Effects of *Dryopteris filix-mass* roots on biochemical parameters

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Abstract

Dryopteris filix-mas (L.), is an important member of the family Dryopteridaceae and used for different purposes such as in the treatment of rheumatoid arthritis, malaria, inflammation, worm infestation, uterine bleeding, internal hemorrhage, fever, carbuncles, mumps and sores in traditional medicine. Keeping in views its medicinal benefits along with toxic effects, the extract of *Dryopteris filix-mass* (L) was administered in present study in rabbits to evaluate its effects on different biomarkers. Animals were randomly selected to assign in any of three groups (10 animals per group) as G1 (control maintained on normal saline 0.5ml), G2 (low dose of 1mg) and G3 (high dose of 5mg). Animals received drugs orally for 90 days and after that blood was collected according to the protocol. The results indicates that *Dryopteris filix-mass* increased RBC (10.7±1.2), MCH (20.1), WBC (10×10^9±0.37) and Platelet count (426×10) and decreased creatinine (0.79) with significant difference ($p \leq 0.05$) at high dose of 5mg/kg but negligible or no effect on Hematocrit, MCV, MCHC, HBAIC, PT, INT, APTT, Random blood glucose levels and urea. As compare to this, lower dose of 1mg/kg produced slight or no change on these biochemical parameters. Increased in cholesterol HDL ratio, cholesterol , triglycerides, HDL, LDL and VLDL was observed from 3.18±0.08, 19±2.7, 110±3.7, 6±0.07, 12±0.07 and 22±1.5 to 12.5±1.1, 88±9, 404±13, 7±0.6, 28±0.6 and 80±14, respectively at high dose. *Dryopteris filix-mass* is helpful to improve red blood cells and Platelet production and in hyperthyroidism but it should be used with cautions in patients with cardiac arrest, high lipid profile and compromised hepatic and renal function at high doses.

Key words; *Dryopteris filix-mass*, biochemical parameters, medicinal use, lipid profile

Introduction

Blood sample analysis is one of the most commonly employed method to get thorough information about blood cells production, oxygen carrying capacity, hemoglobin and body immune response, therefore, helpful in diagnosis of certain pathological conditions such as anemia, infection, immune-deficiencies and also adverse effects of therapeutic agents (George-Gay and Parker, 2003). In preclinical studies, biochemical analysis of animals treated with crude extract of herbal medicine played also very important role in identifying pharmacological effects or developing toxicological profile of newly introduced drugs (Dar *et al.*,2014).

Dryopteris filix-mas (L.), an important member of the family Dryopteridaceae is also used for medicinal purposes. It is commonly called as male fern or wood fern. It is an evergreen fern growing to 1.2m (4 ft) by 1 m (3 ft 3 in) at a medium. Mostly it is found in fertile and moist forests, especially deciduous forest, other than that it is also found in a wide range of other habitats in including open ground and stone/brick walls in towns (Uwumarongie *et al.*,, 2016). The leaves and root is the edible parts of *Dryopteris filix-mas*. The rhizomes can be eaten raw or cooked as part of a course of therapy for losing weight. In addition to this the leaves and rhizome are used in the treatment of rheumatoid arthritis, malaria, inflammation, worm infestation, uterine bleeding, internal hemorrhage, fever, carbuncles, mumps and sores in traditional medicine. To treat dandruffs the leaves, roots and rhizomes are used (Sekendar *et al.*,, 2012, Soare *et al.*,, 2012).

However, its rhizome is reported to contain some toxic compounds which help to expel out tapeworms such as flavvaspidic acid and phloroglucinol. Has been discovered and antioxidant effects of methanolic extract of leaves of wood fern also highlight its cytotoxic effects (Ali *et al* ,2012). Erhirhie and Ilodigwe (2019) evaluate sub-chronic toxicity of leaf extract of *Dryopteris filix*-mass (L) in pre-clinical studies analyzing different biomarkers and histology of liver and kidney. It was concluded that the extract of the leaves may be hepatotoxic and nephrotoxic but these toxicities were reversible (Erhirhie and Ilodigwe, 2019).

Keeping in views its medicinal benefits and certain toxicity risk, the extract of *Dryopteris filix-mass* (L) was administered in present study in albino rats to evaluate its effects on different biomarkers.

Experiment

Materials and methods:

Plant material:

Dryopteris filix-mass (L) roots were collected from Karachi University, Karachi, Pakistan, in the month of September-October. *Dryopteris chrysocoma* (root) (herbarium number, 001006-09).

Extraction procedure:

The fresh (or dry) plant materials were chopped into small pieces then macerated with methanol and kept for 15 days at room temperature for percolation. The methanol extract was then filtered. After filtration once again methanol was added in the remaining material and kept for 15 days at room temperature for further percolation. Later same procedure was repeated for filtration. The methanol extract was evaporated under reduced pressure in a rotary evaporator to obtain a residue. Two residues were combined together and used for experiments (Jahan *et al*., 2015).
Fresh roots of *Dryopteris filix-mass* (L) were purchased from the Botanical garden of Karachi University. The specimen voucher no DR-2016-A/T was deposited to the herbarium department. All the chemicals used in the experiment were of analytical grade. White albino rats with average weight of 1 KG were purchased from animal house of Dow University of health sciences, Karachi, Pakistan.

Methanolic extract of *Dryopteris filix-mass* (L)

Methanolic extract of *Dryopteris filix-mass* (L) was prepared following maceration method explained by Azubike *et al* (2015) in order to get crude extract (Azubike *et al* ,2015). Fresh roots of the plant were washed, cleaned and air dried at room temperature. Methanolic extract of the plant roots was obtained after crushing roots. Filtrate obtained using Whatman's paper no.4. Methanol was allowed to evaporate and the viscous paste was stored at 5^{o}C until subject to use.

Experimental Animals

Animals were randomly selected to assign in any of three groups (ten animals per group) as G1 (control maintained on normal saline 0.5ml), G2 (low dose of 1mg) and G3 (high dose of 5mg). All animals were kept under recommended guidelines of animal care and fed on commercial food (Saleem *et al*, 2018).

Biochemical Studies

Animals were treated according to the decided doses to the corresponding groups. All the groups received drugs orally for 90 days. After the experimental period, rabbit were slaughter and blood was collected and stored at -80^{o}C for the biochemical analysis which includes complete blood count, thyroid test, cardiac enzyme test, lipid profile, electrolyte, liver function test and protein test.

Statistical analysis

Data was subjected for statistical analysis using one way anova. *p* value<0.05 was considered as significant. Values are expressed as mean ±SD.

Results

Present study evaluated the effects of *Dryopteris filix-mass* on different biochemical parameters. This is important to find toxicity of any agent on hematopoietic system (Bashir *et al*., 2015). Table-1 shows that (dosage/conc/) *Dryopteris filix-mass* increased RBC(10.7±1.2), MCH (20.1), WBC (10×10^{9}±0.37) and Platelet count (426×10) and decreased creatinine (0.79) with significant difference ($p \leq 0.05$) at high dose of 5mg/kg except RBCs but negligible or no effect on Hematocrit, MCV, MCHC, HBAIC, PT, INT, APTT, Random blood glucose levels and urea. As compare to this, lower dose of 1mg/kg produced slight or no change on these biochemical parameters.

Table 1: Effects of *Dryopteris filix-mass* on hematopoietic parameters

Biochemical analysis	G1(control normal saline)	G2 (1mg/kg)	G3(5mg/k)	***P* value Control Vs G2**	***P* value Control Vs G3**	**Percent response (%) Control Vs G1**	**Percent response (%) Control Vs G2**
Haemoglobin	9.51±0.41	9.9±0.41	10.7±1.2	0.032	<0.019	4.1	12.5
RBC	4.3±0.79	4.62±0.79	5.1±1.1	0.306	**0.017**	**6.97**	**18.6**
Hematocrit	31.16±1.1	32.1±1.1	34.1±0.9	0.26	0.06	1.58	8.6
MCV	64.3±1.4	64±1.4	66.2±2.4	0.41	0.057	0.46	2.9
MCH	18.7±1.9	18.1±1.9	20.1±1.5	0.13	0.03	3.7	7.48
MCHC	31.21±1.8	31.01±1.8	31.4±1.9	0.63	0.53	0.67	0.608
WBC	7.52×10^{9}±0.1	8.12×10^{9}±0.1	10×10^{9}±1.4	0.32	0.043	8.0	33.2
Platelet count	342×10^{9}±05.5	356×10^{9}±05.5	426×10±1.2	0.046	0.027	4.09	24.56
HbA1C	4.21±0.1	4.11±0.1	4.06±1.4	0.62	0.353	3.06	4.24
PT (control)	14±0.2	15±0.2	14±2.2	0.83	0.00	7.14	0.00
PT Test	6±0.3	6±0.3	7±0.8	0.00	0.12	0.00	16.6
INT	0.44±0.1	0.46±0.1	0.525±0.9	0.13	0.59	4.54	19.31
APTT control	37±3.3	37±2.5	37±1.6	0.00	0.00	0.00	0.00
APTT Test	59±2.5	60±3.6	58±2.7	0.273	0.27	1.69	1.69
Blood glucose (Random)	92±3.1	95±2.5	101±2.8	0.17	0.06	3.26	9.78
Urea	30±2.6	36±1.6	39±1.2	0.064	0.055	20	30
Creatinine	0.88±2.1	0.8±0.6	0.79±0.9	0.87	0.03	9.01	10.22
Calcium	13.28±1.5	14.25±2.4	15.03±1.9	0.12	0.056	7.3	13.17
Uric acid	0.047±0.5	0.048±0.9	0.05±1.4	0.59	0.012	2.12	6.38

Figure 1 shows effects of *Dryopteris filix-mass* on lipid profile. Maximum effects were observed on cholesterol with drug response 363.25% and minimum on HDL with drug response 16.66%. Increased in triglycerides HDL ratio, cholesterol, triglycerides, HDL, LDL and VLDL was observed from 3.18±0.08, 19±2.7, 110±3.7, 6±0.07, 12±0.07 and 22±1.5 to 12.5±1.1, 88±9, 404±13, 7±0.6, 28±0.6 and 80±14, respectively at high dose. Insignificant effects were observed at low dose.

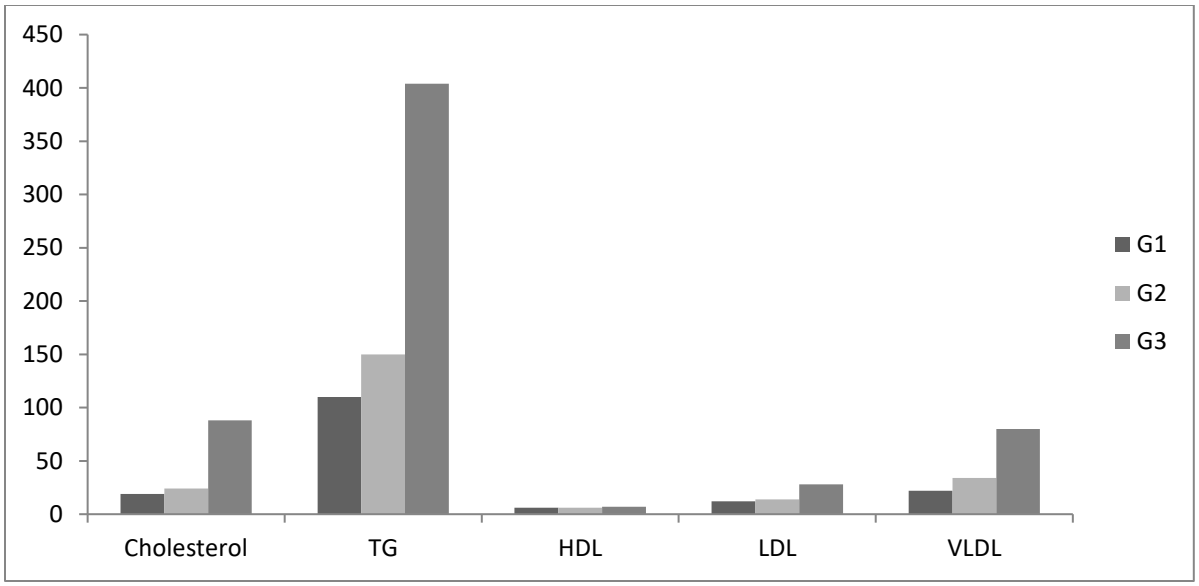


Fig-1: Effects of *Dryopteris filix-mass* on lipid profile

Figure-2 shows effects of *Dryopteris filix-mass* on electrolytes in blood. Maximum effects was observed on bicarbonates with drug response 7.69% and minimum on chlorides with drug response 1.94%. Negligible decrease in sodium (from146±1.22 to 141±14) and chlorides (from103±13 to 101±11) while slight increase in potassium (from4.28±1.22 to 4.6±1.4) and bicarbonates (from2 6±1.02 to 24±9) at $p>0.05$ was observed respectively at high dose. Insignificant effects were observed at low dose.

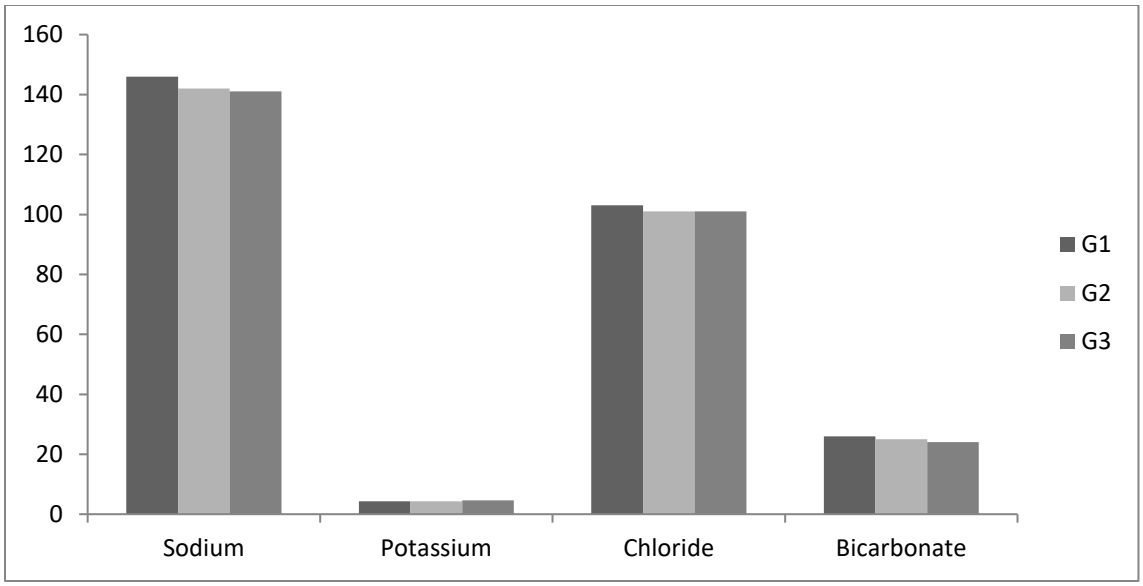


Fig-2: Effects of *Dryopteris filix-mass* on electrolytes

Figure 3 shows effects of *Dryopteris filix-mass* on protein. Maximum effects were observed on A/G ratio with drug response only 10.73% and minimum on total protein with drug response 0.8%. Negligible decreased total protein (9.69±1.2 to 9.61±0.10) and globulin levels (3.5±0.02 to 3.25±0.04) were observed. Significant ($p<0.05$) increased A/G ratio (1.77±0.01 to 1.96±0.03), and albumin (6.19±0.012to 6.36±0.11) was also observed at high dose. Insignificant effects were observed at low dose.

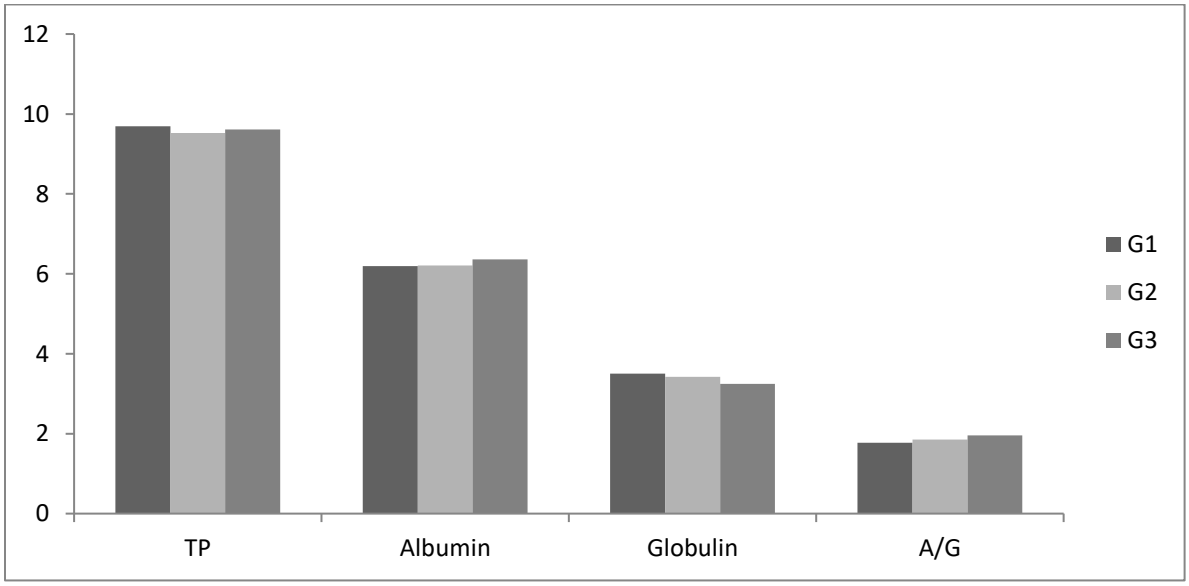


Fig-3: Effects of *Dryopteris filix-mass* on protein

Figure-4 shows effects of *Dryopteris filix-mass* on liver function test. Maximum effects was observed on alkaline phosphate with drug response 100% and minimum on GGT with drug response 15%. Negligible decreased GGT (13±0.2 to 11±0.10) and significant ($p<0.05$). decreased direct bilirubin levels (0.05±0.00 to 0.03±0.00) were observed. Significant ($p<0.05$) increased total bilirubin (0.79±0.01 to 0.8±0.03), SGPT (55±0.70 to 66±0.08) and alkaline phosphate (36±0.08 to 252±10) was also observed at high dose. Insignificant effects were observed at low dose.

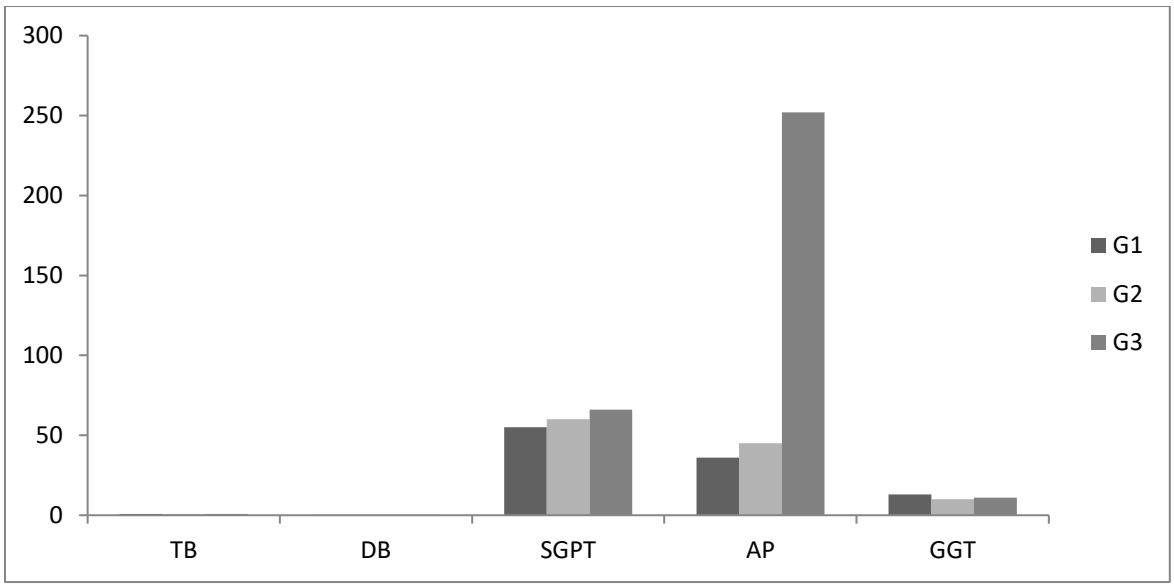


Fig-4: Effects of *Dryopteris filix-mass* on liver function test

Figure-5 shows effects of *Dryopteris filix-mass* on cardiacymes.

Maximum effect was observed on CPK with drug response 132.69% and minimum on LDH with drug response 2.07%. Negligible decreased in LDH (385±22 to 377±12) was observed but significant ($p>0.05$) increased in CPK (from803±23 to 1463±21) and decreased in CK-MB (1168.8±18 to 502±1.4) was observed respectively at high dose. Insignificant effects were observed at low dose.

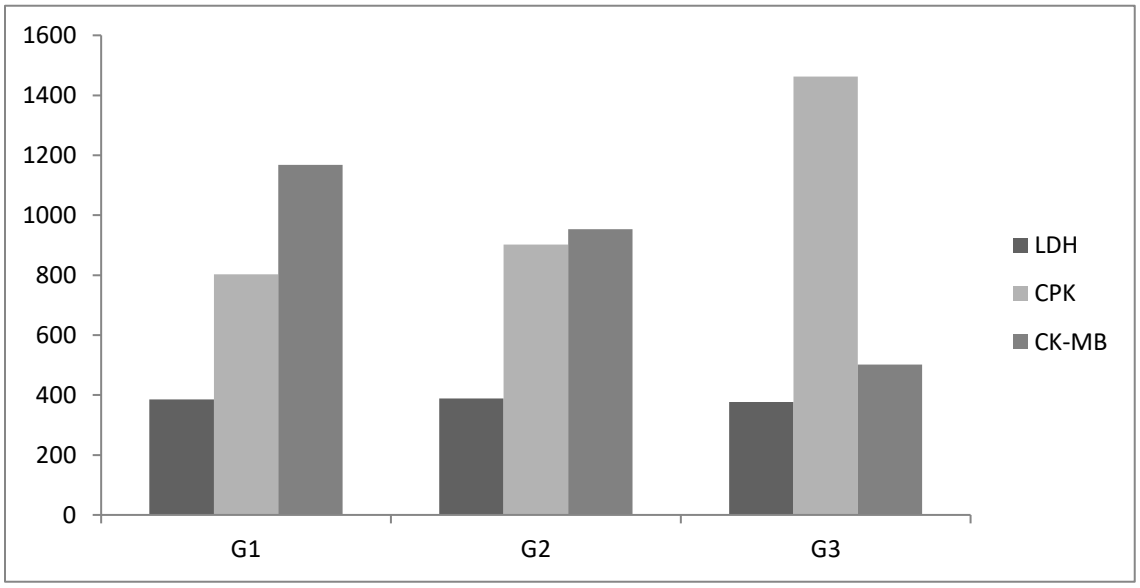


Fig-5: Effects of *Dryopteris filix-mass* on cardiac enzymes

Figure-6 shows effects of *Dryopteris filix-mass* on thyroid hormones. Maximum effect was observed on TSH with drug response 37.5% and minimum on LH with drug response 0.99%. Negligible decreased in FSH (0.104±0.2 to <0.100±0.10) and LH (0.101±0.21 to <0.100±0.11) while increased T3 (0.636±0.02 to 0.697±0.10) was observed ($p>0.05$). Significant ($p<0.05$) decreased T4 (3.11±0.0 to 2.6±0.03) and TSH (0.008±0.02 to 0.005±0.10) was also observed at high dose whereas Insignificant effects were observed at low dose.

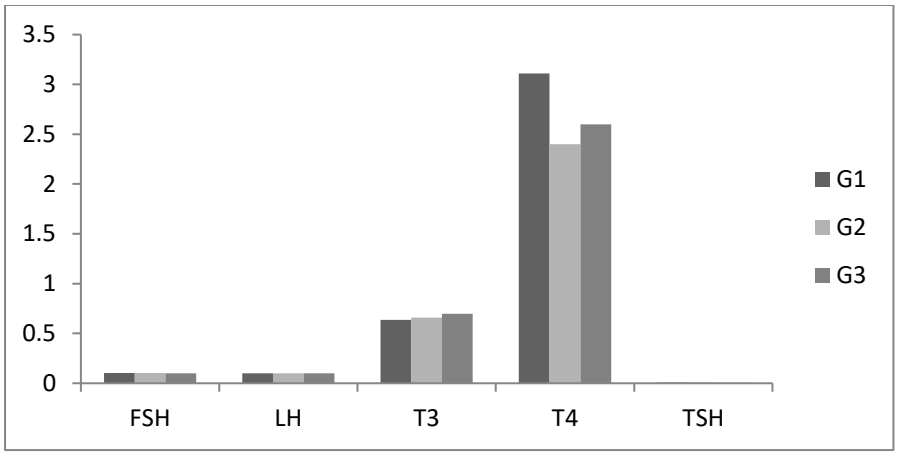
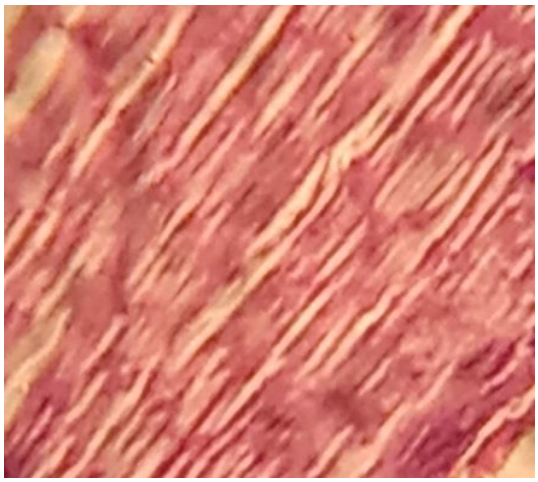


Fig-6: Effects of *Dryopteris filix-mass* on thyroid hormones

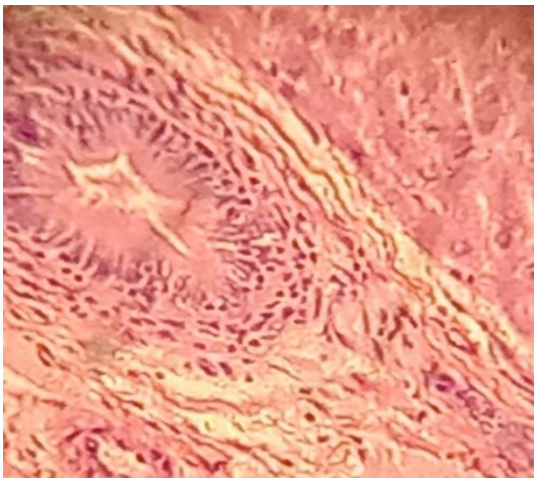
Histopathological slides of *Dryopteris filix-mass* at dose of 1 mg/kg

Heart



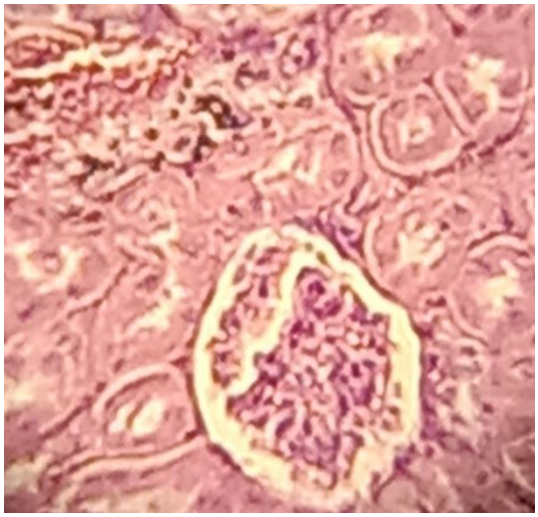
This photomicrograph shows a specimen obtained from the heart of Dryopteris filix 1mg and stained with H&E stain X400. The examination of frozen section revealed it to be normal no necrosis and fibrosis were observed

Liver



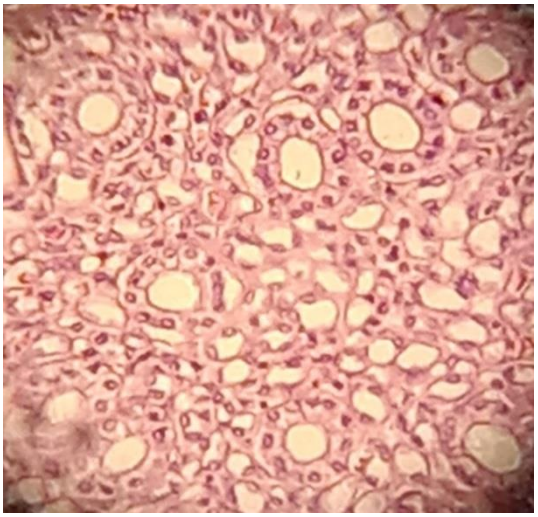
This photomicrograph shows a specimen obtained from the liver of *Dryopteris filix* 1mg and stained with H&E stain X400. The examination of the frozen section showed intact lobular architect 5-7 portal tract identified. Hepatocytes are arranged in cords, mild sinusoidal dilatation is also observed

Kidney



This photomicrograph shows a specimen obtained from the kidney of Dryopteris filix 1mg and stained with H&E stain X100. The examination of the frozen section revealed to be normal no atrophy, no fibrotic changes present in interstitium. variable size glomeruli are seen

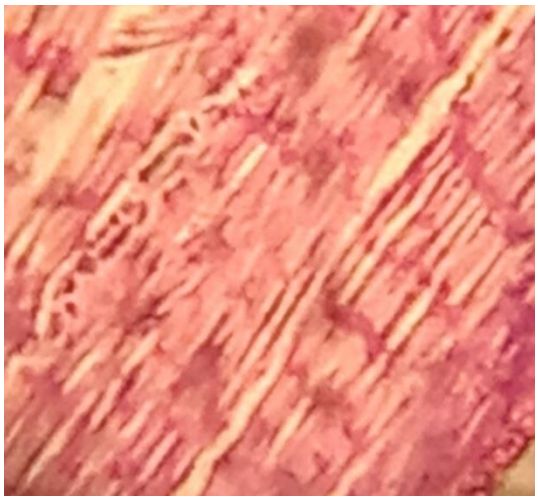
stomach



This photomicrograph shows a specimen obtained from the stomach of *Dryopteris filix* 1mg and stained with H&E stain X100. The examination of frozen section showed intact lamina propria and no infiltration of immune infiltrate is seen

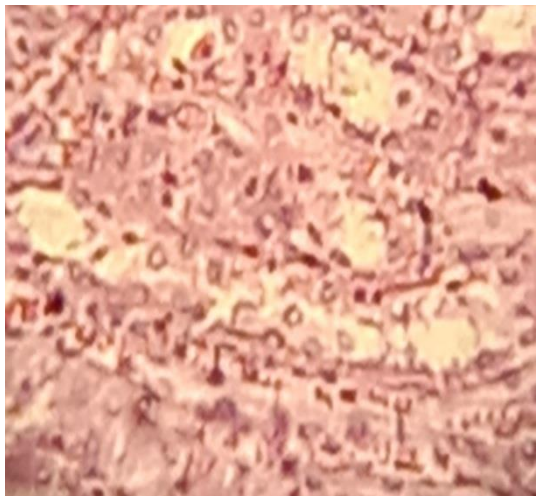
Histopathological slides of *Dryopteris filix-mass* at dose of 5 mg/kg

Heart



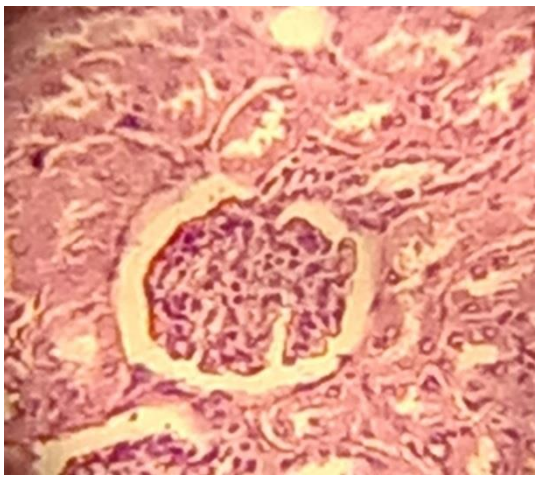
This photomicrograph shows a specimen obtained from the heart of *Dryopteris filix* 5mg and stained with H&E stain X400. The examination of frozen section revealed it to be normal no necrosis and fibrosis were observed

Liver



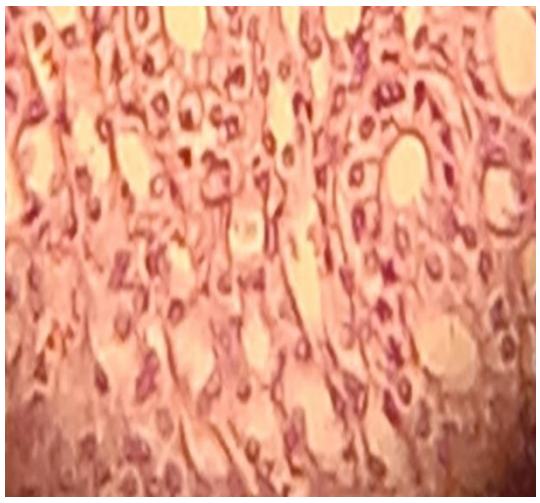
This photomicrograph shows a specimen obtained from the liver of *Dryopteris filix* 5mg and stained with H&E stain X400. The examination of the frozen section showed intact lobular architect 5-7 portal tract identified. Hepatocytes are arranged in cords, mild sinusoidal dilatation is also observed. Mild inflammation is observed

Kidney



This photomicrograph shows a specimen obtained from the kidney of *Dryopteris filix* 5mg and stained with H&E stain X100. The examination of the frozen section revealed to be normal no atrophy, no fibrotic changes present in interstitium. variable size glomeruli are seen

stomach



This photomicrograph shows a specimen obtained from the stomach of *Dryopteris filix* 5mg and stained with H&E stain X100. The examination of frozen section showed intact lamina propria and mild infiltration of immune infiltrate is seen

Discussion

Dryopteris filix-mas (L.) is a plant for medicinal purposes in several diseases (Uwumarongie *et al.*,, 2016). In current study, different biological parameters have been evaluated which can be helpful in developing or maintaining toxicological or pharmacological profile of the medicinal plant. As reported literature indicates that biochemical analysis in preclinical studies is an important tool to observe effects of crude extract (Dar *et al.*, 2014).

Table-1 shows that *Dryopteris filix-mass* increased RBC and Platelet at both doses but decreased WBCs only at high dose. This suggested that *Dryopteris filix-mass* is helpful in improving RBCs or platelet at doses of 1mg and 5mg. Reported study also shows that at very high doses *Dryopteris filix-mass* increased red blood cell production. It is suggested that may be flavonoids and terpenoids help in erythropoiesis (Osano *et al.*, 2016)

The results indicates the decrease in creatinine with significant difference ($p \leq 0.05$) at higher dose. On the other hands, *Dryopteris filix-mass* was also observed to promote urea and uric acid production. This plant slightly increased calcium (Table 1) and other electrolytes levels (figure-1) and protein levels (figure-3). These results suggested that long term intake of this plant particularly at high doses can causes alteration in renal function (250mg of extract), altered electrolytes and protein levels are reported in literature (Erhirhie *et al.*, 2019). Hence, current study shows that doses at 1mg and 5 mg are safer than reported doses of 250 mg.

Figure-4 shows effects of *Dryopteris filix-mass* on liver function test. Maximum effects were observed on alkaline phosphate with drug response 100%. Studies showed that high ALP levels enhances the tendency to progress towards the advance stage of liver disorders such as liver cancer, lymphoma or infiltrative disease (Gotardo et al., 2000, Gopal and Rosen, 2000). Significant ($p<0.05$) increased total bilirubin, SGPT and alkaline phosphate were also observed at high dose. Insignificant effects were observed at low dose. These results showed that higher doses of *Dryopteris filix-mass* may be hepatotoxic as higher levels of bilirubin, SGPT and ALP (figure 4) may be the indication of early stage of hepatic disorder or injury such as primary

sclerosis, cholangitis, bile duct obstruction or chronic liver cirrhosis. These conditions require ultrasound confirmation for diagnosis (Velayudham and Farrell, 2003, Betro and Edwards, 1973).

Figure-1 shows effects of *Dryopteris filix-mass* on lipid profile. Maximum effects were observed on cholesterol with drug response 363.25% and minimum on HDL with drug response 16.66%. Therefore, its intake by cardiac patients should be continuously monitored due to triglycerides and cholesterol association with coronary disorders (Kayode et al., 2016). In addition to this, increased cardiac enzymes level may also suppress the cardiac activity.

Figure-6 shows effects of *Dryopteris filix-mass* on thyroid hormones. Maximum decreased was observed on TSH with drug response 37.5%. Hence this plant with medicinal benefits can be useful in hyperthyroidism (Vijayalkshmi and Kumar, 2013). Over all this study gives a therapeutics dose range of *Dryopteris filix-mass* which could be beneficial for medicinal purpose.

Conclusion

Dryopteris filix-mass is helpful to improve red blood cells Platelet production and in hyperthyroidism, but it should be used with cautions in patients with cardiac arrest, high lipid profile and compromised hepatic and renal function at high doses.

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