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Role of Oral Supplementation of Damiana (*Turnera diffusa*) Reduces the Renal Toxicity, Apoptosis and DNA Damage Associated with Amitriptyline Administration in Rats

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Major depressive disorder and anxiety disorders are two mental diseases that are treated with amitriptyline (AMT). AMT treatments induced liver, heart and testes toxicity; As a result, the purpose of this study was to determine the preventative and therapeutic role of damiana (Dam) as adjuvant herbal therapy against AMT induced heart injury in rats. Six groups of 36 rats (male albino) were randomly assigned; first one is control, second is Dam, third was AMT, fourth was Dam+AMT, fifth was AMT+Dam and sixth was AMT self-healing. A significant elevation in creatinine, urea, sodium (Na+), Chloride (Cl+), renal injury, DNA damage and apoptosis in treatment rats with amitriptyline and self-healing group as related to control and damiana groups. Conversely; potassium (K+) and calcium (Ca++) were a significant decrease in AMT and self-healing groups as compared with control. Treatment of AMT with Dam (Co and Post) revealed a modulation and improvement of renal toxicity with best result in co- treatments than post treatments. As a result, AMT treatments encouraged changes in kidney functions and structure and the post-treatments of AMT with dam modulates these alterations.

Keywords: Amitriptyline; Damiana; Kidney; DNA damage; P53.

Depression is a psychoneurotic issue described by mental and utilitarian action.[1] Each medication used to treat depression called antidepressant medication and it works by adjusting synthetic uneven characteristics of synapses inside the brain.[2,3] Antidepressants are a class of medications used to treat the side effects of burdensome issues Chemical lopsided characteristics could likewise be chargeable for changes in the state of mind and behavior.[4,5]

Amitriptyline is a tricyclic stimulant (TCA) used to treat various psychological sicknesses incorporating significant burdensome problem and nervousness issue, and less generally consideration shortage hyperactivity jumble and bipolar issue, different purposes incorporate

counteraction of headaches, therapy of neuropathic agony, for example, fibromyalgia and postherpetic neuralgia, and less ordinarily insomnia.[6,7] AMT is known to inhibit serotonin and norepinephrine reuptake at the presynaptic level, increasing the clustering of the two synapses at the synaptic interface.[8] As of late, AMT was viewed as an inhibitor of mitochondrial capacities that are answerable for the development of ROS and for setting off the modified cell demise or apoptosis.[9-11] Amitriptyline treatment actuated oxidative pressure and apoptosis in a few tissues, including the mind, in a portion subordinate way.

Alternative medicines for the treatment of a number of human disorders have been the subject of several pieces of research.[12-15] Several phytochemicals were presented to be threatened and possibly valuable therapeutically.[16-18] Damiana (Turnera diffusa Willd) is a restorative plant generally utilized as an energizer, diuretic and Spanish fly; it is additionally regularly utilized for the arrangement of mixtures and alcohols, and in the development of corrective items, cleansers, gelatins, chocolates, and in conventional medicine.[10,19,20] It has been proposed that damiana has a lot of natural balms and cancer prevention agents, especially flavonoids, which could be answerable for its restorative properties.[21,22] Due to late revelations of new metabolites in and expected utilization of damiana, there is rising attention, especially from of the clinical and drug enterprises, on its way of life and business corruption.[23,24] Dam has been shown to have a protective effect against exploratory hepatotoxicity by regulating the ultra-structures of liver cells and affecting the presentation of liver enzymes.[25]

As a result, the purpose of this research was to look at the effects of Damiana (Dam) against the apoptosis, DNA damage, and toxicity induced by Amitriptyline (AMT) administration in kidney.

MATERIALS AND METHODS

Damiana's aqueous extracts production

Dam dried leaves were shacked to powder and soaked in boiled water for 24 hours at 37C then extract and stored at -30˚C in the dark until used.[11]

Experimental animals

36 rats (male albino) were brought from the Giza NRC, Egypt, at the age of 10-12 weeks and weighing roughly 150 g. The Ethical local committee agreed to design the experiment according to the guidelines of the Faculty of Science, Tanta University with the approval of the Institutional Animal Care and Use Committee (IACUC-SCI-TU-0049). Six groups of 36 rats (male albino) were randomly assigned; first one is control, second is Dam (80 mg Dam/kg body weight/daily/ orally) for 4 weeks, third was AMT (70 mg amitriptyline/kg body weight/daily/ intraperitoneally injected) for 4 weeks, fourth was Co-treated Amitriptyline with Damiana (Dam+AMT), fifth was post-treated Amitriptyline with Damiana (AMT+Dam) and sixth was AMT self-healing. The rats were starved overnight at the end of the experiment. Each group's rats were euthanized with sodium pentobarbital to allow for full dissection.

Blood and serum samples

Sera were taken and centrifuged at 4000 rpm for 12 Min to separate it and to determine different blood parameters.

Electrolytes and kidney functions Biomarker

Levels of urea and creatinine were assessed according to Patton and Crouch[26] and Bowers and Wong[27] correspondingly. Electrolytes levels were assessed according to Abd Eldaim et al.[28]

Comet Assay

A comet assay approach developed from earlier available journals was used to analyse and quantify DNA damage in tissue of the kidney.[29]

Histopathological examination

According to Tousson;[30] kidney tissues were removed and promptly fixed in neutral formalin at 10% concentration for 2 days before being processed for paraffin sectioning and H&E staining.

Immunohistochemical detection of P53 immunoreactivity

Conferring to Tousson et al.[31,32], apoptotic P53 in kidney was distinguished through the Avidin-Biotin-Complex method.

Statistical Analysis

Current data were offered as means with standard - error of main. One-way ANOVA was used to parallel data between the amitriptyline group and the other five groups at p0.05, the P value was significant. The analysis was carried out with the help of a Graphpad prism.

RESULTS

Changes in kidney functions and electrolytes in different groups

When AMT and self-treated AMT groups were associated to control and Dam groups, urea, creatinine, Na+, and Cl- levels were significantly higher in the groups of AMT and self-healing AMT. When comparing the groups of AMT and self-treated AMT to the groups of control and Dam, serum potassium (K+) and calcium ions (Ca++) were significantly lower in the AMT and AMT self-cured groups (Figure 1).

When AMT treated with Dam (co and post) groups were associated with AMT and AMT self-healing groups, serum urea, creatinine, Na+, and Cl- levels showed a substantial decrease. When compared to amitriptyline and self-treated AMT

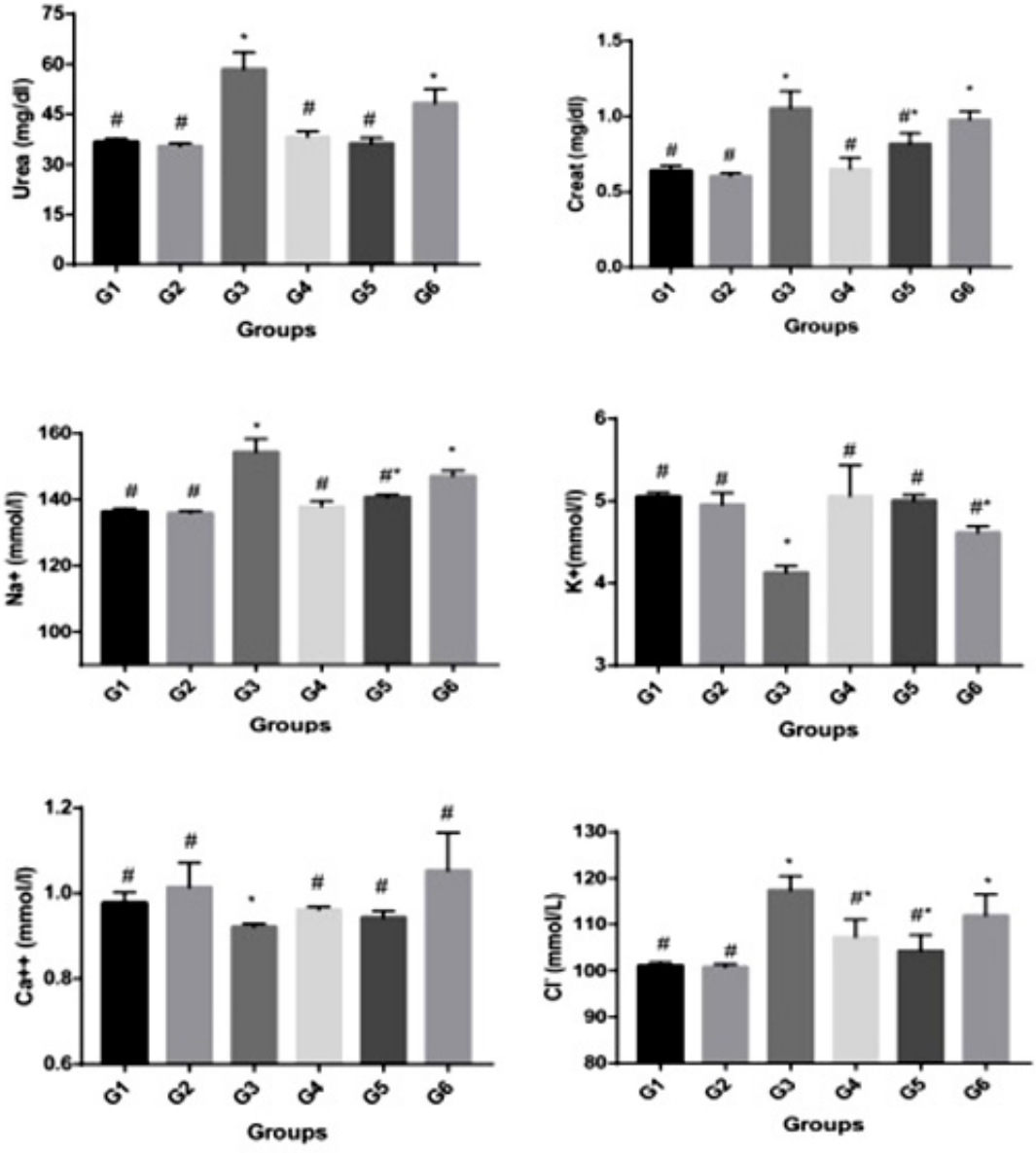


Figure 1. Deviations in levels of urea, creatinine, Na+, K+, Ca++ and Cl- in dissimilar groups. Where G1, control; G2, Dam; G3, AMT; G4, Dam+AMT; G5, AMT+Dam; G6 , AMT self-healing. (*) and (#) Significant difference compared to control and AMT respectively

Table 1. Kidney DNA damage with Comet assay constraints achieved by image analysis in all group cells

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
G1	1.5	98.5	$1.40^{\#}$±0.13	1.49	2.09
G2	3	97	$1.52^{\#}$±0.14	1.63	2.48
G3	23	77	8.11*±0.38	5.37	43.55
G4	7	93	$3.40^{\#*}$±0.15	2.41	8.19
G5	14.5	85.5	6.07*±0.25	3.83	23.25
G6	8	92	$3.75^{\#*}$±0.17	2.62	9.83

Significant difference from the control group (G1) at *p<0.05. Significant difference from amitriptyline group (G3) at #p<0.05. where, G1, control group; G2, Damiana group; G3, Amitriptyline group; G4, co- treated group; G5, post-treated group; G6, self-treated groups.

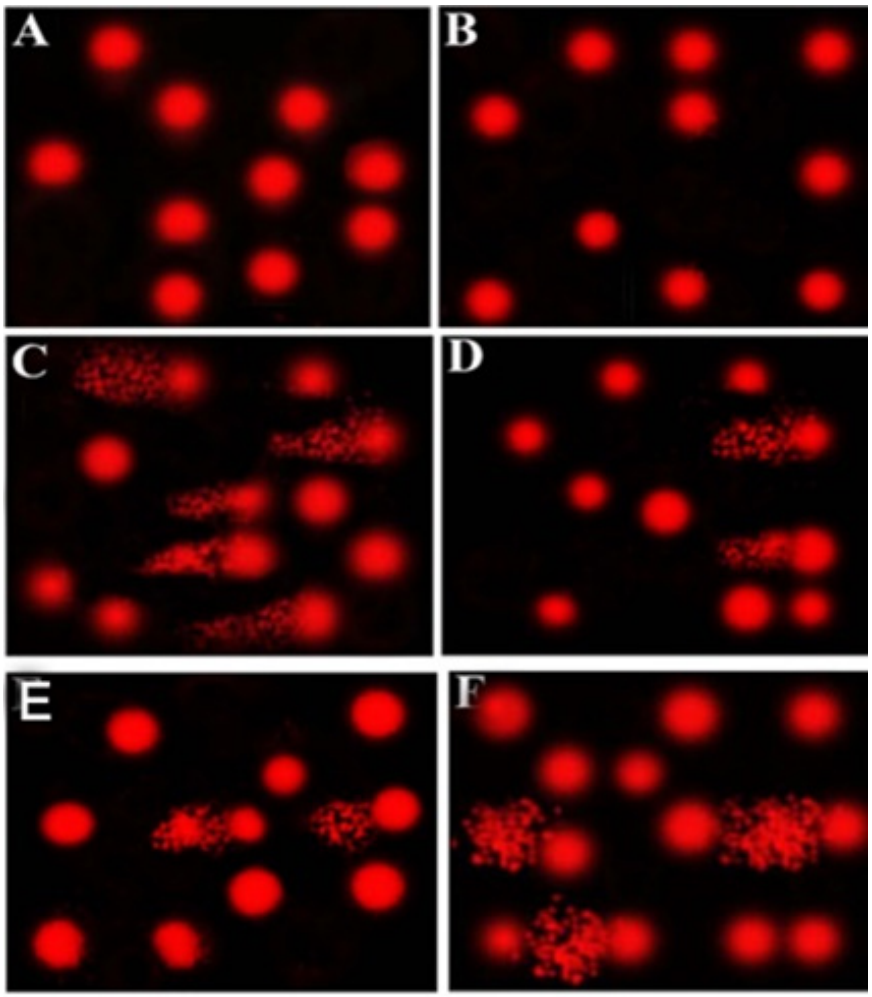


Figure 2. Kidney DNA damage photomicrographs. A, control; B, Dam; C, AMT; D, Dam+AMT; E: AMT+Dam, F, AMT self-healing

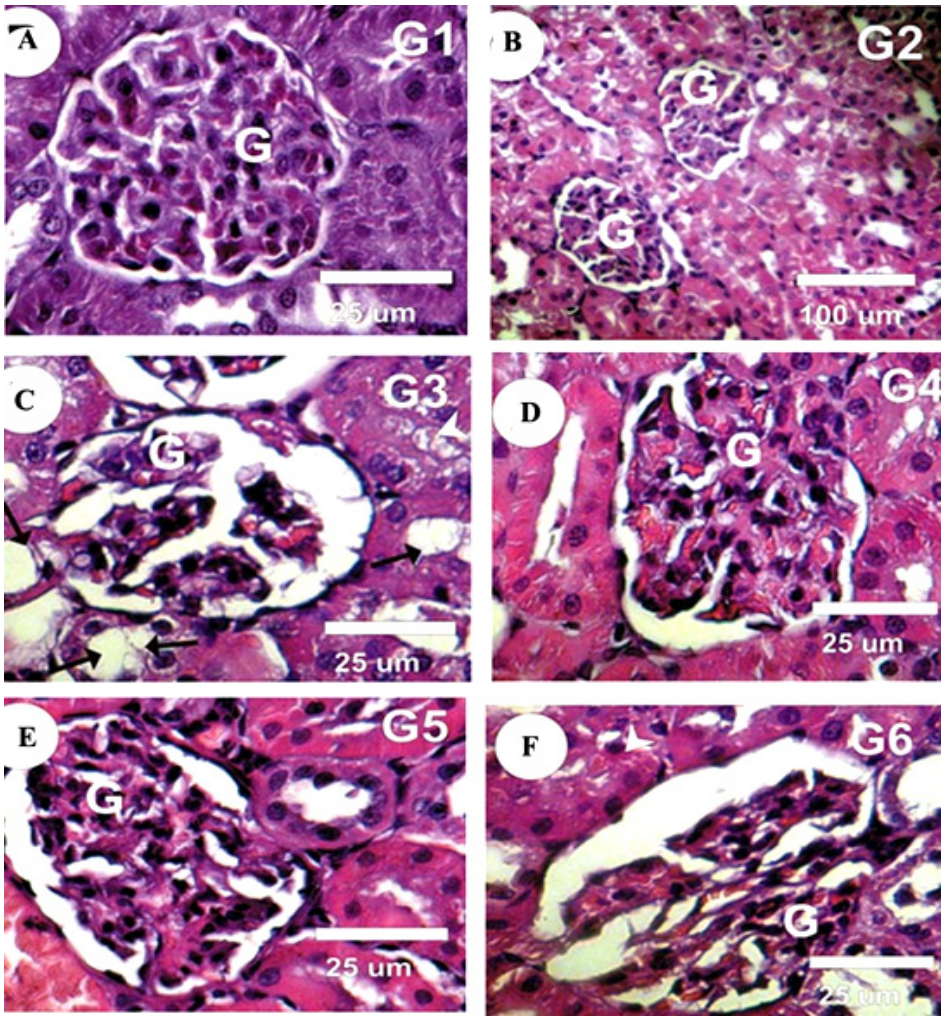
groups, co-treated AMT and post-treated AMT with Dam groups showed a substantial rise in K+ and Ca++ levels (Figure 1).

DNA damage in kidney

Figure (2) and tables (1) revealed kidney DNA damage in tissues. When associated with the control and Dam groups, the AMT group confirmed elevation in DNA damage (P 0.05), as seen by increases in tail length, tail DNA percent, and tail moment. This enhanced kidney DNA damage was reduced in Dam+AMT, AMT+Dam, and self-cured.

Kidney histopathology results

Our results revealed that; Malpighian corpuscles and both proximal and distal convoluted tubules are normal in both control and Dam kidneys (Figures 3A&3B). In contrast; Malpighian corpuscles lost their characteristic configuration, and renal tubules with wide lumen, moderate haemorrhage, mild to moderate atrophic glomerulus and degenerated epithelium, were seen in kidney of AMT and in AMT self-healing groups (Figures 3C&3F). However; kidney in Dam+AMT and AMT+Dam revealed a good improvement with



Figures (3A-3F). Photomicrographs of rat kidney slices stained with H&E A&B: Normal histological architecture of the glomeruli (G) and renal tubules in control (G1) and Dam (G2) kidney sections. C&F: AMT (G3) and AMT self-cured (G6) kidney demonstrated significant cortical tubular epithelial degeneration (arrow heads), localised tubular epithelial necrosis (White arrows), and atrophic glomerulus. D&E: Kidney sections Dam+AMT and AMT+Dam showed significant improvement, with only minor vacuolization in tubular cells.

only negligible vacuolization in renal tubules as compared to AMT and in AMT self-healing groups (Figures 3D&3E).

P53 immunohistochemical changes in Kidney

Immunohistochemistry staining of kidney from the control (G1) and Dam (G2) groups revealed minor positive expression of P53 (grade 2) in the glomeruli and renal tubules (Figures 4A&4B). The glomeruli and renal tubules in the kidneys of AMT (G3) and AMT self-healing (G6) groups showed a heavy positive expression (grade 5) for P53 (Figures 4C&4F). In kidney sections of rats in Dam+AMT (G4) and AMT+Dam (G5), revealed moderate and mild to moderate positive expressions for P53 (grades 4&5 respectively) (Figures 4D&4E).

DISCUSSION

The kidney is a compound cylindrical organ worried about the significant capacity of excretion.[33,34] It removes foreign items, discharges urea and other nitrogenous wastes, and maintains homeostasis by managing the organisation,

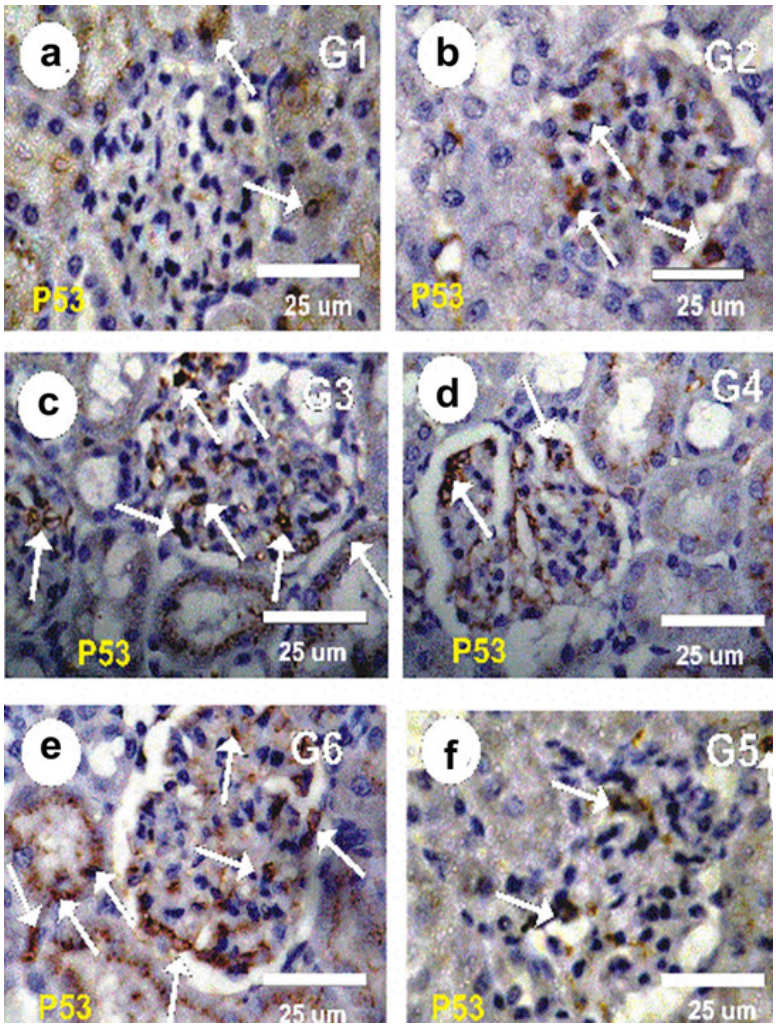


Figure (4A-4F). Kidney section Photomicrograph in rat stained with apoptotic P53. **A&B**: Minor positive (arrows) reactions for P53 were detected in control and dam treated groups. **C&F:** Heavy positive (arrows) reactions (grade 5) for P53 in kidney of AMT (G3) and AMT self-healing (G6). Dam+AMT (G4) and AMT+Dam (G5), revealed moderate (arrows) and mild to moderate positive (arrows) expressions for P53 (grades 4&5 respectively)

volume, and strain of blood.[35] Medications are a common cause of kidney impairment, often known as nephrotoxicity or, in severe cases, renal disappointment. This suggests renal damage, and plasma creatinine levels were found to be elevated in relation to the histological findings. The study assumes that antidepressant medication will have an impact on the vital organs. As a result, these effects must be considered while administering antidepressants to depressed patients.

In the current study; a critical expansion in creatinine, urea, Na+ and Cl- and a marked decline in K+ and Ca++ levels was identified in after treatments with AMT when differentiated from control. Current outcomes were steady with Tousson et al.[36] who announced that; amitriptyline instigated an expansion in sodium particle levels and reduction in potassium particles level. In the current review, expanded urea, and creatinine levels mirror the finding of renal failure.[37,38] Moreover, raised blood urea is known to be related to expanded protein catabolism in well evolved creatures and additionally the change of alkali to urea because of the expanded blend of arginase chemical engaged with urea creation. The rise in serum urea and creatinine levels in AMT-treated rodents is considered a critical marker of renal brokenness and might be connected with metabolic aggravations in liver capacity, as urea is the final result of protein catabolism. Besides, xenobiotics escalate the corrosive secretory capacity of kidney and change the vehicle of sodium.[39] This is reliable with the current outcomes where Na+ and K+ were changed in rodents treated with AMT. organization of Dam shields the kidney capacities from AMT inebriation as demonstrated by a huge rebuilding of serum urea, creatinine, and electrolytes.

Various human chronic diseases have been connected with the changed oxidative pressure, created either through the expansion of the free extreme age as well as a compromised cancer prevention agent level in the objective cells and tissues.[40] These free revolutionaries produced prompt DNA strand breaks and causes oxidative alteration of the DNA bases.[41] DNA harms were performed with comet measure in kidney tissues. In the ongoing review; Amitriptyline actuated a huge expansion in kidney and liver DNA harm ($P < 0.05$) that was demonstrated by expansion in tail length, tail DNA% and tail second when contrasted with ordinary control and damiana gatherings. This expanded DNA harm was decreased in co-treated (G4), post-treated (G5), self-healing (G6) with consumption harm in G4 and G6. Our outcomes concur with Tousson et al.[10] and Hasan et al.[11] who find that; amitriptyline made DNA damage in testes and liver respectively and the treatments with damiana modulate this DNA damage.

Apoptosis is a significant cycle for holding cell numbers under control, without influencing cell replication augmentation notwithstanding it stops the phone cycle at G1 and G2 if there should be an occurrence of DNA harm, permitting the actuation of DNA fixing proteins.[42-44] In the ongoing review; apoptotic P53 articulations were fundamentally expanded in kidney tissues in amitriptyline bunch as contrasted and control recognized amitriptyline incited apoptosis, additionally treatment amitriptyline with damiana balances these progressions in P53.

CONCLUSION

AMT treatments encouraged changes in kidney functions and structure and the post-treatments of AMT with dam modulates these alterations.

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