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Acute and Subacute Toxicity studies on Siddha Herbo-mineral Anti-arthritic formulation "Pooneeru Diravagam" in experimental animal models

Review Article

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Abstract

Ethno pharmacological Relevance: 60% of the world's population depends on traditional medicine [1]. It is in use for primary health care and general health care among rural, urban, semi urban in both developed and developing countries parallel to the usage of western medicines. Pooneeru diravagam is an indigenous Siddha medicine [2]. There is a huge cry, tall fake claims regarding the safety and efficacy of Siddha formulations. The present study is aimed to evaluate the safety of Pooneeru diravagam by determining any toxicity changes through acute and sub-acute toxicity studies per oral administration in swiss albino rats.

Materials and Methods: Acute toxicity study was carried out as per OECD Guideline-423 in healthy swiss albino female rat weighing 200–245 gm. The Study was carried out in three female rats under fasting condition; signs of toxicity were observed for every one hour for first 24 hours and every day for fortnight from the beginning of the study. Sub acute toxicity study was carried out as per OECD guidelines-407 in swiss albino rats of either sex weighing 220–245 gm of three groups of 6 rats each (Three male and three female) at two dosage levels 0.2 ml and 0.4 ml of 28 days continuous drug administration (oral route).

Results: The animals were sacrificed on the 29th day and various blood biochemical parameters haematological and clinical signs were measured. The organ morphology such as kidney, liver, heart, lungs, spleen, pancreas, brain, ovaries and testes were processed for Histopathological study. The results of sub-acute toxicity on 29th day did not show any evidence of changes. Physiological, Hematological as well as Histopathological parameters remained unaltered when compared with control animals throughout the dosing period.

Conclusions: From the results it is concluded that usage of pooneeru diravagam at the dosage of 0.4 ml/kg p.o is safe for the population suffering from rheumatoid arthritis.

Keywords: Herbo-Mineral Formulation; Pooneeru Diravagam; Rheumatoid Arthritis; Siddha; and Toxicity Studies.

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Background

60% of the world's population depends on traditional medicine [3]. It is in use for primary health care and general health care among rural, urban, semi urban in both developed and developing countries parallel to the usage of western medicines. Siddha System is one of the oldest systems of medicine in India [4].This system is enriched with flora, fauna, and mineral resources [5]. Pooneeru diravagam is an indigenous Siddha medicine. The present study investigated the acute and subacute toxicity of *Pooneeru Diravagam* (PD), a herbomineral antiarthritic formulation. The pooneeru diravagam is a distillate contains the extract of purified dry powders of Sodium chloride impura, asphaltum, sodium carbonate, potassium nitrate, potassium chloride, potassium carbonate, sodium chloride, sodium biborate, fuller's earth, Ammonium chloride, Alumen alum, sodium hydroxide, salt of *Achyranthes aspera, Ferula asafoetida* and *Citrus limon* juice [6]. This medicine is prepared as per the Classical Siddha literature.

Materials and Methods

Animals

In an Acute toxicity study was carried out as per OECD Guideline-423 in healthy swiss albino female rat weighing 200–245 gm [7]. Sub acute toxicity study was carried out as per OECD guidelines-407 in swiss albino rats of either sex weighing 220–245 gm of three groups of 6 rat each (Three male and three female) were selected and kept under standard laboratory conditions. These animals were allowed to free access to standard pellet diet and water ad libitum. They were housed in the poly vinyl chloride cages (PVC) cages. This study protocol was approved by the Institutional Animal Ethical committee (IAEC) No. CLBMCP /0731/01/1415, C.L.Baid metha foundation for pharmaceutical education and research, Jyothi nagar, old mahabalipuram road, Thorapakkam, Chennai-96, Tamil Nadu, India.

Acute Toxicity studies [8]

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development, Guideline-423. This Study was carried out at three female rats under fasting condition, signs of toxicity was observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

Justification for Dose Selection

The results of acute toxicity studies in Swiss albino rats indicate that *PD* was nontoxic up to the maximum dose level of 0.4ml/kg body weight. On the basis of these results, the doses selected for the study are 0.2ml/kg, 0.3 ml/kg and 0.4ml/kg body weight. The oral route was selected for use because oral route is the proposed therapeutic route.

Preparation and administration of dose

PD was added to distilled water to obtain concentrations of 200 mg/ml. It was administered to animals at the dose levels of 0.1 ml/kg, 0.2 ml/kg, 0.3ml/kg and 0.4 ml/kg respectively. The suspension was freshly prepared for every two days upto 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Sub acute toxicity studies (Repeated dose oral toxicity study)

Sub acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development, Guideline-407. The animals were randomly distributed to three different groups such as control, low dose and high dose with 6 rats (Three male and three female) each. The animals were fasted overnight and the drug was administered at two dose level of 0.2 ml and 0.4 ml/kg of body weight for 28 day continuous drug administration (oral route). The parameters like clinical signs such as body weight, assessments of posture, Signs of convulsion limb paralysis, body tone, lacrimation, and salivation, changes in skin colour, Piloerection, defecation, sensitivity response, locomotion, muscle gripness, rearing, and urination were observed. Hematological parameters were determined using Hematology analyzer, Bio chemical parameters were determined using auto analyzer, Gross necropsy: All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach, Testis were recorded; Histopathology: Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach, Testis were fixed in 10% formalin for routine Histopathological examination. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50ºC and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with haematoxylin and eosin.

Statistical analysis

All the values are expressed as mean ± S.E.M. The data were statistically analyzed by one-way ANOVA followed by Dunnet-t test. P values < 0.05 were considered significant.

Results

Acute toxicity studies

At the dose levels of 0.1, 0.2, 0.3, 0.4 ml/ kg no behavioral changes observed at every dose level and body weight was normal (Table 1).

Table 1: Effect of test drug on body weight and behavioral changes of albino rats exposed to *PD* for 28days.

GROUP	DAY
Body weight	Normal
Assessment of posture	Normal
Signs of convulsion limb paralysis	Normal
Body tone	Normal
lacrimation	Normal
salivation	Normal
Change in skin colour	Normal
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Normal
Urination	Normal

Repeated dose oral toxicity study

Body weight, Food and water consumption: Body weight gain (Table 2) was found to be normal throughout the dosing period of 28 days. When compared the treatment groups with control. The parameters checked are,

Effect of test drug on Hematological analysis

The results of hematological investigations (Table 3) was conducted on day 29, revealed following significant changes in the values of various parameters investigated when compared with those of control, low dosage and high dosage groups respectively. There is a significant increase in RBC, WBC, PCV, MCV, MCV, MCH, MCHC parameters and decrease in the platelet count and blood sugar level and BUN of low dosage group (0.2ml/kg) when compared to control group. But similarly slight decrease in RBC, WBC, PCV, MCV, MCV, MCH, BUN parameters of high dosage group were obtained for animals in the dose group of 0.4 ml/kg. Increase in MCH values were obtained for animals in dose groups administered 0.2 and 0.4 ml/kg group sacrificed on day 29. However, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

The results of lipid profile (Table 4) was conducted on day 29, revealed following significant changes in the values of various parameters investigated when compared with those of control, low dosage and high dosage groups respectively. There is mild increase in total cholesterol, Serum triglyceride level, and serum LDL levels of low dosage group (0.2ml/kg) but similarly slight decrease in serum HDL and serum VLDL cholesterol levels. Whereas, serum creatinine remains same and total cholesterol level is increased in animals of high dosage group (0.4 ml/kg) when compared to control group.

There is a decreased levels of Serum total protein, SGOT levels and slight increase in SGPT levels of both low dosage and high dosage groups when compared to control group as mentioned in the Table 5.

Table 2. Effect of test drug on Body weight and Food consumption of albino rats exposed to *PD* for 28days.

Grouping	FOOD (g/Day/rat)	Body weight (g)
CONTROL		
MEAN	21.5	233.3
SD	1.975	8.238
SE	0.8062	3.363
LOW DOSE		
MEAN	30.17	232.5
SD	2.137	3.782
SE	0.8724	3.544
HIGH DOSE		
MEAN	23.83	230.2
SD	5.456	5.981
SE	2.227	3.442

Values are MEAN ± S.E.M. (Dunnett test).where N=6 and ns P>0.05 Vs control group.

Table 3. Hematological parameters after 28 days treatment with *PD* in rats.

GROUPING	Total RBC count ($\times 10^6$µl)	Total WBC count ($\times 10^6$µl)	Platelet count ($\times 10^3$µl)	Packed cell volume (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Blood Sugar-R (mg/dl)	BUN (mg/dl)
CONTROL									
MEAN	7.167	7.667	572.8	44.83	56.83	23.17	35.33	76.33	14.83
SD	1.329	1.033	5.913	4.535	3.971	5.037	4.633	4.502	4.07
SE	0.5426	0.4216	2.414	1.851	1.621	2.056	1.892	1.838	1.662
LOW DOSE									
MEAN	9.033	10.65	570.8	48.83	62.33	25.83	43.17	74.67	14.33
SD	0.8524	1.759	5.037	2.041	4.59	5.115	3.371	5.046	2.338
SE	0.348	0.7182	2.256	0.8333	1.874	2.088	1.376	2.06	0.9545
HIGH DOSE									
MEAN	8.533	7.483	565.8	44	56.67	21	44.67	69.67	13.5
SD	0.8165	2.05	6.676	5.254	4.082	4.604	4.885	1.506	3.391
SE	0.3333	0.8368	2.725	2.145	1.667	1.88	1.994	1.6146	1.384

Values are MEAN ± S.E.M. (Dunnett test).where N=6 and ns P>0.05 Vs control group.

Table 4. Effect of test drug on lipid profile of albino rats exposed to *PD* for 28days.

GROUPING	Serum Creatinine (mg/dl)	Serum Total Cholesterol (mg/dl)	Serum Tri-glycerides Level (Mg/dl)	Serum HDL Cholesterol (Mg/dl)	Serum LDL Cholesterol (Mg/dl)	Serum VLDL Cholesterol (Mg/dl)
CONTROL						
MEAN	0.7333	96.33	49.5	28.83	46.33	36.83
SD	0.1862	3.983	4.461	1.329	5.279	3.601
SE	0.07601	1.626	1.821	0.5426	2.155	1.47
LOW DOSE						
MEAN	0.7333	105.5	50.17	25.67	47.17	33
SD	0.1874	4.97	4.834	2.733	4.535	3.578
SE	0.0876	2.029	1.973	1.116	1.851	1.461
HIGH DOSE						
MEAN	0.6167	103.5	42.5	29	42.5	35.67
SD	0.1856	4.848	4.183	5.177	3.391	4.502
SE	0.0942	1.979	1.708	2.113	1.384	1.838

Values are MEAN ± S.E.M. (Dunnett test). where N=6 and ns P>0.05 Vs control group.

Table 5. Effect of test drug on Serum enzyme and Protein of albino rats exposed to *PD* for 28days.

Grouping	Serum total Protein (g/dl)	Serum Albumin (g/dl)	SGOT (AST) (IU/ml)	SGPT (ALT) (IU/L)
CONTROL				
Mean	5.817	2.667	245.3	59.5
SD	0.7195	0.6186	8.779	6.442
SE	0.2937	0.2525	3.584	2.63
LOW DOSE				
MEAN	4.867	2.733	215.2	70.5
SD	0.7118	0.432	3.656	6.025
SE	0.2906	0.1764	1.493	2.46
HIGH DOSE				
MEAN	4.133	2.5	212.3	70.17
SD	0.6501	0.4817	3.445	1.329
SE	0.2654	0.1966	1.406	0.5426

Values are MEAN ± S.E.M. (Dunnett test). where N=6 and ns P>0.05 Vs control group.

Table 6. Effect of test drug on and Hemoglobin blood Leukocyte count of albino rats exposed to *PD* for 28days.

GROUPING	HB mg/dl	Neutrophills (%)	Lymphocytes (%)	Esinophills (%)	Monocytes (%)	Basophills (%)
CONTROL						
MEAN	15.6	69.33	33	1.533	0.8833	0
SD	1.356	7.146	4.69	0.4227	0.2137	0
SE	0.5538	2.917	1.915	0.1726	0.08724	0
LOW DOSE						
MEAN	16.5	64	36	1.783	0.7667	0.3333
SD	3.728	5.367	6.033	0.2137	0.216	0.5164
SE	1.522	2.191	2.463	0.08724	0.08819	0.08819
HIGH DOSE						
MEAN	14.83	68.5	39.33	1.333	0.6167	0.1667
SD	3.189	4.324	6.713	0.6713	0.1835	0.4082
SE	1.302	1.765	2.741	0.2741	0.07491	0.1667

Values are MEAN ± S.E.M. (Dunnett test).where N=6 and ns P>0.05 Vs control group.

Table 7. Effect of Test drug on Mortality rate of the study of albino rats exposed to *PD* for 28days.

TREATMENT	Mortality observed for the duration of 1- 28 days
GROUP I - CONTROL	NIL
GROUP II- LOW DOSE	NIL
GROUP III- HIGH DOSE	NIL

Table 8. Effect of Test drug on organ morphology of albino rats exposed to PD for 28days.

GROUPING	KIDNEY	LIVER	HEART	LUNGS	SPLEEN	PANCREAS	BRAIN	OVARIES	TESTIS
GROUP I - CONTROL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
GROUP II- LOW DOSE	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
GROUP III- HIGH DOSE	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL

The haemoglobin blood leukocyte count of albino rat's shows significant increase in hemoglobin level in low dosage group whereas mild decreased levels of blood leukocytes as shown in table 6.

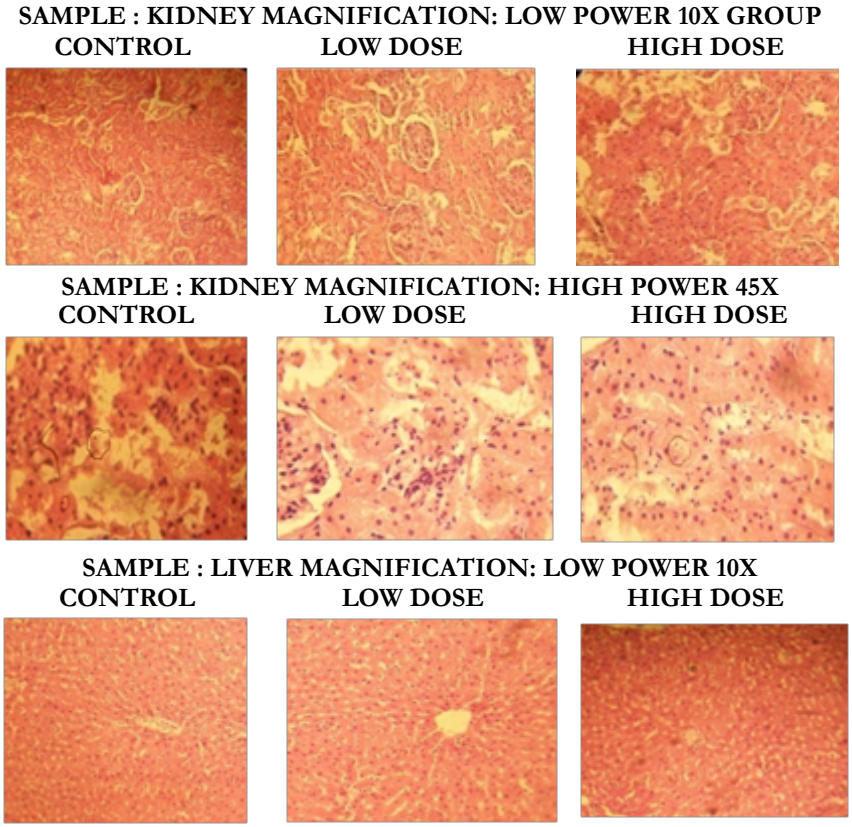
No mortality was observed for the duration of 1-28 days in all the three groups as seen in table 7.

The organ morphology of albino rats such as kidney, liver, heart, lungs, spleen, pancreas, brain, ovaries and testis were normal in all the three groups when exposed to PD for 28 days as seen in table 8.

Histopathological analysis of Sub-Acute toxicity study of albino rats exposed to *PD* for 28 days

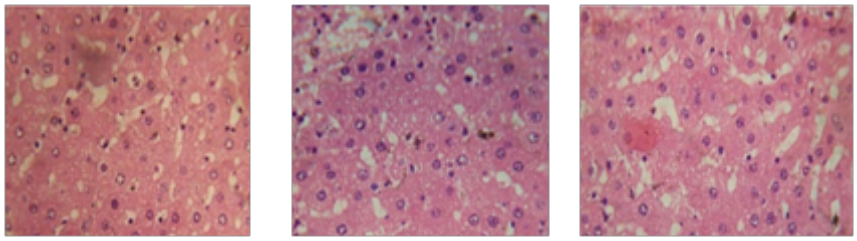
(Figure 1 A-I) for histopathology there were no changes to be observed.

Figure 1(A-I). Histopathological Analysis of *PD* (Kidney,Liver, Heart,Lungs,Spleen, Pancreas, Brain, Ovaries and Testis).



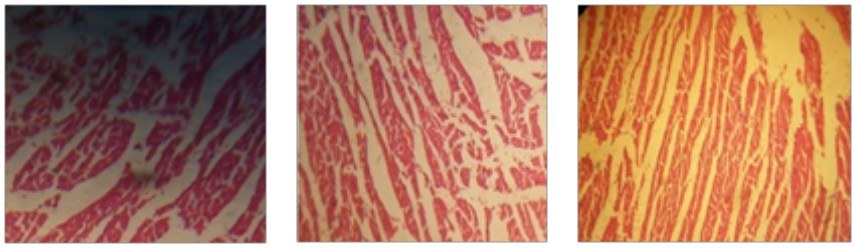
SAMPLE : LIVER MAGNIFICATION: HIGH POWER 45 X

CONTROL **LOW DOSE** **HIGH DOSE**



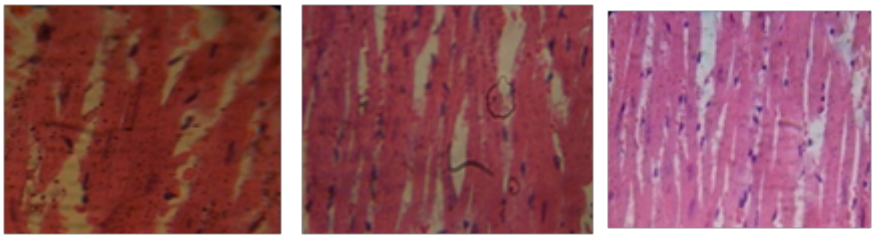
SAMPLE :HEART MAGNIFICATION: LOW POWER 10X

CONTROL **LOW DOSE** **HIGH DOSE**



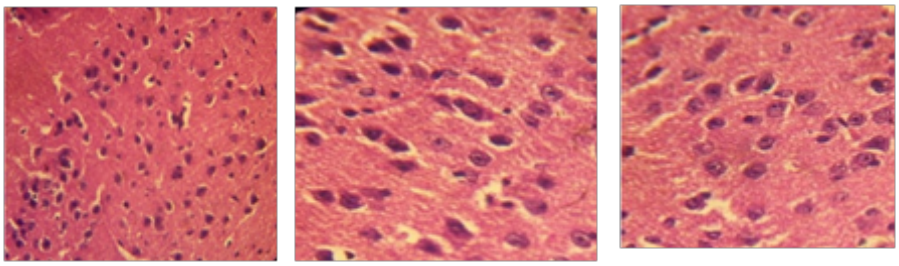
SAMPLE :HEART MAGNIFICATION: HIGH POWER 45X

CONTROL **LOW DOSE** **HIGH DOSE**



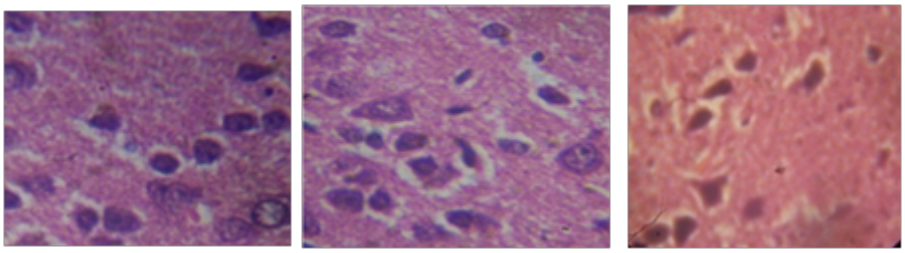
SAMPLE : BRAIN MAGNIFICATION: LOW POWER 10X

CONTROL **LOW DOSE** **HIGH DOSE**



SAMPLE : BRAIN MAGNIFICATION: HIGH POWER 45X

CONTROL **LOW DOSE** **HIGH DOSE**



PATHOLOGIST REPORT

Sample	Observation
Kidney	shows normal arrangement of nephrotic bundle in all the three groups
Heart	No signs of lesion or infract was observed in all the three groups
Liver	Lumen of hepatic veins appears normal. No signs of necrosis.
Brain	normal histology with regular neuronal alignment further there was no considerable observation of signs of edema or degeneration

Discussion

In acute toxicity study, no mortality was observed up to the maximum dose level of 0.4ml/kg b.wt of PD administered orally, which the single high dose is recommended by OECD gudelines-423 for testing the acute toxicity. Thus in this study PD does not cause any adverse acute toxicity. The changes in the body weight have been used as an indicator of adverse effects of PD and chemicals. Since there are no apparent changes in animal behavior, body and organ weights at low and high dosage groups of the treated rats when compared to control group, the present results suggest that at the oral doses administered, the PD is nontoxic. Hematological analysis conducted at the end of the dosing period on 29th day, revealed no abnormalities attributable to the treatment. Biochemical analysis conducted at the end of the dosing period on 29th day, revealed no abnormalities attributable to the treatment. Also, there were Mild changes in RBC, WBC, PCV, MCV, MCV, MCH, MCHC parameters, which indicates that PD may not be toxic and no major effects on circulating blood cells.

The increase in the hemoglobin level might be due to the presence of ferrous sulphate and increased absorption of iron by presence of lemon juice (vit-c) in PD and the increase in WBC level may indicate the impact of PD in immune system of treated groups. All the animals from control and all the treated dose groups up to 0.4ml/kg survived throughout the dosing period of 28 days. No signs of major or significant intoxication were observed in animals from low and high dosage groups during the dosing period of 28 days. Animals from all three treated dose groups exhibited no weight gain throughout the dosing period of 28 days. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days. In the subacute toxicity study, the Pooneeru diravagam treated groups did not show any significant changes in body weight increment at weekly intervals when compared to the control group. The weights of the liver, kidney, and heart were unaltered in the experimental groups compared with control group.

There were no significant changes in any liver function parameters such as SGOT, Serum total protein, Serum albumin, ALT compared to control group. Increase in these parameters would have indicated hepatocyte damage. The normal levels of blood urea and serum creatinine indicate that the test drug did not interfere with renal function and that renal integrity was observed. The hematological and biochemical parameters (LFT &RFT) did not show any significant changes in the PD treated groups when compared to the control group. There is no mortality in all the three groups till 28 days were observed. The PD treated groups did not show any significant changes in the body weight increment, indicating that it did not have any adverse effects on body weight, which is used to assess the response to therapy of drugs. The organ (liver, kidney, and heart) weights in the test drug treated groups remains normal, indicates that PD was not toxic in these vital organs. Furthermore, Histopathological examination of selected organs (heart, liver, kidney, spleen, brain, pancreas adrenals, ovaries and testis) from treated and control animals showed normal architecture, suggesting no detrimental changes seen.

Conclusion

Based on these findings, PD can be considered safe, as it did not cause either any lethality or adverse changes with general behavior of rats in acute and sub acute toxicity study up to the dose of 0.4ml/kg b.wt. in swiss albino rats. The present findings suggest that PD is nontoxic since no marked changes in hematological, biochemical, and Histopathological parameters were observed. Thus, at normal therapeutic doses, PD is considered to be safe for long-term treatment in Rheumatoid arthritis. So, it can be concluded that the *Pooneeru diravagam* can be prescribed for therapeutic use in humans with the dosage recommendations of up to 0.4ml/kg/p.o.

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