyl *et al.*, 2000), and damage of DNA in testes (Yang *et al.*, 2005). These researches proved acrylamide as a potential xenobiotic. So, a special concern about protection/amelioration of AA induced reproductive toxicity arises.

Previous studies states that liver and kidney of AA intoxicated rats showed degeneration in hepatocytes, obstructions of blood vessels in hepatic portal area vacuolation in renal tubules and degeneration of epithelial lining followed by rupture of renal cells (Mahmood *et al.* 2015).

On the other side, natural herbs and medicinal plants are being used as a chemotherapeutic agents from ancient times against xenobiotic-induced injuries due to their antioxidant activities (Ajith *et al.*, 2007; Sak, 2012). Many of these herbs contains potent antioxidants in the form of phenols that act as counter agent against xenobiotic accelerated damages. Tea is considered as a rich source of dietary components with remarkable benefits to human health. Epidemiological as well as clinical studies proved that green tea (black tea to a lesser extent) reduce the risk of chronic diseases (Tsuneki *et al.*, 2004). These beneficial effects of green tea have been attributed due to its polyphenols, the potent antioxidants.

Polyphenols in green tea (*Camellia sinensis*) are well known for their antimutagenic and anticancer activities (Dreosti, 1996). Polyphenols existing in green tea are familiar as catechins and are of great pharmacological importance (Mckenna *et al.*, 2000). It is an established fact that the catechins are biological antioxidants due to their free radical hunting properties. It is observed that they can scavenge both hydroxyl and superoxide radicals (Nanjo *et al.*, 1999; Zhao *et al.*, 2001).

Keeping in mind the above-mentioned findings, the present investigations were carried out to investigate the preventive effect as well as protective potential of green tea against acrylamide induced organ toxicity in mice.

MATERIALS AND METHODS

Chemicals and Reagents: Acrylamide monomer dry crystals, 98% purity (Depew Fine Chemical Co., Ltd. China), Folin-Ciocalteu's reagent (Merck Chemicals), sodium carbonate, sodium acetate and Glacial acetic acid to make acetate buffer, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), ascorbic acid, FeCl₃ (Sigma Aldrich), ethanol, HCl, green tea leaves (From local market Lahore, Pakistan), ELISA kit (Invitrogen, by Thermo Fisher Scientific, USA) and distilled water.

Green Tea Extract Preparation: Leaves of green tea were obtained from a general tea store. Green tea aqueous extract was prepared by soaking 15g of tea leaves in 1L distilled water and temperature was maintained at 90±3°C for 5 minutes. Afterwards, solution was cooled at room temperature and filtered. The filtrate contained the required concentration of green tea extract i.e. 1.5% (w/v) (El-Beshbishy, 2005).

Estimation of Total Phenolic Content in Extract: Total phenolic content (TPC) was measured by method described by Kaur, *et al.*, 2015. In short, 1.0 ml freshly prepared green tea aqueous extract was mixed with 5.0 ml of diluted (10 folds) Folin-Ciocalteu reagent and 4 ml sodium carbonate solution (7.5% w/v). Mixture was mixed and kept at room temperature about an hour and absorbance was measured by spectrophotometer at 760nm. Using Gallic acid as reference standard, a curve was plotted by various known concentrations of Gallic acid vs absorbance. Standard curve used to estimate polyphenols in the green tea extract. Phenolic content results were expressed as gallic acid equivalents (GAE) mg/ 100g of plant material of green tea (Kaur *et al.*, 2015).

Estimation of Antioxidant potential in Green Tea Extract through FRAP Assay: Antioxidant potential of prepared extract was determined by FRAP assay, Benzie and strain (1996) method was opted with little modifications. In short, 100µl extract was mixed with 3ml fresh FRAP reagent (Acetate Buffer 300Mm with pH 3.6 + TPTZ (2,4,6-Tripyridyl-S-triazine) + FeCl₃ 20 mM, mix these reagents in 10:1:1 ratio to prepare FRAP solution) vortexed and absorbance was noted at 593nm. Then samples were placed in water bath at 37°C and after 4 minutes second absorbance was noted to calculate change in absorbance. A blank was run with FRAP reagent only. A standard curve was obtained by using known concentrations of Ascorbic Acid (100µM - 1000µM) verses their respective absorbance. FRAP values of samples were determined by using equation of this standard curve, simply putting absorbance value of each sample. Results are expressed in µM Fe (II)/ 100g of plant material.

Animal Husbandry: The experiment was conducted on eighty adult male Swiss albino mice (8 weeks old) having weight (26±2g), in Animal House, Department of Zoology, University of the Punjab, Lahore, Pakistan. The animals were kept in clean iron cages and maintained at 28±2°C, 50-60% relative humidity and 13h/11h light dark cycle. The experiment was performed with compliance of EU Directive 2010/63/EU, of 24 November 1986 (86/609/EEC) and guidelines approved (D/1023/UZ dated 04-09-18) by local ethical committee on animal experimentation. Conspicuous clinical symptoms of toxicity, mortality and morbidity of all mice were observed and recorded twice a day throughout the experimental period.

Experimental Groups and Dose Administration: Various concentrations of acrylamide i.e. 20µg/g, 40µg/g and 80µg/g B.W. of mice were prepared in distilled water so that 0.1 ml contained the respective doses of acrylamide.

Eighty male albino mice were placed into 8 groups (N=10) following completely randomization design (CRD). Control Group (C) animals were treated with only distilled water for thirty days. Mice of dose Groups AA-I, AA-II and AA-III were administered with acrylamide 20µg/g, 40µg/g and 80µg/g B.W of mice, respectively for thirty days regularly. Dose and antidote groups AA-I+GE, AA-II+GE and AA-III+GE were treated with subsequent doses of AA along with green tea extract (GE), as an antidote, so the antidote group (GE) was treated with green tea extract only. The extract, used as an antidote (GE) was a replacement of water as an exclusive source of drinking fluid throughout the whole experiment for 30 days.

Body Weight Measurements and Dissection: Body weights of mice were measured at the start of experiment, day 0 (before dose administration) and at the end of experiment (before dissection). Data were recorded in the whole numbers. Recorded data were processed for Mean \pm SEM.

Animals were euthanized after about 24 hours of last dose administration according to the planned protocols and ethical guidelines. Required tissues (liver, kidneys and testes) and blood samples were collected for further processing.

Serum Biochemical Analysis: Blood samples for biochemical assay were taken in EDTA containing tubes from intracardiac puncture. After centrifugation of blood, separated serum was stored at -80°C until assayed. Serum was assayed for renal (urea and creatinine) and hepatic (ALT, ALP, bilirubin) function tests and for testosterone by ELISA kit, according to manufacturer's instructions.

Morphological and Morphometric Observations: The sacrificed male mice liver, kidneys and testes were dissected out and observed by placing under binocular microscope and were then macro photographed with camera Panasonic TZ15. Morphological and morphometric studies involved wet weight measured by the digital balance and length and width were recorded by digital Vernier Caliper.

Preparation of tissues for histopathological examination: Liver, kidneys and testes were processed for wax embedded serial sectioning in Developmental Biology Laboratory, University of the Punjab, Lahore. Transverse sections of 5-micron thickness were cut with the help of rotary microtome. Slides were stained using Eosin-hematoxylin stains following histological techniques (Bancroft and Gamble, 2008). The sections were analyzed by Light Microscope, SWIFT MD3500, Japan.

Statistical Analysis: Whole numerical data like mice body weight, tissue weights, liver and kidney enzymes and hormone were subjected to analyze statistically through one-way analysis of variance (ANOVA) and Tukey's multiple range test to assess the level of significance and differences among the groups, using SPSS 19 software. $P \leq 0.05$ was considered significant.

RESULTS

Antioxidant power and Total phenolic content in Green tea extract: Antioxidant activity and phenolic content are the basic parameters regarding quality of the green tea. Green tea extract used in this experiment showed high ferric reducing ability showing its antioxidant power as well higher content of total polyphenols indicates its detoxifying ability against free radical generated toxicity. A stronger correlation between phenolic content and antioxidant power (FRAP values) was observed. Pearson's correlation coefficient was $r^2 = 0.931$

Body and organ weight: Mice body weights were recorded before, during and on dissection day. The results showed that body weight was significantly ($p \leq 0.05$) reduced in AA treated groups (AA-I, AA-II and AA-III) as compared to control group. But the average body weight of mice in AA +Antidote treated groups were comparable with control and Antidote groups (GE) except in AA-III+GE group in which absolute body weight significantly ($p \leq 0.05$) lowered against control (Table 1).

The average weight and size of testes showed a significant decrease ($p \leq 0.05$) in AA treated groups while significant increase in the average weights and size of liver and kidneys in AA treated groups. But in case of AA+GE and GE groups, average weights and size of testis, liver and kidneys were comparable to control group except in higher concentrations of AA + GE group (Table1).

Blood Biochemical Analysis: Serum testosterone and ALP levels lowered significantly ($p \leq 0.05$), and level of the Urea, Creatinine, ALT and bilirubin levels significantly increased in mice treated with AA when compared with the control. Levels of all parameter again returned to normal level, when mice intoxicated with acrylamide were treated with green tea extract. This indicated the protective effects of green tea extract against the toxic effects of acrylamide. But when green tea extract co-administered with high concentration of AA, it could not completely guard AA toxic effects and biochemical toxicity at level of significance ($p \leq 0.05$) in AA-III+GE group (Table 2).

Table 1. shows positive potential of green tea leaves extract on morphometric parameters in AA intoxicated male albino mice.

Dose group ^A Parameter	Cont.	AA Dose Group			Antidote + Dose Group			GE
		AA-I	AA-II	AA-III	AA-I +GE	AA-II +GE	AA-III + GE	
Body weight (g) (Mean ± SEM)	27.40±0.13	27.00±0.17	26.88±0.07	26.60±0.10	27.44±0.10	27.48±0.09	27.10±0.009	27.42±0.05
Day-30	30.12±0.31 ^c	26.40±0.10 ^b	25.90±0.18 ^a	24.78±0.17 ^a	29.96±0.08 ^c	29.57±0.12 ^c	26.38±0.014 ^b	29.00±0.15 ^c
% change in body weight (g)	9.92	-2.22	-3.64	-6.84	9.18	7.61	-2.65	5.76
Organ weight(mg) (Mean ± SEM)	1214.20±14.63 ^a	1292.60±17.32 ^a	1441.00±13.41 ^b	1812.00±15.82 ^c	1223.40±13.02 ^a	1240.00±6.68 ^a	1257.90±7.17 ^a	1216.20±3.16 ^a
Liver								
Kidney	226.00±5.91 ^a	242.40±4.42 ^b	254.00±5.98 ^c	263.60±5.51 ^c	231.50±1.93 ^a	234.90±1.07 ^a	239.50±1.19 ^b	223.20±1.21 ^a
Testis	90.00±0.660 ^c	75.80±0.64 ^b	62.80±0.71 ^b	54.20±0.71 ^a	88.80±1.23 ^c	87.50±0.50 ^c	63.10±0.71 ^b	89.10±0.99 ^c
Liver (mm±SEM)								
Length	23.06±0.43	22.11±0.23	22.14±0.17	21.23±1.1	23.13±0.12	22.43±0.19	21.01±0.17	23.16±0.05
Width	14.51±0.24	14.43±0.65	13.83±0.85	13.01±0.76	14.87±0.54	14.67±0.12	13.98±0.94	14.56±0.01
Kidney (mm±SEM)								
length	9.00±0.011 ^a	9.89±0.06 ^{bc}	10.54±0.009 ^c	11.74±0.012 ^c	9.10±0.023 ^a	9.07±0.004 ^a	9.70±0.085 ^b	9.06±0.012 ^a
width	4.53±0.009 ^a	5.14±0.111 ^b	5.48±0.008 ^{bc}	6.25±0.010 ^c	4.63±0.049 ^a	4.63±0.064 ^a	5.02±0.042 ^b	4.54 ±0.041 ^a
Testis								
length (mm±SEM)	5.17±0.098 ^{bc}	4.13±0.116 ^a	3.91±0.013 ^a	3.70±0.015 ^a	5.07±0.042 ^b	4.97±0.128 ^b	4.36±0.020 ^{ab}	5.30±0.013 ^c
width (mm±SEM)	3.66±0.211 ^c	3.25±0.080 ^b	2.17±0.014 ^a	2.03±0.009 ^a	3.41±0.013 ^c	3.38±0.011 ^{bc}	3.07±0.016 ^b	3.48±0.020 ^c

Values in cells having no common letters in superscript are significantly different from each other at $p \leq 0.05$. **Δ: For explanation see materials and method**

Table 2 showing the protective role of green tea extract on enzymes and hormones in AA intoxicated male albino mice.

Dose Group ^A Parameter	Cont.	AA Dose Group			Antidote + Dose Group			GE
		AA-I	AA-II	AA-III	AA-I + GE	AA-II +GE	AA-III + GE	
Liver Functional test (LFT)								
ALT (U/L)	64.92±0.28 ^a	97.06±0.25 ^b	103.86±0.39 ^c	162.52±0.33 ^d	65.80±0.38 ^a	66.60±0.68 ^a	70.00±0.47 ^{ab}	65.20±0.44 ^a
Bilirubin (mg/dl)	0.76±0.05 ^a	0.87±0.004 ^b	0.89±0.004 ^{bc}	0.95±0.006 ^c	0.77±0.004 ^a	0.78±0.003 ^a	0.86±0.009 ^b	0.77±0.008 ^a
AST(U/L)	36±0.12 ^a	39±0.11 ^a	49±0.09 ^b	76±0.32 ^c	35±0.15 ^a	41±0.18 ^a	56±0.28 ^b	34±0.11 ^a
ALP (U/L)	146.00±0.47 ^d	106.00±0.74 ^c	87.00±0.87 ^b	55.00±0.37 ^a	144.20±0.59 ^d	144.00±0.47 ^d	125.40±0.54 ^c	145.40±0.33 ^d
Renal function test (RFT)								
Urea (mg/dl)	34.18±0.40 ^a	51.32±0.39 ^b	54.24±0.38 ^b	97.94±0.25 ^c	35.80±0.57 ^a	36.26±0.44 ^a	45.70±1.01 ^b	35.00±0.47 ^a
Creatinine (mg/dl)	0.47±0.004 ^a	0.51±0.003 ^a	0.60±0.006 ^b	0.88±0.005 ^c	0.48±0.004 ^a	0.48±0.006 ^a	0.50±0.003 ^a	0.47±0.001 ^a
Testis Hormones								
Testosterone (ng/dl)	3.42±0.006 ^c	0.50±0.009 ^b	1.79±0.006 ^{ab}	0.7±0.008 ^a	3.41±0.009 ^c	3.40±0.009 ^c	3.39±0.002 ^c	3.41±0.006 ^c

Δ: For explanation see materials and methods

Values in cells having no common letters in superscript are significantly different from each other at $p \leq 0$.

Histopathological observations: Administration of different doses of acrylamide (AA-I, AA-II and AA-III) caused liver injuries and induced vacuolations, necrosis, congestion in hepatic portal vein, lysed blood cells in portal vein, hemorrhages, pyknotic nuclei, hypertrophy, sinusoidal dilations, and cytoplasmic degenerations. However, histopathology of mice liver after treatment of acrylamide along with green tea extract (AA-I+GE, AA-II+GE and AA-III+GE) reversed these defects mostly and presented almost normal histological structures of liver except those who were treated with high dose of acrylamide along with green tea extract. Whereas mice treated only with green tea extract (GE) showed normal histology of liver comparable with controls (Fig. 1).

Labels: H: Hepatocytes, N: Necrosis, DS: Dilated sinusoidal space, S: sinusoidal space, CV: Central vein, CCV: Congested Central Vein, CD: Cytoplasmic

degeneration, SH: Swollen hepatocytes V: Vacuolation, HN: Hypertrophic Nuclei, HG: Haemorrhage

Histopathological examination of the kidney tissues of control mice showed normal structure of glomeruli and tubules in the cortex. While exposure to different doses of acrylamide (AA-I, AA-II and AA-III) caused multiple histopathological defects including glomerulonephritis, vacuolation, glomerulosclerosis, tubular dilation, tubular hyperplasia, proximal convoluted tubular degenerations, epithelium degeneration, hemorrhages, pyknotic nuclei, swelling and cellular rupture and hyalinization. However, histopathology of mice after treatment with acrylamide and green tea extract (AA-I+GE, AA-II+GE and AA-III+GE) protected kidney tissues and presented normal sections of kidney comparable with control (Fig. 2).

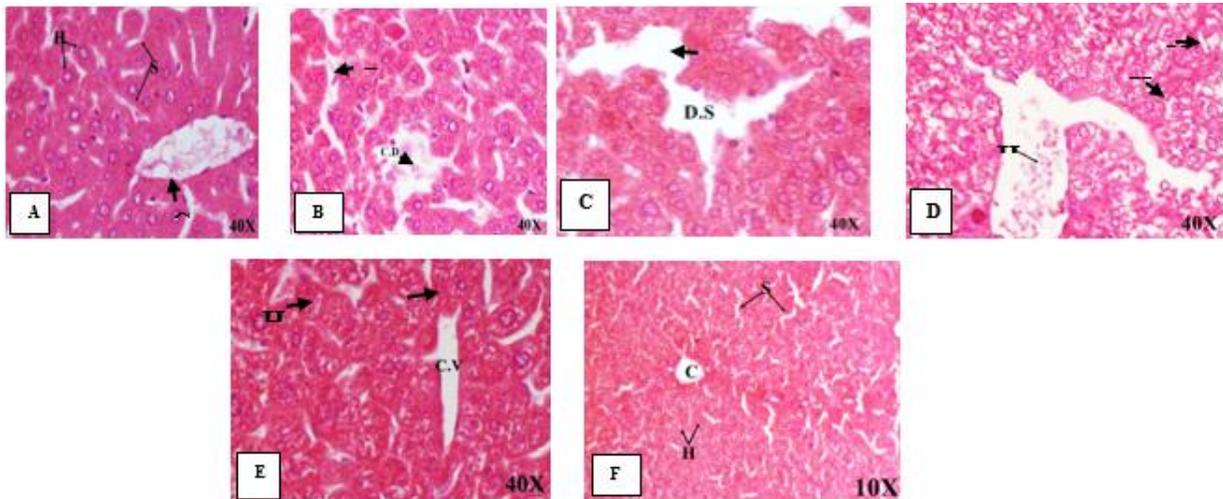


Figure 1: Microphotographs of Liver recovered from the AA, AA+ green tea extract and green tea extract treated groups of mice. A:Control, B: AA-I, C:AA-II, D:AA-III, E:AA-III+GE, F:GE

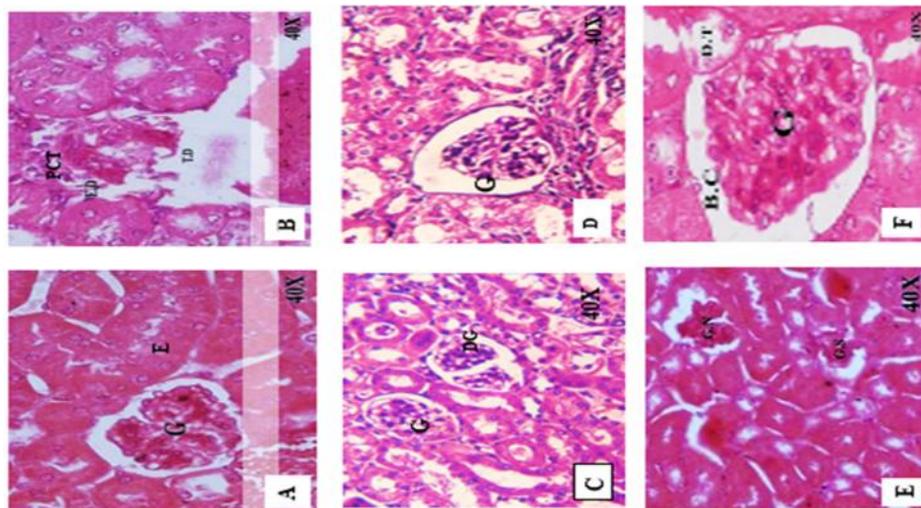


Figure 2: Microphotographs of Kidney recovered from the AA, AA +green tea extract and green tea extract treated groups of mice. A:Control, B: AA-I, C:AA-II, D:AA-III, E:AA-III+GE, F:GE

Labels: G:Glomeruli, E: Epithelial cell lining, GS: Glomerulosclerosis, TD: tubular degeneration, GN: glomerulonecrosis, PCT: proximal convoluted tubules, DT: Distal Tubules, ED: Enothelial degeneration, BC: Bownman’s Capsule, FH: Focal haemorrhage

Exposure to different concentrations of acrylamide (AA-I, AA-II and AA-III) showed various testicular damages including testicular hemorrhages, exfoliations, tubular degenerations and deforming shapes

of tubular structures, oligospermia, leydig cells degenerations, apical sloughing and hypertrophied nuclei in testis. However, histopathology of mice testis after the treatment of different doses of acrylamide along with green tea extract reversed most of these defects except in mice testes that were treated with higher concentration of acrylamide along with green tea extract. Whereas, mice treated only with green tea extract exhibit normal histological sections of testis just like control (Fig. 3).

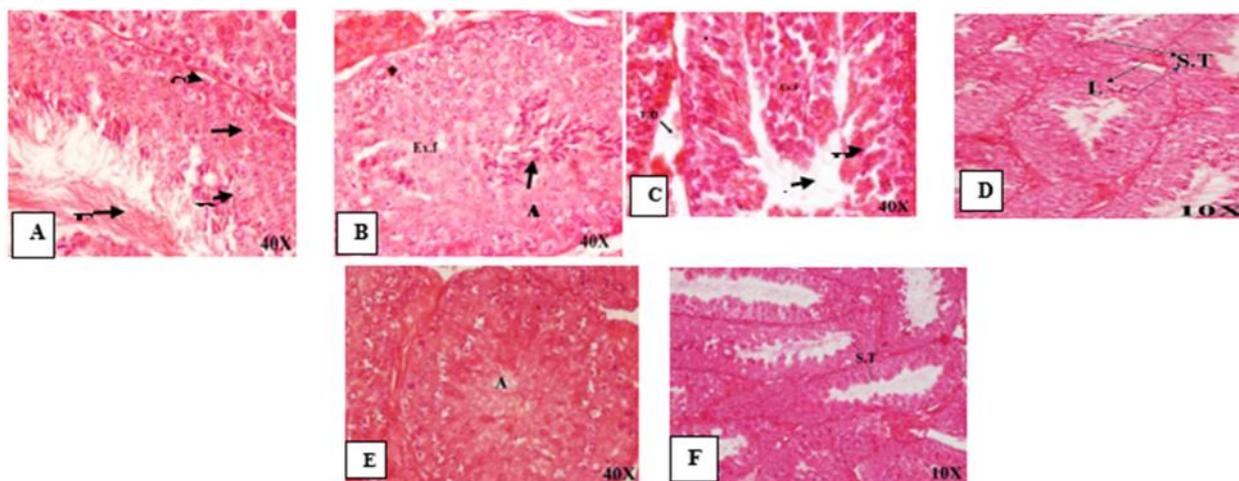


Figure 3: Micrphotographs of Testes recovered from AA, green tea extract +AA and green tea extract treated groups of mice. A:Control, B: AA-I, C:AA-II, D:AA-III, E:AA-III+GE, F:GE.

Labels: S: spermatogonia., ST: seminiferous tubules., TD: tubular degeneration., AS: Aspermia.,ExF: exfoliation., ES: sperms, RS: Spermatid, SC: Sertoli cell HG: haemorrhage

Lesion scoring: Histopathological defects were seen less pronounced when low dose of acrylamide is administered

in mice and frequency of defects increased with the increment of acrylamide dose. The frequency of lesion reversed when green tea extract is co-administered with acrylamide dose except at highest concentration. (Table 3).

Table 3. Summary of histopathologic lesions in liver, kidney and testis in various experimental groups.

	Control	(AA treated Group)			(AA + GE treated group)			GE
		AA-I	AA-II	AA-III	AA-I+GE	AA-II+GE	AA-III+GE	
Liver (section examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hepatocellular degeneration	0	6	9	12	2	2	7	1
Pyknotic nucleus	0	2	5	7	1	3	7	0
Congested portal vein	0	1	3	6	0	1	5	0
Tubular degeneration	0	3	6	11	2	1	3	0
Dilated sinusoidal spaces	0	2	4	7	1	1	2	0
Kidney (section examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Glomerulonecrosis	0	1	0	3	1	1	4	0
Glomerulosclerosis	0	0	1	5	0	4	5	0
Pyknotic nucleus	0	2	5	7	2	1	3	0
Glomeruonephritis	0	0	1	3	0	1	0	0
Tubular degeneration	0	7	10	3	2	4	5	1
Testes (section examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Exfoliation	0	4	6	9	0	1	4	0
Aspermia	0	3	5	7	1	2	6	0
Tubular degeneration	0	1	7	10	2	2	3	1
Necrosis	0	3	5	9	1	4	3	0

DISCUSSION

Acrylamide (AA), due to its wide range uses and its presence in widely consumed items its recurrent exposure to general population is obvious. Oxidative destruction is the chief mechanism of toxicity that predominantly takes place through production of free radicles that eventually cause damage to membranes and related tissues.

Body mass is a sensitive and visual indicator of xenobiotic produced adverse effects. In the current study, body weight of mice reduced rather than increasing after acrylamide exposure, anyhow; weight gain in groups treated with acrylamide and green tea was also decreased but not prominently. These outcomes were similar with Rawi *et al.*, (2012). Accordingly, Hogervorst *et al.*, (2007) also reported body weight reduction in acrylamide treated human beings. This was probably due to the loss of appetite and hence reduced rate of weight gain explained by a study on male rats (Wang *et al.*, 2015)

In accordance with the study by Yang *et al.*, (2005), the testes weight significantly reduced when mice were treated with acrylamide. In our observations various testicular damages observed after tissue histopathology, which consequently disrupted spermatogenesis. Present results states that acrylamide strongly affects the male reproductive function. These findings are supported by Yassa *et al.*, (2014). They reported the atrophied seminiferous tubules as well as degeneration of leydig cells and exfoliations.

Exposure to acrylamide reduced the testosterone levels of mice. Current results are in alliance with Yang *et al.*, (2005) who proved that testosterone levels severely altered in rats treated with acrylamide. Song *et al.*, (2008) reported that the sperm development is badly affected by acrylamide and affects the activity of enzymes and endocrine function of the testis. Leydig and sertoli cells are basic cell types playing critical role in spermatogenesis and steroidogenesis. Degeneration and hypoplasia of these cells in fact will be the reason of low endocrine function and spermatogenic arrest.

Liver act as a detoxification center, so more vulnerable organ towards xenobiotic. The liver weight of mice increased when treated with acrylamide that is in accordance with the finding of Kalipci *et al.*, (2012). Increase in liver weight was perhaps Intra cellular and extra cellular fluid retention as well as hypertrophy of the hepatocytes due to toxic insults. Exposure to different doses of acrylamide causes liver injury and induced vacuolations, necrosis, congestion in hepatic portal vein, breakdown of blood cells in portal vein, hemorrhages, pyknotic nuclei, hypertrophy, necrosis, sinusoidal dilations, and cytoplasmic degeneration. Above results are in accordance with Kedam *et al.*, (2012), who reported that acrylamide treatment induced vacuolations and necrosis in liver hepatocyte, hypertrophy and

pyknosis in nuclei of liver of chicks. This is a well observed fact that AA produce free radicals that leads to tissue injuries. Findings of Veenapani *et al.*, (2010) explained similar histopathological lesions in liver following AA administration. Green tea in this study was selected as protective agent due to its free radical scavenging activities.

Our study showed the ALT and bilirubin levels of liver increased whereas ALP levels decreased in the acrylamide treated mice. Allam *et al.*, (2010) reported that the increase in ALT of acrylamide generally reflect cellular damage. These findings are consistent with study of Zhang *et al.*, 2013, who reported that the increase in ALT, increased the permeability and damage or necrosis of hepatocytes. The decline in ALP levels of acrylamide treated mice is in accordance with Allam *et al.*, (2010). These alterations in biochemical parameters of liver are due to the decline in the antioxidant defenses in the liver. Actually, AA produced free radicals deteriorate the structure of poly unsaturated fatty acids in the plasma membranes, that disrupt the cellular integrity hence increase in liver injury markers.

Exposure to different doses of acrylamide affects badly the renal profile, increased the weights of kidneys and introduced numerous histopathological defects including glomerulonephritis, vacuolation, glomerulosclerosis, tubular dilations, tubular hyperplasia, proximal convoluted tubular degeneration, epithelium degeneration, focal hemorrhages, pyknotic nuclei, swelling and cellular rupture and hyalinization. Ozturan-Ozer *et al.*, (2014) reported the vacuolations in kidney of rats intoxicated with acrylamide. The results are also in consistent with Hammad *et al.*, 2013, who reported the glomerulonephritis, degeneration of proximal convoluted tubule and glomerulosclerosis.

The urea and creatinine levels of kidney increased in the acrylamide treated mice. These results are in agreement with Khalil and Abd El Aziem (2005) who reported the alterations of urea and creatinine levels after acrylamide treatment. In normal circumstances, urea is filtered by the kidney glomerulus from blood/plasma. Then it returns to the blood through kidney tubules, and most of it is excreted via urine. However, if the kidney is malfunctioning, then sufficient urea cannot be removed from plasma. so high level of urea in plasma is considered as biomarker for kidney functioning

Antioxidants in the form of phytochemicals are the key factors against oxidative stress induced by reactive oxygen species causing organ toxicity. Working on this principle, green tea extract showed protective effect to a great extent by bringing the body and organ weights, size, enzymes and serum testosterone levels towards its normal range in comparison with the controls and attenuated the histopathological defects of acrylamide liver, kidney and testis. Yassa *et al.*, (2014) confirmed these results. The strong health promoting

effect of green tea can be attributed to the antioxidant activity of the catechins that are the main constituents of green tea (Yang *et al.*, 2005), whereas the phenolic contents increases the excretion of detoxified metabolites resulting from xenobiotic metabolism (Weisburge, 1999). But the toxicity induced by higher concentrations of acrylamide did not ameliorate by green tea extract. This novel finding indicated the marked elevated concentrations of acrylamide beyond certain limits cannot be enervated by green tea extract significantly. This showed the limitation of green tea extract in term of its anti-oxidative power.

Conclusions: The current study showed that AA induced toxic effects in male mice, especially in terms of hepatic, renal and testicular injuries and reproductive, endocrine functioning. Sideways green tea aqueous extract was proved as an efficacious antioxidant that prevented and rectified acrylamide toxicity. It is therefore, recommended that we should avoid overcooked carbohydrates rich foods, smoking and acrylamide containing products for health perspectives and intake of green tea is beneficial if someone ingested these products

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