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Research Article

In vivo studies: multi-disciplinary action of *Digitalis purpurea* Linn. extract in rabbits

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ABSTRACT

In continuation to our studies on *Digitalis purpurea* Linn., now we report that on oral introduction of its extract in low concentration (25mg/ml) for 90 days in rabbits (male & female), most of the blood parameters were found normal except platelet count (elevated; 508.5 ± 0.836 in comparison to control group). In kidney function test uric acid (0.063 ± 0.007) and globulin (2.605 ± 0.0083) levels were declined while others were raised in the male test group whereas only globulin (5.87 ± 0.01) level was found elevated in female treated group and the rests were at the lower side. In cardiac enzymes evaluation, CPK level was elevated in both genders (male = 630.5 ± 0.836 ; female = 927.5 ± 0.836) whereas variations in other enzymes were also observed. In both genders, HDL level was found raised (male = 21.167 ± 1.036 ; female = 14 ± 0.632) while in other lipid profile parameters, variation was found between both genders. SGPT was found raised in both genders (male = 191.5 ± 0.836 ; female = 83.5 ± 0.836) whereas variation was observed in rest of liver enzymes results of both genders. Histo-pathology results at a low doses of *D. purpurea* extract for a period of 90 days are completely in accordance of blood parameters. No effects were found on heart, stomach, liver and kidney tissues in comparison to control group. Furthermore, in CCl₄ toxicity induced test this drug showed hepatoprotective action. From our previous and present investigations, it is concluded that the drugs have anthelmintic, insecticidal, molluscicidal, antioxidant, BP stabilizing, diuretic, anti-urolithic, analgesic, anti-inflammatory and hepatoprotective properties and may be utilized for the preparation of different medicines.

Keywords: Blood biochemistry; CCl₄ toxicity; *Digitalis purpurea*; haematology; histopathology.

INTRODUCTION

Digitalis purpurea belongs to the family Scrophulariaceae and are distributed in Europe, Western Asia and the Mediterranean region (Mehlika et al. 2009). *D. purpurea* is a valuable drug in treatment of irritable heart along with palpitation due to overwork, heart strain, arrhythmia, moderate degrees of ventricular dilatation and cardiac asthenia (Bhowmik et al. 2010).

For the last ten years our group is working on this plant to explore its hidden medicinal properties now we are reporting it's some *in vivo* effects on rabbits' kidney, stomach, heart and liver. This plant is rich in cardiac and steroidal glycosides, volatile oil, fatty matter, starch, sugar, gum (Nesher et al. 2007; Lungeanu et al.

1963), mineral content (Boron, Chromium, Manganese, Cobalt, Nickel, Copper, Arsenic and Lead (Negi et al. 2012).

MATERIAL AND METHODS

Plants collection

D. purpurea mother tincture (Willmer Schwabe, Germany; Lot no. 2010207) was purchased from homeopathic drug suppliers. The extract obtained was stored in cool, dry place for further studies.

Chemicals & Reagents

All the chemicals and reagents used were of analytical grade and purchased from Merck (Germany).

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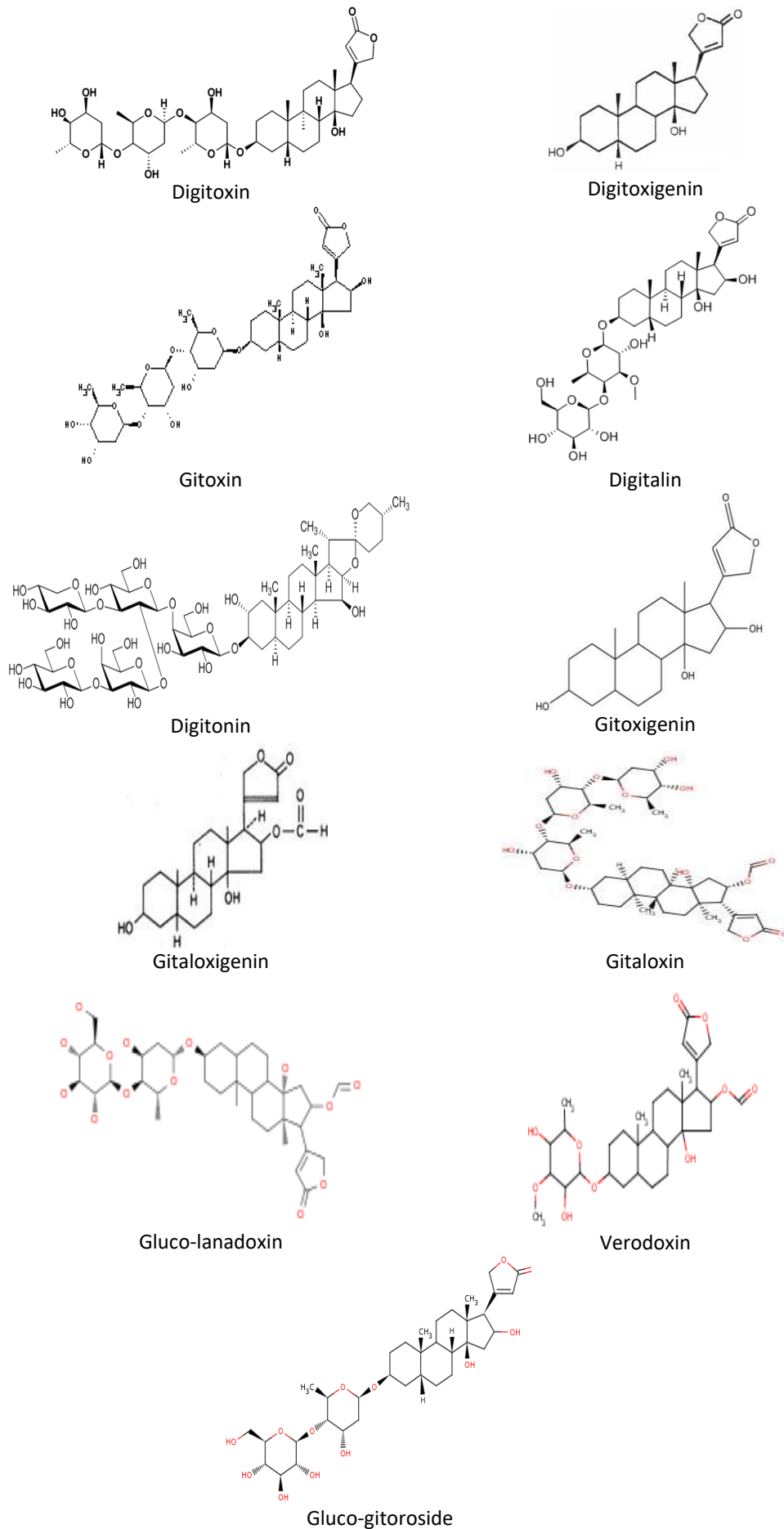


Figure 1: Cardiac glycosides present in *Digitalis purpurea* Linn

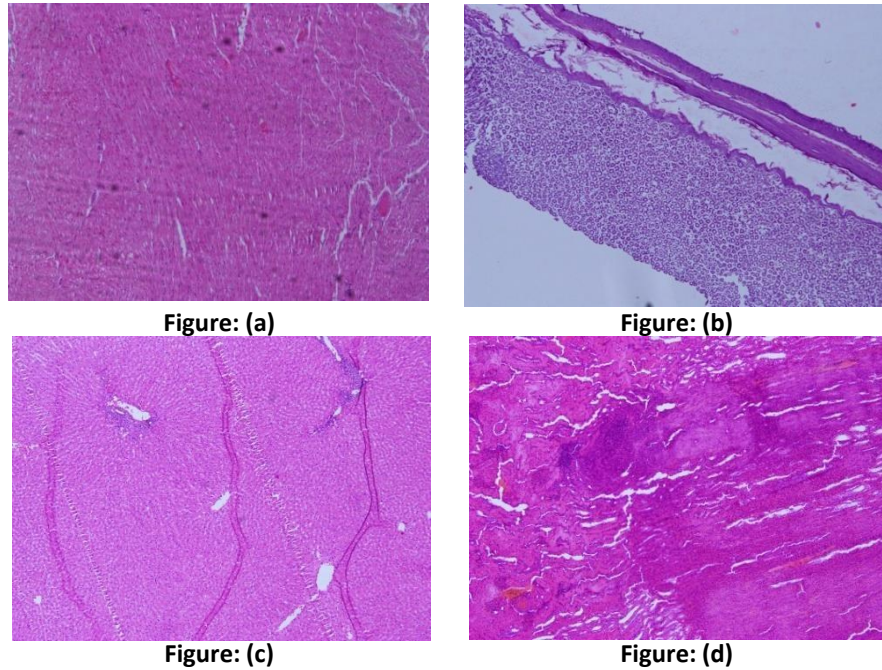


Figure 2: a, b, c, d Shows the Histo-pathology of Group II treated with *D. Purpurea* extract for 90 days

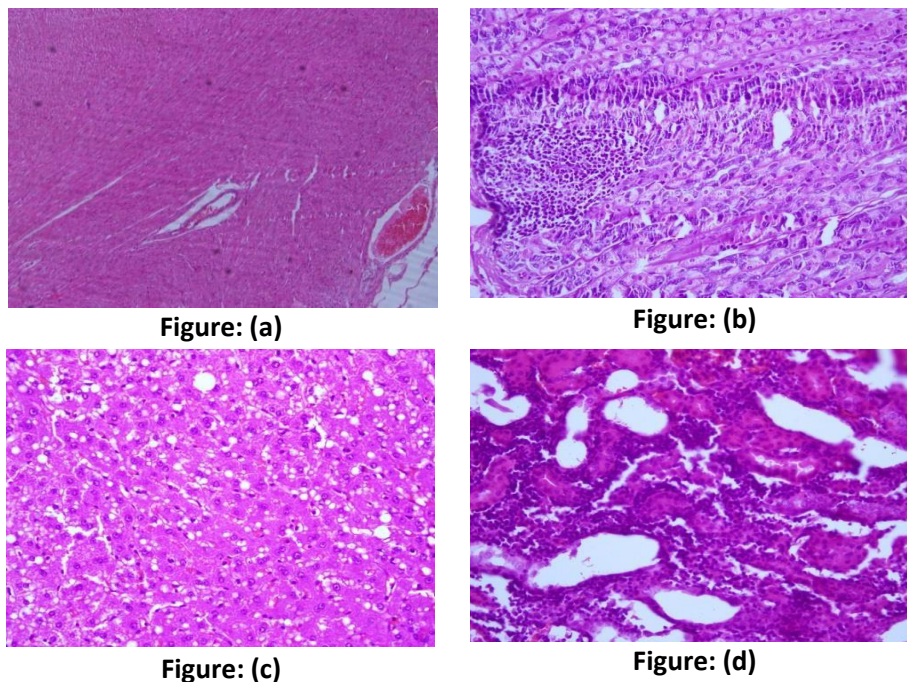


Figure 3: a, b, c, d Shows histo-pathology of group IV treated with *D. purpurea* extract for 90 days, then injected CCl₄ six hours prior to dissection

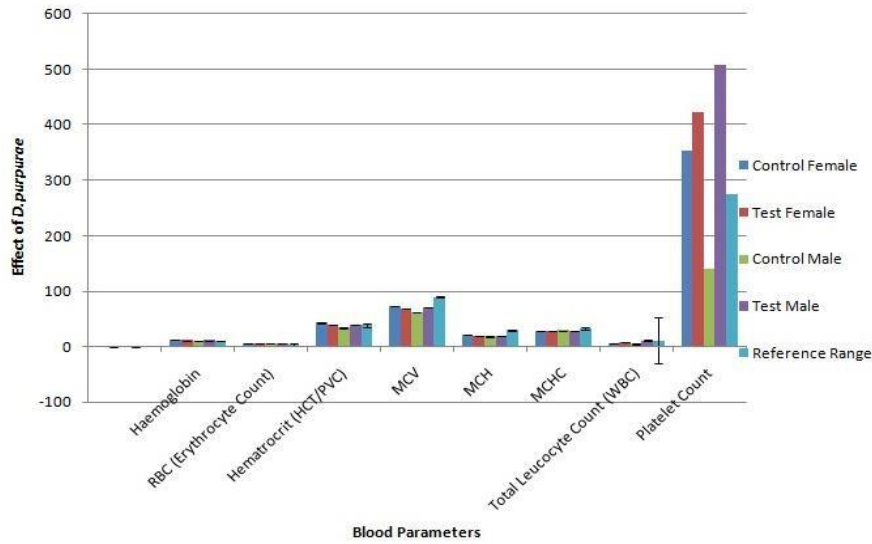
Experimental Animals

The rabbits of both sexes, having 1.5kg weight were purchased from Animal House of Dow University of Health Sciences (DUHS), Karachi and kept in animal house for a period of 15 days to acclimatize. Male and female rabbits were kept in separate cages and fed with their normal diet and water. Their weights were checked on random basis. The drug was administered at the interval of 24 hours for a period of 3 months. The blood of the rabbits was taken by cardiac puncture at the end of 3 month.

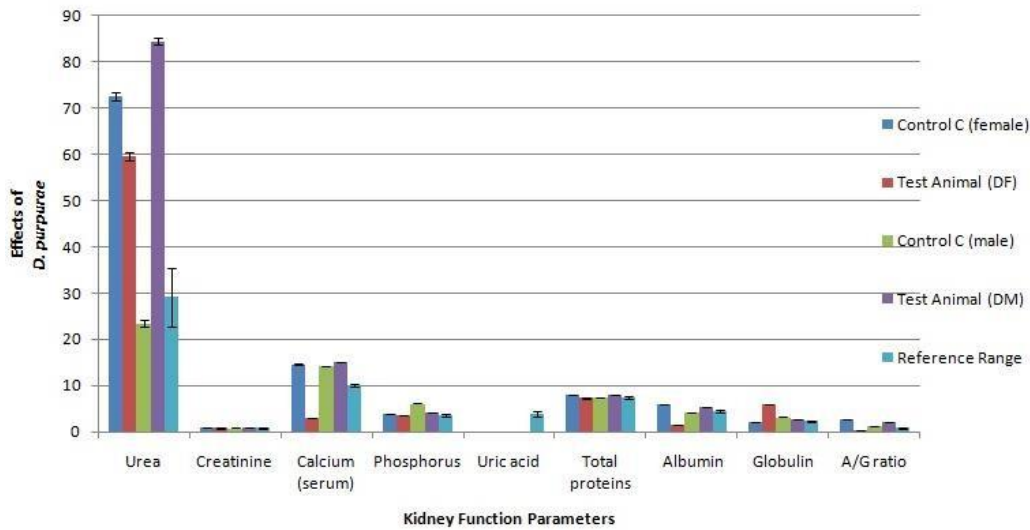
Animal grouping and drug dosing for hematological and biochemical evaluation

Four groups were made (male control – 6 rabbits), (female control – 6 rabbits), (male test (DM) – 6 rabbits) and (female control (DF) – 6 rabbits). Male and female control groups were given distil water, while test groups DM and DF were given 25mg/kg *D. purpurea*. All the administrations were given orally.

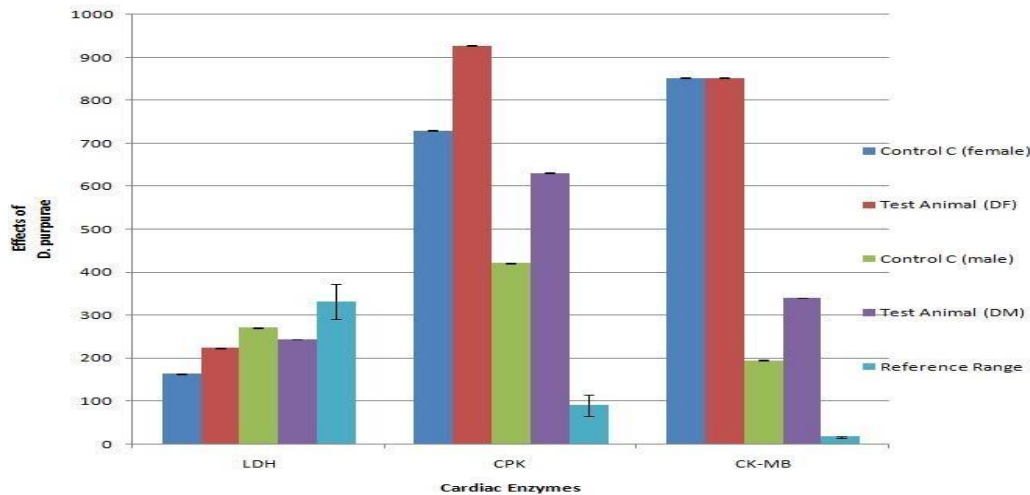
The treatment continued for 90 days. Blood (6 ml) was collected by cardiac puncture with 10 ml sterile syringe



Graph 1: Shows the effect of *D. Purpureae* extract on the blood parameters of rabbit in comparison with the control



DF = Female rabbits treated with *D. purpurea* extract; DM = Male rabbits treated with *D. purpurea* extract
Graph 2: Shows the effect of *D. Purpureae* extract on the Kidney function parameters of rabbit in comparison with the control



DF = Female rabbits treated with *D. purpurea* extract; DM = Male rabbits treated with *D. purpurea* extract
Graph 3: Shows the effect of *D. purpurea* extract on the Cardiac enzymes of rabbit in comparison with the control

Table 1: Shows the effect on Complete Blood Count of Rabbits with and without D. Purpurea extract. A dose of 25 mg/kg was given each day for 1.5 months

Blood Parameter	Control Female	Test Female (DF)	Control Male	Test Male (DM)	Reference Range
Hemoglobin	12.15±0.0836	11.08±0.11	10.05±0.0836	11.1±0.105	10.75±0.689
RBC (Erythrocyte Count)	5.895±0.00836	5.668±0.013	5.485±0.00836	5.525±0.00836	3.916±0.277
Hematocrit (HCT/PVC)	42.835±0.0739	38.95±0.0836	34.2±0.0632	38.75±0.0836	38.67±1.932
MCV	72.416±0.0658	68.8±0.0632	62.5±0.836	69.85±0.0836	89±3.183
MCH	20.835±0.0739	19.25±0.0836	18.15±0.0836	19.95±0.0836	30.167±1.180
MCHC	28.783±0.0658	28.25±0.0836	29.05±0.0836	28.25±0.0836	32.5±0.836
Total Leucocyte Count (WBC)	6.05±0.0836	8.05±0.0836	5.5±0.0632	10.783±0.0658	11±1.673
Platelet Count	353.5±0.836	423.5±0.836	140.5±0.836	508.5±0.836	275±41.83

DF = Female rabbit treated with drug; DM = Male rabbit treated with drug

Table 2: Shows the effect on Kidney Function Parameters of Rabbits with and without D. Purpurea extract. A dose of 25 mg/kg was given each day for 1.5 months

Biochemical Parameters	Control C (female)	Test Animal (DF)	Control C (male)	Test Animal (DM)	Reference Range
Urea	72.5±0.83	59.5±0.836	23.5±0.83	84.5±0.83	29.167±6.39
Creatinine	0.85±0.008	0.78±0.01	0.85±0.0083	0.83±0.01	0.8167±0.127
Calcium (serum)	14.59±0.063	2.895±0.0083	14.17±0.0083	15.03±0.01	10.03±0.318
Phosphorus	3.825±0.068	3.595±0.0083	6.195±0.0083	4.206±0.009	3.53±0.318
Uric acid	0.0175±0.004	0.014±0.0019	0.165±0.0083	0.063±0.007	3.916±0.639
Total proteins	8±0.02	7.27±0.01	7.495±0.0083	7.95±0.016	7.467±0.347
Albumin	5.83±0.013	1.393±0.0096	4.305±0.0083	5.285±0.0083	4.5±0.28
Globulin	2.153±0.0096	5.87±0.01	3.185±0.0083	2.605±0.0083	2.35±0.146
A/G ratio	2.715±0.0083	0.245±0.0083	1.35±0.016	2.035±0.0083	0.75±0.052

DF = Female rabbits treated with drug; DM = Male rabbits treated with drug

Table 3: Shows the effect on Cardiac Enzymes Parameters of Rabbits with and without D. Purpurea extract

Biochemical Parameters	Control C (female)	Test Animal (DF)	Control C (male)	Test Animal (DM)	Reference Range
LDH	163.5±0.836	223.5±0.836	270.5±0.83	243.5±0.836	331.67±40.34
CPK	729.5±0.83	927.5±0.836	421.5±0.83	630.5±0.836	90.33±25.03
CK-MB	852.5±0.83	852.5±0.836	194.5±0.83	340.5±0.836	16.67±2.46

DF = Female rabbits treated with drug; DM = Male rabbits treated with drug

using 1mg/1ml EDTA as anticoagulant for the determination of blood and biochemical parameters.

Animal grouping and drug dosing for histopathological examination

Four groups were made namely group I (positive control), group II (male test group without CCl₄), and group III (Negative control) and group IV (male test group with CCl₄)

Group 1 (Positive control): Six animals were kept as male positive control. Water and food was provided to the animals during the whole period of experiment.

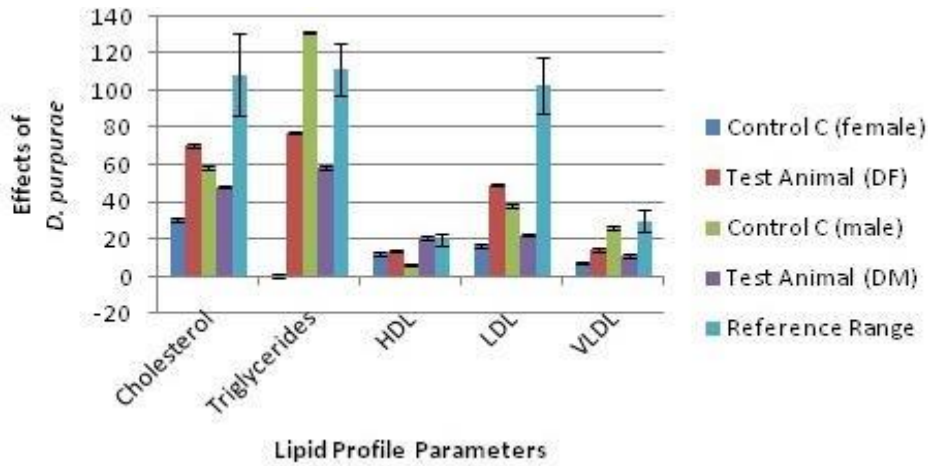
Group 2 (without CCl₄ group - male test group): Six animals were administered 0.025gm of Test drug ex-

tract, water and food was provided to the animals during the whole period of experiment.

Group 3 (Negative control): Six animals were kept as male negative control. Water and food was provided to the animals during the whole period of experiment.

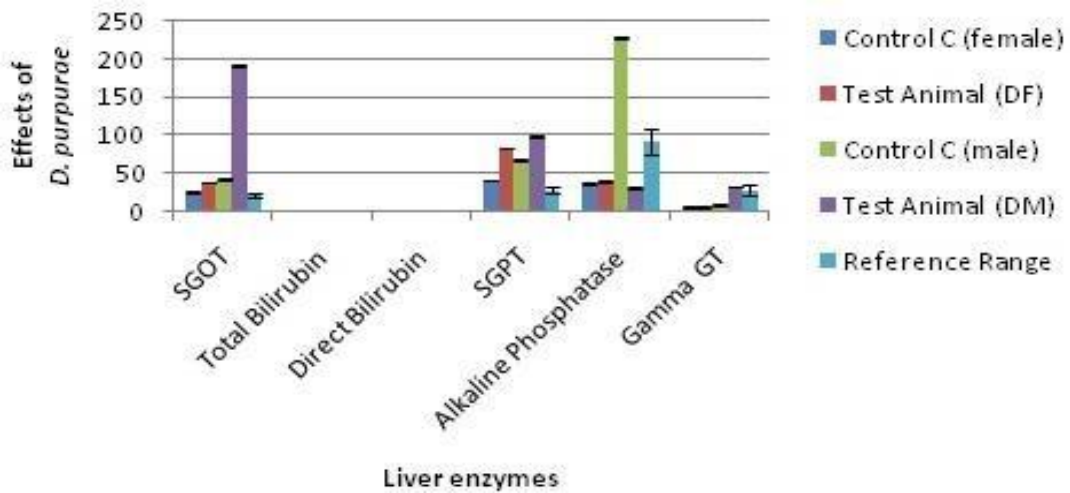
Group 4 (with CCl₄ group - male test group): Six animals were administered 0.025gm of Test drug extract, water and food was provided to the animals during the whole period of experiment.

The animals were sacrificed at the end of 90 days after taking out blood through cardiac puncture technique for the above mentioned tests. Carbon tetrachloride was injected 6 hours before taking blood for carrying out liver function test to group III & IV by cardiac puncture and sacrificing (Lucas *et al.* 2004). Animal studies



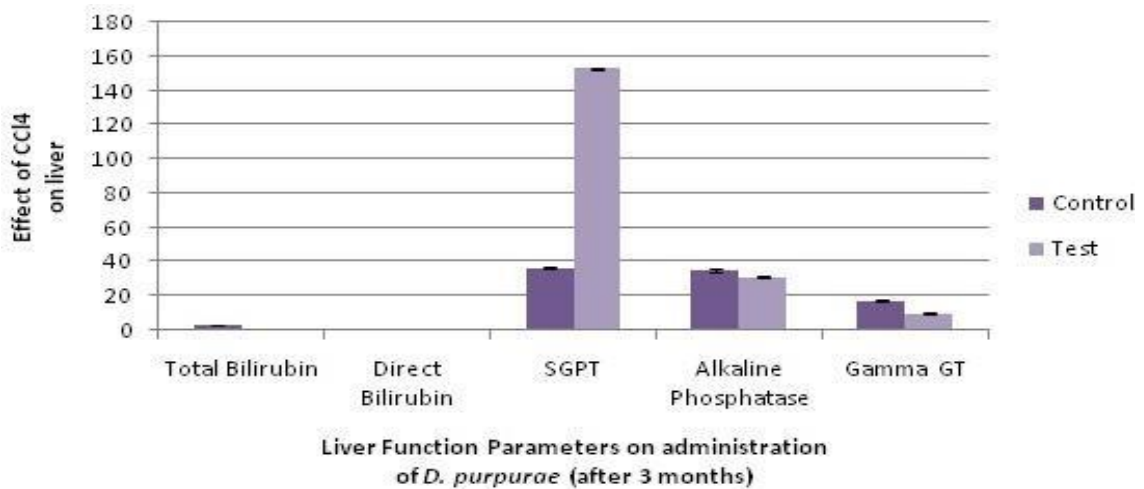
DF = Female rabbits treated with *D. purpurea* extract; DM = Male rabbits treated with *D. purpurea* extract

Graph 4: Shows the effect of *D. purpurea* extract on the Lipid profile parameters of rabbit in comparison with the control



DF = Female rabbits treated with *D. purpurea* extract; DM = Male rabbits treated with *D. purpurea* extract

Graph 5: Shows the effect of *D. purpurea* extract on the Liver enzymes of rabbit in comparison with the control



DF = Female rabbits treated with *D. purpurea* extract; DM = Male rabbits treated with *D. purpurea* extract

Graph 6: Shows the effect of injection of CCl₄ six hours prior to dissection on liver enzymes of Group 4 rabbits that were treated with *D. purpurea* (25mg/day) for 90 days.

were carried out according to Ethical Principles and Guidelines for Experiments on Animals formulated jointly by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences.

Hematological evaluation

Total erythrocyte counts were counted using a Neubauer chamber under a light microscope at 40 x 10 magnifications. Blood samples were diluted to 200 times by Hayem's reagent before counting. Blood hemoglobin concentration was determined using a Sahli's hemometer. Micro Wintrobe hematocrit tubes and hematocrit centrifuge were used to determine the (PCV). Total leucocyte counts were detected using a Neubauer chamber under a light microscope at 10 x 10 magnification after diluting blood samples to 10 times with Turk's solution. Mean erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) for particular blood samples were calculated using hematological data as mentioned by Burnett *et al.* (2006); differentiation of leucocytes was carried out according to IVANOVA 1983 Determination of the relative abundance of all the cell types was carried out by counting total of 200 blood cells (Zorriehzahra *et al.* 2010).

Biochemical evaluation

Serum samples were obtained by centrifugation of blood at 1300xg for 15 min. The Menarini Classic Chemistry Analyzer was used to determine the calcium (Ca), phosphorus (P), blood urea, creatinine, total bilirubin, total protein, albumin, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), cholesterol, glucose, amylase, and gamma-glutamyl transferase (GGT). The globulin concentration was determined by subtracting the albumin Concentration from the total protein concentration (Amadori *et al.* 1997)

Histo-pathological Analysis

The liver, kidney, heart and stomach tissues were dehydrated separately with ethanol of graded concentrations. The tissues were passed through xylene solution to clear the ethanol and facilitate molten paraffin wax infiltration (55°C). After that, they were treated with paraffin wax and cast into blocks sections of 5µm thickness were cut with microtome. These were later placed on clean glass slide.

The sample slides were subsequently stained in haematoxylin-eosin and examined under a light microscope, photomicrographs of the samples were recorded using an Olympus Research Microscope (model BX51) (Aliyu *et al.*, 2007, Bunchaoren *et al.* 2012).

Statistical analysis

Results of the study were presented as a mean plus or minus standard error of mean (Mean ± SEM). Differences between control and treatment

groups were analyzed by student t-test (Snedecor and Cochran 1967).

RESULTS

Moderate increase in hemoglobin (11.1±0.105), RBC count (5.525±0.00836), hematocrit (38.75±0.0836), MCV (69.85±0.0836), MCH (19.95±0.0836) and total leucocyte count (10.783±0.0658) was found. Platelet count (508.5±0.836) was significantly elevated. While MCHC level (28.25±0.0836) was slightly decrease (Table 1, Graph 1)

Slight decline in hemoglobin (11.08±0.11), RBC count (5.668±0.013), hematocrit (38.95±0.0836), MCV (68.8±0.0632), MCH (19.25±0.0836) and MCHC (28.25±0.0836) levels were found in female test group treated with *D. purpurea* in comparison to the respective control female group. However, elevation was observed in white blood cells (8.05±0.0836) and platelet count (423.5±0.836) of the test group as compared to the control group (Table 1, Graph 1).

The levels of urea (84.5±0.83), serum calcium (15.03±0.01), total protein (7.95±0.016), albumin (5.285±0.0083), A/G ratio (2.035±0.0083) were raised while creatinine (0.83±0.01), phosphorus (4.206±0.009), uric acid (0.063±0.007) and globulin (2.605±0.0083) were declined in male test group treated with *D. purpurea* extract in comparison to control male group (Table 2, Graph 2).

Globulin (5.87±0.01) level was slightly raised. While the rest of the kidney function parameters were lowered in female test group treated with *D. purpurea* extract; Urea (59.5±0.836), creatinine (0.78±0.01), serum calcium (2.895±0.0083), phosphorus (3.595±0.0083), uric acid (0.014±0.0019), total protein (7.27±0.01), albumin (1.393±0.0096), A/G ratio (0.245±0.0083) when compared with its respective control group (Table 2; Graph 2) CPK (630.5±0.836) and CK-MB (340.5±0.836) enzymes were raised, while LDH (243.5±0.836) levels were lowered in the test group treated with *D. purpurea* extract in comparison to male rabbit's control group (Table 3, Graph 3).

LDH (223.5±0.836) and CPK (927.5±0.836) levels were elevated while CK-MB (852.5±0.836) enzyme level was lowered in the female test group treated with *D. purpurea* as compared to its female control group (Table 3, Graph 3).

HDL (21.167±1.036) levels were raised while the rest of the lipid profile parameters, Cholesterol (48.5±0.836), Triglycerides (58.5±0.836), LDL (22.5±0.836) VLDL (11.167±1.036) were declined in the male treated group with *D. purpurea* extract in comparison to its respective male control group (Table 4, Graph 4).

Triglycerides (77.5±0.836), HDL (14±0.632), LDL (49.5±0.836), VLDL (14.5±0.836) levels were raised while the cholesterol level was lowered in female

Table 4: Shows the effect on Lipid Profile Parameters of Rabbits with and without *D. Purpurea* extract

Biochemical Parameters	Control C (female)	Test Animal (DF)	Control C (male)	Test Animal (DM)	Reference Range
Cholesterol	30.5±0.83	70.5±0.836	58.5±0.83	48.5±0.836	109.16±22.24
Triglycerides	0.5±0.83	77.5±0.836	131.5±0.83	58.5±0.836	111.67±13.68
HDL	12.5±0.83	14±0.632	6.5±0.83	21.167±1.036	19.67±3.18
LDL	16.5±0.83	49.5±0.836	38.5±0.83	22.5±0.836	103.33±15.14
VLDL	7.5±0.83	14.5±0.836	26.5±0.83	11.167±1.036	30±5.83

DF = Female rabbits treated with drug; DM = Male rabbits treated with drug

Table 5: Shows the effect on Liver Enzymes Parameters of Rabbits with and without *D. Purpurea* extract. A dose of 25 mg/kg was given each day for 1.5 months

Biochemical Parameters	Control C (female)	Test Animal (DF)	Control C (male)	Test Animal (DM)	Reference Range
SGOT	26.5±0.83	38±0.632	42.5±0.83	191.5±0.836	21.83±3.11
Total Bilirubin	0.275±0.0083	0.225±0.00836	0.265±0.0083	0.28±0.0063	1.75±0.083
Direct Bilirubin	0.021±0.005	0.035±0.0083	0.041±0.0065	0.065±0.0083	0.029±0.0008
SGPT	41.5±0.83	83.5±0.836	68.5±0.83	98.5±0.836	27.5±4.18
Alkaline Phosphatase	37.5±0.83	40.5±0.836	228.5±0.83	31.5±0.836	91.67±17.30
Gamma GT	6.5±0.83	6.5±0.836	9.5±0.83	33±0.632	29.16±6.39

DF = Female rabbits treated with drug; DM = Male rabbits treated with drug

Table 6: Shows the effects on histological specimens of male rabbits with and without *D. Purpurea* extract

Specimen	Control Male (C - male)	Test Male DM
Heart	No significant pathology is seen.	No significant pathology is seen.
Stomach	No significant pathology is seen.	No significant pathology is seen.
Liver	Mild portal inflammation and periportal fibrosis.	Mild portal inflammation and periportal fibrosis.
Kidney	Chronic nonspecific pyelonephritis.	Chronic nonspecific pyelonephritis.

Table 7: Shows the effect on Carbon tetrachloride on Liver Enzymes of Rabbits with and without *D. Purpurea* extract

Liver Function Test Parameters	Control (M±SEM)	Test (M±SEM)
Total Bilirubin	2.88±0.007	0.08±0.0063
Direct Bilirubin	0.12±0.0063	0.02±0.0056
SGPT	36.5±0.836	153±0.632
Alkaline Phosphatase	35±0.632	31±0.632
Gamma GT	17±0.632	10±0.632

treated group with *D. purpurea* extract in comparison to control female rabbits group (Table 4, Graph 4).

All the liver enzymes, SGOT (191.5±0.836), Total Bilirubin (0.28±0.0063), Direct Bilirubin (0.065±0.0083), SGPT (98.5±0.836), Gamma GT (33±0.632) were raised, while alkaline phosphatase (31.5±0.836) level was lowered in the male treated group with *D. purpurea* extract when compared to its respective male control group (Table 5 Graph 5).

There was slight decline in total bilirubin level (0.225±0.00836). However, SGOT (38±0.632), direct bilirubin (0.035±0.0083), SGPT (83.5±0.836) and alkaline phosphatase (40.5±0.836) parameters were found

raised in *D. purpurea* extract treated female test group when compared with its female control group (Table 5, Graph 5).

In male group treated with *D. purpurea* extract, no substantial pathology was found in heart and stomach tissues. Mild portal inflammation and peri-portal fibrosis was observed in liver tissues whereas chronic nonspecific pyelonephritis was seen in kidney tissues (Table 6, Figure1).

Group 4 treated with *D. purpurea* extract for three months were administered carbon tetrachloride 1.5ml before taking out blood via cardiac puncture for LFT. Only SGPT level was found significantly raised (153±0.632). While rest of the liver enzymes; total bi-

Table 8: Shows the effects on histological specimens of female rabbits with and without *D. Purpurea* extract. a dose of 25 mg/kg was given each day for three months. Carbon tetrachloride was given IM prior to dissection

Specimen	Control female (C – female)	Test Female DF
Heart	No significant pathology is seen.	No significant pathology is seen.
Stomach	No significant pathology is seen.	Chronic nonspecific gastritis. No H Pylori.
Liver	No significant pathology is seen.	Mild portal inflammation and peri-portal fibrosis with focal steatosis and centri-lobular hepatocytic degeneration.
Kidney	No significant pathology is seen.	Moderate ATN (acute tubular necrosis) and mild tubule-interstitial nephritis. No evidence of granuloma or malignancy is seen

DF= female rabbits treated with *D. Purpurea*

lirubin (0.08 ± 0.0063), direct bilirubin (0.02 ± 0.0056), alkaline phosphatase (31 ± 0.632) and gamma GT (10 ± 0.632) were found lowered as compared to the control group (Table 7, Graph 6).

Histo-pathology of group IV treated with *D. purpurea* extract for 90 days, then injected CCl₄ six hours prior to dissection

a) Microscopic Examination of Heart

Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined.

b) Microscopic Examination of Stomach

Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Foci of lymphocytic infiltrate are seen at places forming lymphoid aggregates. Underlying sub mucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No *H. Pylori*. No evidence of metaplasia or dysplasia is seen.

c) Microscopic Examination of Liver

Sections show liver tissue with overall preserved lobular architecture. Portal tracts are mildly dilated with lymphocytic infiltrate and minimal fibrosis. Foci or macrovesicular steatosis seen Centrilobular hepatocytic degeneration also noted, No siderosis, No cholestasis, No evidence of granuloma or malignancy is seen.

d) Microscopic Examination of Kidney

Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Moderate degree of acute tubular necrosis and mild tubulointerstitial nephritis are seen. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen in any of the sections examined.

The Histo-pathology of Group II treated with *D. purpurea* extract for 90 days

a) Microscopic Examination of Heart

Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined.

b) Microscopic Examination of Stomach

Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined.

c) Microscopic Examination of Liver

Sections show liver tissue with overall preserved lobular architecture. Portal tracts are mildly expanded with lymphocytic infiltrate and minimal fibrosis. No significant lobular inflammation seen. No siderosis No cholestasis, No evidence of granuloma or malignancy is seen.

d) Microscopic Examination of Kidney

Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Lymphocytic infiltrate is seen in the tubule-interstitial compartment. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen in any of the sections examined.

No substantial pathology was observed in heart tissues of the group 4 treated with *D. purpurea*. Chronic nonspecific gastritis was found in stomach tissues. In liver tissues, mild portal inflammation and peri-portal fibrosis with focal steatosis and centri-lobular hepatocytic degeneration was seen. Whereas, in kidney tis-

sues, moderate ATN (acute tubular necrosis) and mild tubule-interstitial nephritis (Table 8, Figure 2).

DISCUSSION

As we have already mentioned that this research work is a part of our detailed studies on

D. Purpurea. This plant has potent anthelmintic, molluscicidal, insecticidal and anti-oxidant activity (Ahmad et al. 2013 a, b; Ahmad et al. 2014). Here we are reporting the *in vivo* studies on blood parameters, kidney function parameters, cardiac enzymes and histopathology of the sensitive organs to a drug for example liver, stomach, kidney etc.

It is a well-known drug of cardiac failure and is also reported as cardio tonic (promotes and stimulates the activity of heart muscle tissues), improves blood flow to the kidneys and aids in removing any obstructions there thus improving urination (Lindholm et al. 2002). Side by side it is poisonous and fatal in high doses due to the presence of cardiac glycosides especially digoxin and digitalin (Figure 3) (McGuffin et al. 1997). Now referencing to our histopathology results they support it in this regard but at low doses (Figure 1 & 2).

In case of blood parameters, no significant changes in blood biochemistry and histopathology were found. Our results showed no malignancy in the tissues of heart, liver, kidney and stomach upon treated with 25 mg of *D. Purpureae* for 90 days alone and in the group treated with *D. Purpureae* (25mg) for 90 days and then administered CCl₄ six hours prior to dissection. Negligible cell degenerative changes were observed in the liver and kidney tissues of rabbit as compare to high doses. *D. Purpureae* treated group injected CCl₄ preserved the cellular architecture that shows its protective property from damaging (Rabadia et al. 2014).

From our results it can be concluded that *D. Purpurea* is a multidisciplinary action drug for example it balances the blood parameter, provides protection properties to kidney, stomach and liver, and maintains the cardiac enzymes. It also causes toxicity in high or over doses.

REFERENCES

Ahmad M, Farah-Saeed, Mehjabeen, Noor-Jahan. 2013a. Anthelmintic and molluscicidal activities of some medicinal plants. *Int J Adv in Pharma Res.* 4(7); 1984-1994.

Ahmad M, Farah-Saeed, Mehjabeen, Noor-Jahan. 2013b. Evaluation of Insecticidal and Anti-oxidant activity of selected medicinal plants. *Journal of Pharmacognosy and Phytochemistry.* 2(3); 146-148.

Ahmad M, Farah-Saeed, Mehjabeen, Sherwani SK, Noor-Jahan. 2014. Evaluation of anti-microbial potential of some medicinal plants. *Int J Phytomedicine.* 6; 115-121.

Aliyu R, Adebayo AH, Gatsing D, Garba IH. The effects of ethanolic leaf extract of *Commiphora Africana*

(Burseraceae) on rat liver and kidney functions. *J. Pharmacol. Toxicol.* 2007; 2: 373-379.

Amadori M, Archetti IL, Frasnelli M, Bagni M, Olzi E, Caronna G, Lanteri M. An immunological approach to the evaluation of welfare in holsteinfrisian cattle. *J. Vet. Med.* 1997; 44: 321-327.

Benli M, Yiğit N, Geven F, Güney K and Bingöl Ü. 2009. Anti-microbial activity of endemic *Digitalis lamarckii* from Turkey. *Indian J. Exp. Biol.* 47; pp: 218-221.

Bhowmik D, Chiranjib, Kumar KPS. 2010. Traditional herbal drugs: digitalis and its health benefits. *Int J Pharm Biomed Sci.* 1(1); pp: 16-19.

Buncharoen W, Saenphet S, Chomdej S. Evaluation of biochemical, hematological and histopathological parameters of albino rats treated with *Stemonaaphylla* Craib. extract. *J Med Plants Research.* 2012.6(27): 4429-4435.

Burnett N, Mathura K, Metivier KS, Holder RB, Brown G, Campbell M. An investigation into hematological and serum chemistry parameters of rabbits in Trinidad. *World Rabbit Sci.* 2006; 14: 175 – 187.

Frohne D, Pfänder HJ. Poisonous plants, 2.edn, London: Manson Publishing Ltd., 2004.

Hiermann A, Kartnig T, Seligmann O, Wagner H. 1977. Flavonoids in the leaves of *Digitalis lanata*. *Planta Med.* 32 (1); pp: 24-26.

Ivanova NT. Atlas of fish blood cells. *Moskva, izd. Legkajai piščevajapromyšlennost.* 1983: 75. (In Russian)

Kelly RA, Smith TW. Pharmacological treatment of heart failure. In: *Goodman & Gilman's The Pharmacological Basis of Therapeutics.* 9th ed. New York, NY: McGraw-Hill; 1996:809–838.

Lindholm P, Gullbo J, Claeson P. 2002. Selective cytotoxicity evaluation in anticancer drug screening of fractionated plant extracts. *J Biomolecular Screening.* 7(4); pp: 333–340.

Lopez-Lazaro M, Palma De La Pena N, Pastor N, Martin-Cordero C, Navaro E, Cortes F, Ayuso MJ, Toro MV. 2003. Anti-tumour activity of *Digitalis purpurea* L spp. heywoodii. *Planta Med.* 69 (8); pp: 701-704.

Lucas RL, Lentz KD, Hale AS. Collection and preparation of blood products. *Clinical Techniques in Small Animal Practice.* 2004; 19(2): 55-62.

Lungeanu I, Calcandi V, Calcandi I. 1963. Kariologische und phytocem ischeunter suchungenuber *Digitalis purpurea* L. ssp. Heywoodii. *Die Naturwissenschaften.* 21; pp: 673-674.

Mc Guffin M, Hobbs C, Upton R, Goldberg A. 1997. *American Herbal Products Association's Botanical Safety Handbook.* Boca Raton, FL: CRC Press.

Mehlika B, Nazife Y, Fatmagü G, Kerim G, Umit B. 2009. Antimicrobial activity of endemic *Digitalis lamarckii* Ivan from Turkey. *Indian J. Exp. Biol.* 47; pp: 218-221.

Navarro E, Alonso PJ, Alonso SJ, Trujillo J, Perez C, Toro MV, Ayuso MJ. 2000. Cardiovascular activity of a

- methanolic extract of *Digitalis purpurea* spp. heywoodii. *J Ethnopharmacol.* 71; pp: 437-442.
- Navarro E, Alonso SJ, Trujillo J, Gomez ME, Ayuso MJ. 1994. Pharmacological effects of glycosides from *Digitalis heywoodii*. *Methods and Findings.* 16 (1); pp: 119.
- Negi JS, Bisht VK, Bhandari AK, Sundriyal. 2012. Determination of mineral contents of *Digitalis purpurea* L. and *Digitalis lanata* Ehrh. *J Soil Sci & Plant Nutr.* 12 (3); pp: 463-469.
- Nesher M, Spolansky U, Rosen H, Lichtstein D. 2007. The endogenous digitalis-like compounds - A new family of steroid hormones. *Life Sci.* 80; pp: 2093.
- Nikolov P, Avramov N, Boiadzhiev TS, Kirrov D. 1962. Some experimental data on the anti-inflammatory action of *Digitalis lanata*. *Nauchni Tr. Vissh. Med. Inst. Sofia.* 41; pp: 1-16.
- Rabadia J, Hirani U, Kardani D, Kaneria A. 2014. Hepato protective activity of aqueous extract of *Digitalis purpurea* in Carbon tetrachloride induced hepatotoxicity in albino rats. *Asian J Biomed & Pharma. Sci.* 4(34); 64-71.
- Reddy BA. 2010. Digitalis therapy in patients with congestive heart failure. *Intl J Pharmaceutical Sci. Rev. & Res.* 3 (2); pp: 90-95.
- Snedecor GW and Cochran WG. *Statistical Methods.* Sixth edition. Ames, Iowa: The Iowa State University Press. 1967; p: 423.
- Zorriehzahra MJ, Hassan MD, Gholizadeh M and Saidi AA. 2010. Study of some hematological and biochemical parameters of Rainbow trout (*Oncorhynchus mykiss*) fry in western part of Mazandaran province, Iran. *Iranian J Fisheries Sciences.* 9 (1); pp: 185-198.