Specific Target Organ Toxicity (STOT) After Acute Exposure of Dinotefuan And Its Attenuation With Vitamin C Supplementation

Hafiz Muhammad Ahmad

University of Veterinary and Animal Sciences

Munazza Raza Mirza

Dr Panjwani Center for Molecular Medicine and Drug Research

Muhammad Shahbaz Yousaf

University of Veterinary and Animal Sciences

Sehrish firyal

University of Veterinary and Animal Sciences

Muhammad Muddassir Ali

University of Veterinary and Animal Sciences

Rahat Naseer (✉ rahat.naseer@uvas.edu.pk)

University of Veterinary and Animal Sciences <https://orcid.org/0000-0002-8618-2495>

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Abstract

The present study was designed to evaluate the effect of 2-methyl-2-nitro-3-guanidine on non-target mammalian species after acute exposure and its probable. Forty-five four weeks old *Sprague Dawley* rats of mixed sexes having mean body weight (80 ± 20 g) were randomly divided into three groups fifteen each, namely C (control), E(exposed), and V (exposed and vitamin supplemented) group. The E and V groups were exposed to 2000 mg/kg of body weight via gauge. The vitamin C was administrated by dissolving 400mg/350ml of water and given *ad libitum* (LD50). Eight hours after dosing 5ml blood was collected by cardiac puncture under sedation. After 48 hours five rats were selected randomly from each group, anesthetized, euthanized, and dissected. Mortalities in each group were taken into account to reduce decretory mortality. Different body tissues, liver, kidney, heart, and bones were separated and preserved in formalin for further analysis. Complete blood count was conducted along with markers of liver kidney and heart function. Bone characteristics and histopathology of soft tissues were carried out. Results showed significant disturbance in CBC and other biomarkers of liver kidney and heart function in the E group. Vitamin supplementation although improved the picture but non-significantly. Histopathology showed signs of necrosis in the exposed group with no improvement in the C group. The weight/Length index and the robusticity index were also found to follow similar trends. The present study was cautiously concluded that even single high dose exposure can cause tissue damage in non-target species.

Introduction

Neonicotinoids in the class of neurotoxic insecticides which are highly systemic have a very unique mode of action and broad-spectrum efficacy. They irreversibly bind with the nicotinic receptors of acetylcholine, blocking the nerve impulses in insects. On the contrary, they are claimed to be less toxic towards mammals, fishes, and birds (Tomizawa and Casida 2005). Although many nicotinoids like imidacloprid and clothianidin show very strong toxicity towards pollinating insects, particularly to honey bees (*Apis mellifera* L.) there market share showed a steady rise and it is 24 % of total insecticides turnover (Jeschke et al. 2010).

Before being registered, the formulated pesticides are currently undergoing various tests to evaluate the risk that these molecules pose to honey bees. In the European Union, the guidelines of the European and Mediterranean Plant Protection Organization No. 170 (OEPP / EPPO, 2001) and the relative risk-assessment procedure are generally followed, but their efficiency for systemic insecticide has recently been questioned, with particular reference to neonicotinoids (Halm et al. 2006).

The dinotefuran [1-methyl-2-nitro-3-(tetrahydro-3-furyl methyl) guanidine], is the most advanced neonicotinoid insecticide and is widely used for crop protection due to its less toxic effect on human health. It is utilized as a high potential insecticide to control various sucking and plant bugs like insects like beetles, whiteflies, green rice leafhoppers, and aphids due to its capability to block the nicotinic

acetylcholine receptors unlike the other insecticides (Bass et al. 2011) ; (Hem et al. 2012). Presently, it has been popularized and connected in various strategies throughout the planet (Rahman et al. 2015).

It demonstrates brilliant properties i.e. rapid take-up and translocation in plants, high insecticidal action, safe for the atmosphere and people(Watanabe et al. 2011), steady towards soil condition, and a half-life of 50–100 days (Morrissey et al. 2015). Even though neonicotinoid insecticides are expected to have destructive and harmful effects on animals, there is minimal animal experimental information available to understand their impact and mechanism of action (Yoneda et al. 2018). The present study is designed to assess the systemic effect of acute exposure of dinotefuran on different mammalian tissues.

Materials And Methods

2.1. Chemicals and Reagents:

Dinotefuran [1-methyl-2-nitro-3-(tetrahydro-3-furyl methyl) guanidine] supplied by four brothers, Vitamin C(Merck), Formalin Buffer (Reidel-de Haen) Ethanol (VWR), distill Water, Diethyl Ether (Merck Germany)

2.2. Methodology:

Fifteen Sprague Dawley rats of mixed sexes having weight about 80 ± 20 g and aged 4 ± 0.5 weeks were safely housed as by EU guidelines of animal research at the animal housing facility center of UVAS, Lahore. They were safely kept in stainless steel cages at ambient temperature ($21 \pm 2^\circ\text{C}$) and 50% humidity providing basic diet and water *ad libitum* for about 12 hours to adjust them and afterward were randomly divided into 3 different groups i.e. 5 rats in each group. Group C was our control group, Group E was the pesticide exposure group, and Group V was exposed to pesticide and supplemented with vitamin C. Group C and V were gauged 2000 mg/kg of body weight. Group V was also supplemented provided with Vitamin C (4ml of Vit C dissolved in 350 ml of water).

After 12 hours, 5 ml of blood was collected directly from the heart puncture of each rat. Serum was extracted from 3.5 ml of blood and the remaining 1.5 ml of blood was collected in EDTA vials.

After 48 hours, no mortality was recorded, but among all the groups, four rats were showing the symptoms of lethargy and disinterest in food. All rats were given anesthesia and then slaughtered for organ extraction. The organs under study (liver, kidney, heart, and bone) were preserved in formalin buffer until used for further analysis (Cui et al. 2009). All the procedures were carried out humanly as by the institutional code of animal research.

Histopathology of liver, kidney, and heart was performed according to the procedure of (Cui et al. 2009). The removed left tibia was cleaned and incubated in boiling water for 10 minutes by following the method proposed by (KOCABAĞLI 2001).

The bone weight / length index and robusticity index were determined by the following formulae:

Weight / Length Index (mg/mm) = Weight (mg) / Length (mm) (Seedor et al. 1991)

Robusticity Index (mm/mg^{1/3}) = Bone Length (mm) / Cube root of bone weight (mg) (KOCABAĞLI 2001)

All the observations were collected in triplicate. Thus, the obtained data were statistically analyzed through analysis of variance (ANOVA) and the values were calculated by using SPSS software. The significant difference of each parameter was statistically measured by using a significance level of $\alpha = 0.05$.

Results And Discussion

No mortality and no weight loss were recorded after 48 hours of exposure. Similar results were reported while using other Neonicotinoids like *Imidacloprid* (10 and 20 mg/kg/ 60 days) (Vohra et al. 2014) alone and in combination with *Fipronil*. Although these studies reported chronic low dose exposure. dose chronic as reported by (Chakroun et al. 2017).

3.1 Blood Toxicity:

Complete Blood Count (CBC) of all the fifteen rats which were sampled after 12 hours was carried out. The results of each parameter were pooled to assess the overall blood picture. The mean values of WBCs, MID cells (infrequently existing rare cells having a connection with monocytes, basophils, eosinophils), and granulocytes (GRA) were found to decrease significantly ($p \leq 0.05$) while significant Lymphocytosis (LYM) was found ($p \leq 0.05$) in the experimental group concerning control group. Results also showed a significantly positive effect of vitamin C supplementation as shown in table no. 1.

This reduction in leukocytes and their types may be attributed to the production of an oxidatively stressed environment resulting in the lysis of the individual cells resulting in depressed hematopoiesis (Chatterjee et al. 2014). However, a transitory rise in lymphocyte count may be due to body defense (Bassini-Cameron et al. 2007) (Ghazi et al. 2012). As it was an acute trial, so only short-term data is available and shared (Moid et al. 2014). A significant reduction in the number of leukocytes and its various types, except lymphocytes, and significant promotion in the number of lymphocytes (lymphocytosis) in adult Sprague Dawley rats (weighing about 200g) were reported by (Nair et al. 2010) in a study of sublethal toxicity of orally applied cypermethrin.

Table no. 2 explains that mean ($\pm$ SD) values of RBCs, HGB, HCT, and RDWc were decreased in the experimental group as compared to the control group. On the other hand, mean ($\pm$ SD) values of MCV, MCH, and MCHC were increased in the intoxicated rat group as compared to the control group. The presence of Vitamin C has reduced the toxicity effect of dinotefuran on RBCs and its related parameters. Several factors have been reported for this reduction in erythrocyte count and hemoglobin concentration like internal hemorrhage (Enan and EE 1983) or depletion in the synthesis of hemoglobin or due to an elevation in the destruction of hemoglobin (Karmakar et al. 2000) (Lal et al. 2011) (Chatterjee et al. 2014). Blood cells affected by toxic substances like insecticides may be responsible for hemolysis (Kataria et al.

2016). Reduced level of GSH and elevation of lipid peroxidation can cause cell lysis. Similar results were well reported with cypermethrin (Kataria et al. 2016) and imidacloprid (Jasper et al. 2012).

Table no. 3 shows that mean ($\pm$ SD) values of PLT and PCT % were decreased in the intoxicated rat group as compared to the control group. But mean ($\pm$ SD) values of MPV, PDWc, and PLCR % were increased in the experimental group as compared to the control group. In this case, Vitamin C also showed its antioxidant and inhibitory effect against the toxic effect of dinotefuran.

Bone marrow is highly responsive to toxic substances like insecticides that are responsible for aplasia, dysplasia, and impairment in cell function (Subramanian 2019). This decrease in platelet number may be due to the impact of insecticide on bone marrow cells either at their synthesis level or their developmental level, resultantly hematopoiesis becomes slow or stops (Chatterjee et al. 2014). A raised mean platelet volume (MPV) level indicates high blood clotting ability. As a result of which there will be more chances of thrombosis, stroke, or cardiovascular disorders (Moid et al. 2014).

Previous results also revealed that sub-acute (28 days) toxicity of diazinon (20 mg/kg/day) and imidacloprid in male BALB/c mice and orally administrated female mice for 24 hours was responsible for depletion in platelet number (Zeinali et al. 2018). In another study, when different concentrations of imidacloprid (25% 50%, and 75% of LD50) (Kataria et al. 2016) and (Moid et al. 2014).

3.2. Liver Toxicity:

After 48 hours of the trial period, liver histopathology and LFT (Liver Function Test) were performed to evaluate the liver-related disorders and extent of liver damage. Table no. 4 explains the mean ($\pm$ SD) values of hepatic-related serum parameters like alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin total. These values were found significantly increased in the experimental group as compared to the control group. Vitamin C showed a significant (antioxidant) effect on mean values of hepatic-related serum parameters.

The elevated level of these enzymes may be due to the interruption of insecticide in the biosynthesis of these enzymes along with the changes in the permeability of hepatic cells by degeneration in hepatic tissues. As a result of which normal or regular functions of the hepatic plasma membrane (especially transportation) are interrupted. Therefore, these enzymes transport from the cytosol of hepatic tissues into the bloodstream (Celik and Suzek 2008) (Jadon et al. 2007). The increased enzymatic activities of ALP, ALT, and AST may also be due to loss of hepatic membrane and hepatocellular damage (Chakraborty et al. 2016). Increased levels of ALP, ALT, and AST also indicate tissue damage, necrosis of hepatic tissues (Sathiavelu et al. 2009).

It has already been well documented that the prolonged effect of chlorpyrifos (an organophosphate insecticide), caused (Uzun and Kalender 2013). Imidacloprid (10 and 20 mg/kg/ 60 days) (Vohra et al. 2014) and fipronil are significantly related to increased concentration of liver enzymes although acute exposure requires more study. In both cases, the effect is dose-dependent.

Hepatic tissue extracted from the control group showed normal cytoplasm with no pyknotic nuclei. There were no morphological changes due to hydropic degeneration, cellular swelling, and any necrosis. There was no congestion in sinusoidal capillaries. On the other hand, there was severe hydropic degeneration and coagulative necrosis in the hepatic tissue of the Experiment group (LD50). Severe hydropic degeneration was also seen in the hepatic tissue of the Vitamin group (LD50 + Vitamin C) as shown in figure no. 6.

3.3 Renal and Cardiac Toxicity:

After 48 hours of the trial period, kidney histopathology and RFT (Renal Function Test) were performed to evaluate the kidney-related diseases and extent of renal damage. Table. 5 describes that Serum creatinine and Blood Urea Nitrogen (BUN) levels were significantly increased in the intoxicated rat group as compared to the control group. Here, Vitamin C also showed its antioxidant and protective effect on these kidney-related serum parameters.

Histopathology of a thin film of renal tissue extracted from the control group showed normal shape and space in Bowman's capsule. There was no congestion, pyknosis, karyolysis, or any necrosis in renal tubular epithelial cells. However, there was peritubular congestion, pyknosis, karyolysis, coagulative necrosis, and coagulative necrosis in renal tubular epithelial cells of the Experiment group (LD50). But, histopathology of renal tissues of the Vitamin group (LD50 + Vitamin C) showed mild congestion and coagulative necrosis as shown in figure no. 7.

After 48 hours of the trial period, histopathology of the heart was performed to evaluate the extent of heart damage. Histopathology of a thin film of cardiac tissue extracted from the control group showed normal cardiac fibers and sarcoplasm in cardiac cells. There was no fragmentation of cardiac fibers, congestion, or any necrosis in their cells. However, there was the loss of sarcoplasm and fragmentation of cardiac fibers in cardiac tissues of the Experiment group (LD50). But, histopathology of cardiac tissues of the Vitamin group (LD50 + Vitamin C) showed congestion and mild cellular degeneration as shown in figure no. 8.

These results indicate dinotefuran nephrotoxic potential. It has been reported that insecticide induced variations in kidney function markers (serum creatinine and BUN) along with histopathological changes in renal tissues are due to oxidative damage (Abdel-Daim and Abdeen 2018) because ROS increased production is said to be responsible for the reduction of GFR (Pedraza-Chaverri *et al.* 2000) and subsequently abnormal and deviant function of the glomerulus (Parlakpinar *et al.* 2005). Studies using different insecticides also supported these findings (Abouelghar *et al.* 2020), (Kanu *et al.* 2016), (Jasper *et al.* 2012).

Histopathology of the liver, kidney, and heart showed that dinotefuran also affects the structure of these vital organs. The only control group showed normal structure, remaining all groups including vitamin groups showed significant changes in their structure. Mild toxicity of insecticide was observed in vitamin-taking rat groups. The same results were found by (Kerem *et al.* 2007) after acute toxicity of fenthion (an

organophosphate insecticide). A previous study showed that co-treatment (Vitamin and insecticide) can significantly inhibit imidacloprid-induced changes in the liver of Sprague Dawley rats when they for weeks (Soujanya et al. 2013). The above discussion of biochemical parameters justifies these histopathological results.

3.4. Bone Toxicity:

Mean values ($\pm$ SD) of Weight (mg) and Length (mm), in different rat groups, were decreased in the experimental group as compared to the control group. As a result of which Weight / Length Index (mg/mm) in the experimental rat group was decreased (as shown in table no. 6), while Robusticity Index (mm/mg^{1/3}) in experimental rat groups was increased (as shown in the table no. 7). Again, in this case, Vitamin C shows its inhibitory and protective effect against the toxic effect of dinotefuran. These results explain the decreased density of bone (Monteagudo et al. 1997). There was no data available for the weight/length index and robusticity index of any rat bone against any insecticide or pesticide toxicity.

Conclusion

In light of the above discussion, we concluded that single acute exposure to dinotefuran affects the structure and functions of many vital organs (liver, kidney, heart, and bone) in mammals. It causes biochemical, hematological, histopathological, and bone-related alterations. It means that even short-term exposure to dinotefuran can be dangerous. Vitamin C can play a role in minimizing insecticide toxicity. However, these effects are dose-dependent.

Declarations

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Declaration of interests

On the behalf of all the authors, I declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Raw data is available on request

Consent to participate

All the authors gave full consent to participate

Consent to Publish

All the authors gave consent to publish this data

Plant Reproductively

Not applicable

Clinical trial registration

Not applicable

Author contribution

Dr Rahat Naseer Conceptualize and write lay out

Mr.Muhammad Ahmad Conduct lab trials

Dr.Mnnaza Raza Guide and perform analysis proof read manuscript

Dr Muhammad Shahbaz statistical design

Dr. Sehrish faryal helped and performed animal trials

Dr Muddassir manuscript writing

All the authors declare that data used in this manuscript were generated in-house and no paper mill was used.

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The research got approval from independent ethical committee of university

On behalf of all the authors

Dr. Rahat Naseer

Author /Corresponding author

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Tables

Table No. 1: Mean (± SD) values of WBCs and its types in different rat groups. Control Group = Rat group fed with only normal diet (no exposure of pesticide or vitamin), E-Group = Rat group fed with LD₅₀, V-Group = Rat group fed with LD₅₀ + Vit C.

Parameter	Control Group	E-Group	V-Group
WBC (10 ⁹ / L)	8.62 ^a ± 0.67	4.62 ^b ± 0.94	5.08 ^b ± 1.04
LYM (10 ⁹ / L)	6.93 ^a ± 0.01	8.97 ^b ± 0.59	8.33 ^c ± 0.21
MID (10 ⁹ / L)	0.68 ^a ± 0.18	0.26 ^b ± 0.02	0.36 ^b ± 0.06
GRA (10 ⁹ / L)	0.55 ^a ± 0.03	0.29 ^b ± 0.05	0.31 ^b ± 0.04

Mean values with different subscripts differ significantly (p ≤ 0.05)

Table No. 2: Mean (± SD) values of RBCs and related parameters in different rat groups. Control Group = Rat group fed with only normal diet (no exposure of pesticide or vitamin), E-Group = Rat group fed with LD₅₀, V-Group = Rat group fed with LD₅₀ + Vit C.

Parameter	C-Group	E-Group	V-Group
RBC (10 ¹² / L)	7.63 ^a ± 0.74	5.18 ^b ± 0.64	6.13 ^c ± 0.41
HGB (g/dl)	14.70 ^a ± 0.68	10.70 ^b ± 0.45	11.21 ^b ± 1.09
HCT %	40.84 ^a ± 2.3	30.29 ^b ± 1.52	32.71 ^c ± 1.05
MCV (fl)	52.14 ^a ± 2.62	58.00 ^b ± 2.28	57.54 ^b ± 1.94
MCH (pg)	17.30 ^a ± 1.46	20.60 ^b ± 0.8	19.83 ^b ± 1.17
MCHC (g/dL)	34.13 ^a ± 1.93	35.20 ^a ± 0.71	35.13 ^a ± 0.7
RDWc %	21.55 ^a ± 2.06	15.30 ^b ± 1.22	16.67 ^b ± 2.77

Mean values with different subscripts differ significantly ($p \leq 0.05$)

Table No. 3: Mean ($\pm$ SD) values of Platelets and related parameters in different rat groups. Control Group = Rat group fed with only normal diet (no exposure of pesticide or vitamin), E-Group = Rat group fed with LD₅₀, V-Group = Rat group fed with LD₅₀ + Vit C.

Parameter	Control Group	E-Group	V-Group
PLT (10 ⁹ / L)	554 ^a ± 5.7	459 ^b ± 3.16	507 ^c ± 5.96
PCT %	0.52 ^a ± 0.03	0.27 ^b ± 0.04	0.30 ^b ± 0.03
MPV (fl)	5.80 ^a ± 0.68	6.60 ^a ± 1.09	6.40 ^a ± 0.11
PDWc (10 ⁹ / L)	33.20 ^a ± 1.29	35.20 ^b ± 0.79	34.50 ^{ab} ± 0.84
PLCR %	9.03 ^a ± 0.44	21.17 ^b ± 0.7	20.58 ^b ± 0.96

Mean values with different subscripts differ significantly ($p \leq 0.05$)

Table No. 4: Mean ($\pm$ SD) values of Liver Function Test (LFT) in different rat groups. Control Group = Rat group fed with only normal diet (no exposure of pesticide or vitamin), E-Group = Rat group fed with LD₅₀, V-Group = Rat group fed with LD₅₀ + Vit C.

Parameter	Control Group	E-Group	V-Group
ALP (U/L)	155 ^a ± 4.00	316.8 ^b ± 1.79	275.8 ^c ± 2.59
ALT (U/L)	21.4 ^a ± 2.51	47.8 ^b ± 3.11	43.6 ^c ± 3.05
AST (U/L)	107.4 ^a ± 2.70	167.2 ^b ± 2.17	153.8 ^c ± 3.27
Bilirubin Total (mg / dL)	0.42 ^a ± 0.08	1.88 ^b ± 0.18	1.44 ^c ± 0.05

Mean values with different subscripts differ significantly ($p \leq 0.05$)

Table No. 5: Mean ($\pm$ SD) values of Renal Function Test (RFT) in different rat groups. Control Group = Rat group fed with only normal diet (no exposure of pesticide or vitamin), E-Group = Rat group fed with LD₅₀, V-Group = Rat group fed with LD₅₀ + Vit C.

Parameter	Control Group	E-Group	V-Group
Serum Creatinine (mg / dL)	0.34 ^a ± 0.03	1.11 ^b ± 0.03	0.98 ^c ± 0.04
BUN (mg / dL)	15.4 ^a ± 1.14	28 ^b ± 2.74	26 ^b ± 3.74

Mean values with different subscripts differ significantly ($p \leq 0.05$)

Table No. 6: Weight / Length Index (mg/mm) of left tibia of different rat groups. Control Group = Rat group fed with only normal diet (no exposure of pesticide or vitamin), E-Group = Rat group fed with LD₅₀, V-Group = Rat group fed with LD₅₀ + Vit C.

Parameter	Control Group	E-Group	V-Group
W/L Index (mg/mm)	20.63	16.83	17.13

Table No. 7: Robusticity Index (mm/mg^{1/3}) of left tibia of different rat groups. Control Group = Rat group fed with only normal diet (no exposure of pesticide or vitamin), E-Group = Rat group fed with LD₅₀, V-Group = Rat group fed with LD₅₀ + Vit C.

Parameter	Control Group	E-Group	V-Group
Robusticity Index (mm/mg ^{1/3})	2.229	2.295	2.285

Figures

Figure 1

Comparative analysis of WBCs and its types in different rat groups. E1 = Rat group fed with LD10, E2 = Rat group fed with LD25, E3 = Rat group fed with LD50, V1 = Rat group fed with LD10 + Vit C, V2 = Rat group fed with LD25 + Vit C, V3 = Rat group fed with LD50 + Vit C.

Comparative Analysis of RBCs and Related Parameters

Figure 2

Comparative analysis of RBCs and related parameters in different rat groups. E1 = Rat group fed with LD10, E2 = Rat group fed with LD25, E3 = Rat group fed with LD50, V1 = Rat group fed with LD10 + Vit C, V2 = Rat group fed with LD25 + Vit C, V3 = Rat group fed with LD50 + Vit C.

Comparative Analysis of Platelets and Related Parameters

Figure 3

Comparative analysis of Platelets and related parameters in different rat groups. E1 = Rat group fed with LD10, E2 = Rat group fed with LD25, E3 = Rat group fed with LD50, V1 = Rat group fed with LD10 + Vit C, V2 = Rat group fed with LD25 + Vit C, V3 = Rat group fed with LD50 + Vit C.

Figure 4

Comparative analysis of Liver Function Test (LFT) in different rat groups. E1 = Rat group fed with LD10, E2 = Rat group fed with LD25, E3 = Rat group fed with LD50, V1 = Rat group fed with LD10 + Vit C, V2 = Rat group fed with LD25 + Vit C, V3 = Rat group fed with LD50 + Vit C.

Comparative Analysis of RFT in Different Rat Groups

Figure 5

Comparative analysis of Renal Function Test (RFT) in different rat groups. E1 = Rat group fed with LD10, E2 = Rat group fed with LD25, E3 = Rat group fed with LD50, V1 = Rat group fed with LD10 + Vit C, V2 = Rat group fed with LD25 + Vit C, V3 = Rat group fed with LD50 + Vit C.

A = Normal Liver of Control (40X)

B = Liver of Experiment Group (40X)

C = Liver of Vitamin Group (40X)

Figure 6

Liver Histopathology of different rat groups.

D = Normal Kidney of Control (10X)

E = Kidney of Experiment Group (40X)

F = Kidney of Vitamin Group (40X)

Figure 7

Renal Histopathology of different rat groups.

F = Normal Heart of Control (10X)

G = Heart of Experiment Group (40X)

H = Heart of Vitamin Group (40X)

Figure 8

Histopathology of heart of different rat groups.