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PRECLINICAL ACUTE AND REPEATED DOSE TOXICITY OF *PUERARIA TUBEROSA* (PTWE) ON CHARLES FOSTER RATS

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ABSTRACT: Here we have carried out the acute and repeated dose toxicity study on tubers of *Pueraria tuberosa* water extract (PTWE). For acute toxicity study, rats were orally administered a single dose of 2000 and 5000 mg/kg body weight of PTWE. For repeated dose toxicity study on rats in one set, the different doses were given daily up to 28 days to make general observation and mortality rate. In another set of experiment, group of different doses were sacrificed on 7, 14, 21 and 28 days to assess the biochemical and histological changes. The selected doses were 250, 500, 1000 and 2000 mg/kg b.w. To evaluate it, we have used OECD guidelines 425 and 407 respectively, in both acute and repeated oral toxicity. No adverse effect was observed in all the rats, up to 14 days after treatment with single doses of 2000 and 5000 mg/kg b.w. Slight increase in salivation, mild diarrhea and letharginess were observed on 7^{th} day at dose of 500 and 1000mg/kg, but recovered on 14^{th} day and remain normal up to 28^{th} day. There was 100% survival up to 28 days in rats treated with dose of 1000 mg/kg b.w., but at dose of 2000 mg/kg b.w., mortality was 17 % on 7^{th} day, 50% on 14^{th} days & 100% on 21 days. Based on these datas, the LD_{50} was calculated to be > 5000mg/kg b.w. After considering all the toxic information on PTWE, we could elaborate all its previously mentioned medicinal properties in further clinical studies.

INTRODUCTION: The therapeutic properties of medicinal plants are attributed to presence of various phytomolecules, which makes a natural cocktail of various secondary metabolites. Some of them are biologically active and act in the additive or synergistic manner, but other photochemical may be biologically inert but might be helping in latenciation and bio-availability of these active molecules.

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Recently, several reports indicate that total extract is more effective than the pure photochemical, isolated from the plant extract. Since the activity depends on their absorption and bioavailability to target tissues, so it becomes essential to test the toxicity of these extracts.

Here we have prepared powder extract of *Pueraria tuberosa* Linn. (Fabaceae), commonly known as, Indian kudzu [1] or Nepalese kudzu [2]. It is a climber with woody tuberculated stem [3]. In Sanskrit it is called Swadukanda, Ikshuganha, Kandapalash and in Hindi it is called Vidarikanda [4]. It's the tuberous roots are white, starchy and have a sweet taste. It is rich in steroids, sugars, amino acids and sterols [27] with 64% carbohydrates and 10.9% proteins. As per Ayurvedic texts it is used for treatment of

problems associated to urinary and the reproductive system, as lactogague and general health promoter [3, 5]. In recent past, we have reported its antioxidant [6], anti-infertility [28] anti-inflammatory and nephro-protective potential [7]. It has shown significant protection against acute nephrotoxicity induced by cisplatin and diabetes induced chronic kidney disease.

One of its nephroprotective mechanism has been identified through activation of MMP-9, resulting degradation of accumulated of ECM (extra cellular matrix) [8]. It has also shown inhibition of DPP4, resulting higher level of serum GLP 1 [9, 10, 32] and incretin receptor agonist [11]. Also reported as anti-hypertensive [29] and anti-diabetic property [30].

In the texts of Ayurveda, it is used in powder form and also its water extract as part of decoction of combined medicinal plants (quath). In our animal experiments we have used its extract isolated by organic solvents *e.g.* methanol, ethanol and hydroalcholic mixtures.

Since now we are preparing for its clinical trial, where organic solvents are discuraged, so we have prepared its water extract based semipurified preparation named as (PTWE) for clinical use. Thus the pre-clinical toxicity of this preparation has been done here according to OECD 425 and 407. This kind of work has already been done in medicinal plant as well *e.g.* Purna cantirotaya centuram [12]. This preparation reduces the bulky dose, improves efficacy and patient friendly. Here the moisture content is also reduced to enhance the shelf life and to avoid microbial growth.

Pueraria is rich in daidzin, tuberosin, puerarin, genistein, pterocarpintuberosin, puerarone, coumarin, hydroxytuberosone, anthocyanin, lupinoside, puetuberosanol. A validated RP-HPLC-UV method for quantitative determination of puerarin in *Pueraria tuberosa* tuber extract. They belong to group of flavones, isoflavones steroids and terpenes, which are polyphenolic compounds and attributed to its antioxidant and anti-inflammatory potentials. However, our main aim of this study to assess the safety profile of whole extract, as it is proposed to be used for clinical studies and not to determine the active principle of this preparation.

MATERIALS AND METHODS:

Collection and Identification of Plant Materials: The plant material was purchased Ayurvedic pharmacy, and physically matched with our reference samples, preserved in our laboratory (Ref. no YBT/MC/12/1-2007) and also with the sample preserved in museum of Department of Dravyaguna, Faculty of Ayurveda of our Institute. It was further compared with TLC finger printing with standard sample, which has been characterized earlier in our laboratory by DNA finger-printing, HPTLC and LCMS [13].

Preparation of *Pueraria tuberosa* Water Extract: The 250 gm of PT powder was boiled with 2.5 little of distilled water. When the volume was reduced to 500 ml, it was cooled and filtered through a clean cheese cloth. The filtrate was washed with hexane solvent in a separating funnel and aqueous layer was saved and lyophilized. It was dried in a vacuum desiccation till moisture content was reached to 7%. The yield value of extract was 36% w/w. It was characterized by TLC finger printing.

Preparation of Animals: The rats of inbred colony were purchased from central animal house and acclimated in our laboratory conditions. During this period, the animals were disinfected by giving the metronidazole drug and tetracycline by gavage. These rats were randomly divided in to different groups given in respective tables. The whole study was approved by animal ethical committee of our institute (Ref. no. Dean/2017/CAEC/721). The animal was divided in different groups (6 in each male rats).

The Doses Preparation of PTWE: The (PTWE) was suspended in water with 10% gum acacia, properly mixed in mortar and pestle. The final concentration was fixed to 333.33 mg/ml. 1 - 1.5 ml volume of each dose was fixed depending on the b.w. of animal.

Limit Test: Limit dose is done to determine upper limit of the dose to be tested (2000 - 5000 mg/kg b.w). Limit test was performed. The animal were kept at room temperature was between 25 - 28 ºC, with 12 h light dark cycle. Althought PT preparation are already in clinical use with no reported toxicity, but even than limit test was carried out as (PTWE) was a new preparation.

Since the earlier studies have reported its no toxicity on single dose administration of alcoholic extract up to dose of 227.5 mg/100 g bw [13], so based on % yield ration of these 2 extracts, the dose of PTWE for limit test was fixed as 2000 mg/kg bw. Initially 1 animal was dosed and observed for 72 h. It did not die so same dose was given to another 2 animals and all of them were observed individually for 14 days. Later on same experiment was repeated with dose of 5000 mg/kg b.w. The observations were made 0, 2, 4, 24 h and daily up to 14 days. Only survival is observed upto 14 days. Results are given in **Table 1**.

Main Test: The further confirmation the safety profile, we also conducted the main test. Where different dose of PTWE maximum upto 5000 mg/kg bw in the increasing ratio of 3 fold such as 170 mg, 544 mg, 1740 mg and 5570 mg/kg bw where given to single rat and mortality of rat was observed upto 48 h.

Acute Toxicity Studies: The acute toxic test method is based on biometric evaluations with fixed doses, as per organization for economic co-operation and development 425. [31] It is to determine the adverse effects, if any after oral administration of a single dose or multiple doses, within 24 h. The 14 days observation is made to assess any delayed death or adverse effect. This experiment does not allow to determine the LD_{50}, but it defines the safe exposure ranges. The LD_{50} values are usually obtained with plants widely used as food and/or herbs [14, 15]. The (PTWE) was orally given to overnight fasted animal by gavage using a stomach tube in a group having 3 animals in each. The doses were 2000 and 5000 mg/kg b.w.

All the rat were observed for general behavioral changes; symptoms of toxicity and mortality after treatment for the first 4 (critical) hours, then over a period of 24 h, 48 h 72 h thereafter daily for 14 days. The body weight was determined before initiation of doses and at weekly intervals and finally after 14 days, when humanely killed. The change in body weight were calculated and recorded. All gross pathological changes were recorded in animals died during observational period or humanly killed animals. The microscopic examinations of vital organs were also carried out. The sign of changes and number of mortality in each group along with time of death of individual animals and, and necropsy findings were also recorded separately. The time course of toxic effects and its reversibility were also recorded. B results the PTWE may be classified in the hazard category - 5 *i.e.* 2000 mg/kg < LD_{50} < 5000 mg/kg.

Repeated Dose Toxicity: The repeated dose toxicity study was carried out. Here those animal prepared described above and randomly divided into different group having five animal. Different doses 250, 500, 1000, and 2000 mg/kg bw of PTWE were given to respective groups for 28 days. General observation like change in body wt. lethargic behaviour, diarrhoea, salivation *etc.* were made as described above. The animal, which died during the experiment, were dissected out for detail study otherwise all animals were terminated on 29th day. Blood was collected from brachial by artery puncture. The deferent organs from rat such as liver, kidney, brain, testis ovary, spleen, pancreas, heart, (be specific) were safely dissected out.

The attached tissue was removed in petridish, filled in Ringer's buffer. The clean organs were taken out and dried on a tissue paper and weighed on electronic balance.

Statistical Analysis: Total all findings such as biochemical, body wt variation hematological and some expression were tabulated and analyzed. Data are expressed as the mean ± slandered deviation. The mean value and slandered deviation (SD) were calculated for each variable analyzed by Newman - keulis multiple comparison test of ANOVA to determined significant difference between group at $P<0.05$.

RESULTS:

Limit Test: After the given dose 2000 mg/kg bw of PTWE no animal died in 72 h. Similar result was observed at the dose of 5000 mg/kg b.w. Those it was concluded the (PTWE) is safe on single administration of 5000 mg/kg b.w. and the LD_{50} > 5000 mg/kg bw.

Main Test: Interestingly no animal died on all the given tested doses 170, 544, 1740 and 5570mg/kg bw of PTWE So no any type of adverse effect (salivation, lethargy, coma, diarrhoea *etc.*) was found in the animals.

Acute Toxicity Study:

General observation: In general observation was, not show any adverse effect (salivation, lethargy, diahorea *etc.*) in the rat which is dose was 2000 and 5000 mg/kg b.w. of single oral administration up to 14 days.

Body weight Evaluation: The body wt change was same as untreated controlled rat. There was no stagnation in body wt increase suggesting the single dose of (PTWE) treatment is not inhibiting the normal group of the animals **Table 1**.

TABLE 1: BODY WT VARIATIONS

Acute toxicity study						
S. no	**Dose**	**0 days**	**7th days**	**14th days**	**21st day**	**28th day**
1	Normal control	220 ± 5	230 ± 3.6	235 ± 5	-	-
2	2000 mg/kg b.w.	215 ±5.7	230 ± 5	240 ± 5.6	-	-
3	5000 mg/kg b.w.	220 ± 5.2	220 ± 5.7	240 ± 5.5	-	-
Repeated dose toxicity study						
S. no	**Dose**	**0 days**	**7th days**	**14th days**	**21st days**	**28th days**
1	Normal	150 ±4.0	155 ± 5	170 ±4.3	180 ±4.2	180 ± 5.3
2	250 mg/ kg b.w.	155 ± 5	160 ± 5.6	163 ± 4.6	170 ± 5.5	175 ± 5.5
3	500 mg/kg b.w.	150 ± 4.8	155 ± 4.3	155 ± 5.0	160 ± 4.6	165 ± 5.6
4	1000 mg/kg b.w.	150 ± 5.6	155 ± 5.6	155 ± 4.8	160 ± 5.5	160 ± 5.5
5	2000 mg/kg b.w.	155 ± 4.7	155 ± 4.6	150 ±5.5	140 ± 4.7	

Value are expressed as mean ± SEM, significance with Newman-keuls Multiple comparison test following One Way Anova is evaluated as P*<0.05

Biochemical Evaluation:

Effect of Different Doses of PTWE on Liver Function, Renal Function and Blood Glucose level in Blood: The rat where sacrificed on 14 days to collect blood and different tissue. We observed the significantly increases only in serum SGOT in the dose of 5000 mg/kg b.w. Serum SGPT, urea creatinine and blood glucose where normal and no change where observed in drug treated rat **Table 2** and **3**. All parameters were done using their respective accurex kits.

TABLE 2: EFFECT OF DIFFERENT DOSE OF PTWE ON LIVER FUNCTION AND BLOOD GLUCOSE LEVEL IN BLOOD

Acute toxicity study 425 (n=6)							
		SGOT		**SGPT**		**Blood Glucose**	
S. no.	**Dose**	**0 day**	**14th day**	**0 day**	**14th day**	**0 day**	**14th day**
1	Normal rat	23 ± 2.2	23 ± 2.3	24 ± 2.5	23 ± 3.1	125 ± 3.6	127 ± 4.4
2	2000 mg/kg b.w.	25 ± 1.5	24 ± 2.4	28 ± 3.2	27 ± 2.8	124 ± 3.2	132 ± 3.8
3	5000 mg/kg b.w.	25 ± 2.2	64 ± 2.5	26 ± 2.7	39 ± 2.7	125 ± 2.8	131 ± 4.1
Repeated dose toxicity 407 (n=6)							
		0 day	**28 day**	**0 day**	**28 day**	**0 day**	**28 day**
S. no.	Normal rat	23.0 ± 2.3	23 ± 3.3	28 ± 2.6	29.1 ± 2.4	125 ± 3.7	128 ± 3.7
1	250 mg/kg b.w.	27.3 ± 2.6	40.5 ± 3.2	29 ± 3.4	68.7 ± 3.1	126 ± 4.1	131 ± 4.3
2	500 mg/kg b.w.	25.7 ± 2.8	38.3 ± 2.5	29 ± 3.2	65.1 ± 2.5	131 ± 3.8	129 ± 3.6
3	1000 mg/kg b.w.	26.3 ± 2.9	86 ± 3.8	28 ± 3.5	161 ± 2.4	131 ± 3.4	129 ± 3.2
4	2000 mg/kg b.w.	23.3 ± 2.8	-	26 ± 2.5	-	126 ± 3.2	-

Value are expressed as mean ± SEM, significance with Newman-keuls Multiple comparison test following one way anova is evaluated as P* < 0.05

TABLE 3: EFFECT OF DIFFERENT DOSES OF PTWE ON RENAL FUNCTION

Acute toxicity study 425 (n=6)					
S. no.		**Urea**		**Creatinine**	
	Doses	0 day	14th day	0 day	14th day
1	Normal	49 ± 2.64	50 ± 3.65	0.50 ± 0.03	0.51 ± 0.02
2	2000 mg/kg b.w.	51 ± 3.65	50 ± 2.62	0.54 ± 0.04	0.50 ± 0.03
3	5000 mg/kg b.w.	52 ± 3.23	54 ± 2.43	0.59 ± 0.03	0.69 ± 0.03
Repeated dose Toxicity 407 (n=6)					
		0 day	28th day	0 day	28th day
1	Normal	51 ± 3.34	49 ± 3.23	0.50 ± 0.05	0.51 ± 0.04
2	250 mg/kg b.w.	50 ± 3.45	46 ± 3.42	0.54 ± 0.04	0.51 ± 0.06
3	500 mg/kg b.w.	50 ± 3.24	47 ± 2.83	0.50 ± 0.04	0.52 ± 0.04
4	1000 mg/kg b.w.	52 ± 3.12	50 ± 3.43	0.54 ± 0.06	0.53 ± 0.05
5	2000 mg/kg b.w.	51 ± 2.45		0.52 ± 0.03	

Value are expressed as mean ± SEM , significance with Newman-keuls Multiple comparison test following ONE WAY ANOVA is evaluated as P*<0.05

Haematological Evaluation: Haematology data such as (WBC, RBC, HB and Platelets) are shown in table. Haematological test also was did not show any change at the dose 2000 and 5000 mg/kg b.w. of PTWE after oral administration in given rat **Table 4**.

TABLE 4: HAEMATOLOGICAL TEST

Acute Toxicity study 425 (n=6)				
14 days				
Dose	HB	RBC	PLT 10^5	WBC
Normal	14.6 ± 1.45	9.46 ±1.17	11.23 ± 1.44	10.56 ± 1.24
2000mg/kg b.w.	14.2 ± 1.23	9.31 ± 1.23	10.86 ± 2.21	10.68 ± 2.41
5000mg/kg b.w.	15.9 ± 0.82	10.2 ± 0.86	11.14 ± 2.12	10.25 ± 2.31
Repeated dose toxicity 407 (n=6)				
28 days				
Dose	HB	RBC	PLT 10^5	WBC
Normal	14.9 ± 1.42	9.53 ± 1.67	11.68 ± 1.86	10.25 ± 2.12
250 mg/kg b.w.	13.1 ± 2.01	8.19 ±1.26	10.68 ±1.42	10.58 ± 1.75
500mg/kg b.w.	12.1 ± 1.76	8.1 ± 1.89	10.26 ± 1.87	10.78 ± 2.23
1000 mg/kg b.w.	10.1 ± 1.68	7.14 ± 0.91	9.2 ± 2.11	11.19 ± 2.56
2000 mg/kg b.w.				

Value are expressed as mean ± SEM , significance with Newman-keuls Multiple comparison test following ONE WAY ANOVA is evaluated as P*<0.05

Relative Weight of Organs: The relative organs weight of rats treated with doses 2000 and 5000 mg/kg bw were recorded at the end of study did not show any significant difference as compared with normal control. Shown in **Table 5**.

TABLE 5: RELATIVE WEIGHT OF DIFFERENT ORGANS AFTER SINGLE DOSING WITH PTWE FOR 14 DAYS (n=6), UNIT GRAM

Organ	Normal Rat	2000mg/kg bw	5000mg/kg bw
Body wt	235 ± 5	240 ± 5.6	240 ± 5.5
Lung	1.2 ± 0.24	1.18 ± 0.54	1.41± 0.23
Heart	0.9 ± 0.05	0.9 ± 03	0.9 ± 0.02
Liver	5.1 ± 0.66	4.2 ± 0.72	5.2 ± 0.62
Spleen	0.6 ± 0.02	0.6 ± 0.03	0.7 ± 0.03
Right Kidney	1.52 ± 0.09	1.48 ± 0.07	0.7 ± 0.04
Left Kidney	1.46 ± 0.13	1.41 ± 0.10	1.23 ± 0.18
Stomach	2.12 ± 0.4	2.36 ± 0.6	2.71 ± 0.9
Right Testis	2.6 ± 0.59	2.9 ± 0.42	1.17 ± 0.48
Left Testis	2.1 ± 0.40	2.5 ± 0.32	1.15 ± 0.41
Adrenal Left	0.2 ± 0.04	0.2 ± 0.06	0.2 ± 0.07
Adrenal Right	0.2 ± 0.06	0.3 ± 0.03	0.3 ± 0.05
Urinary Bladder	0.06 ± 0.02	0.06 ± 0.03	0.04 ± 0.03

Value are expressed as mean ± SEM , significance with Newman-keuls Multiple comparison test following ONE WAY ANOVA is evaluated as P*<0.05

Histology: The Histological study of liver & kidney where carried out in haematoxylin and Eosin stain T.S. section under 40 X and 10X magnification in microscope laica. There was no significance changed in liver, kidney were observer.

Histological changes such as no necrosis, in liver hepatocytes cell, and in kidney no glomeruler dilation and tubular necrosis be found in with single dose of 2000 and 5000 mg/Kg b.w. of PTWE here **Fig. 1** and **Fig. 2**.

Repeated Dose Toxicity Study:

Genaral Observation: There was no adverse effect up to the dose 500 mg/kg b.w. At the dose of 1000 mg/kg b.w. the symptoms of excess salivation, mild diarrhoea and lithergness were observed on 7th day.

At the dose of 2000 mg/kg b.w. raised salivation litharginess and diorrhea were observed on 5th day treatment & continued upto 21 days **Table 6** and **7**.

TABLE 6: GENERAL OBSERVATION (1000 mg/kg b.w.)

Observation	0 h	30min	4h	24h	48h	Week 4
Skin & fur	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N
Salivation	N	N	P		P	N
Lethargy	Nil	Nil	Nil	Nil	Nil	NIL
Sleep	N	N	N	N	N	P
Coma	Nil	Nil	Nil	Nil	Nil	NIL
Convulsion	Nil	Nil	Nil	Nil	Nil	Nil
Tremor	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	P	P	P	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil

N= No found, P= present

TABLE 7: GENERAL OBSERVATION (2000 mg/kg b.w.)

Observation	0 h	30min	4h	24h	48h	Week 4
Skin & fur	N	N	N	N	N	
	N	N	N	N	N	
Mucus membrane	N	N	N	N	N	
Salivation	N	N	P	P	P	
Lethargy	Nil	Nil	P	P	P	
Sleep	N	N	N	N	N	
Coma	Nil	Nil	Nil	Nil	Nil	
Convulsion	Nil	Nil	Nil	Nil	Nil	
Tremor	Nil	Nil	Nil	Nil	Nil	
Diarrhea	Nil	P	P	Nil	P	
Mortality	Nil	Nil	Nil	Nil	Nil	

N= No found, P= present

Survival: At the dose of 2000mg/kg b.w. treated animal died & 100% mortality was observed on 28th day. In the first 3 weeks the rat of mortality was 17% on 7th day, 50% on 14th day & 100% was on 21 day.

Body weight Evaluation: In the repeated dose toxicity study body weight of rat increase in PTWE after oral administration of 250, 500,1000mg/kg bw show similar pattern in normal rats. But in dose 2000 mg/kg b.w. body wt of rats was gradually decreases up to 21 days **Table 1**.

Biochemical Evaluation:

Effect of Different Dose of PT on Liver Function Test and Blood Glucose: In the acute and repeated dose toxicity study liver function test was detected by SGOT and SGPT analysis. Dose 25 and 50 mg/100 kg b.w. is safe. Blood glucose level was not increase in all days **Table 2**.

Effect of Different Dose of PTWE on Renal Function Test: In acute and repeated toxicity study, all the doses were found to be safe for kidney functions **Table 3**.

Haematological Evaluation: In repeated dose toxicity, the haematological test also did not showed any significance changes in Hb, PLT, WBC and RBC values at the doses of 250, 500 1000 and 2000 mg/kg bw PTWE after oral administration upto 28 days **Table 4**.

TABLE 8: REPEATED DOSE TOXICITY STUDY: RELATIVE WEIGHT OF DIFFERENT ORGANS AT DIFFERENT TIME INTERVALS AFTER REPEATED ORAL DOSING WITH PTWE FOR 28 DAYS (n=6), UNIT GRAM

S. no.	Organ wt in gm n=6	Normal Rat				250mg/kg b.w.				500mg/kg b.w.			
	Days	7	14	21	28	7	14	21	28	7	14	21	28
1	Bw	155±5	170±4.3	180±4.2	180±5.3	160±5.6	163±4.6	170±5.5	175±5.5	155±4.3	155±5	160±4.6	165±5.6
2	Liver	5.2±0.5	5.1±0.3	5.2±0.7	5.8±0.5	4.3±0.7	5.8±0.6	5.7±0.7	5.6±0.8	4.2±0.6	5.4±0.8	4.1±0.7	4.8±0.7
3	Kidney L	1.1±0.2	1.2±0.3	1.1±0.6	1.1±0.4	1.2±0.5	1.2±0.6	1.1±0.6	0.6±0.8	1.2±0.4	1.2±0.3	1.2±0.6	1.5±0.5
4	Kidney R	1.2±0.3	1.2±0.4	1.1±0.5	1.1±0.4	1.1±0.7	1.1±0.7	1.3±0.8	0.8±0.5	1.2±0.6	1.2±0.7	0.9±0.5	0.9±0.2
5	Heart	1.0±0.2	0.7±0.4	0.9±0.3	1.0±0.3	0.9±0.2	0.7±0.4	0.9±0.5	0.8±0.3	1.0±0.6	1.2±0.4	1.0±0.5	1.0±0.3
6	Testis	1.1±0.4	1.1±0.5	1.1±0.6	1.2±0.7	1.1±0.4	1.1±0.8	1.2±0.4	1.2±0.9	1.1±0.4	1.1±0.6	1.2±0.4	1.8±0.7
7	Jejunum	0.6±0.01	0.6±0.05	0.7±0.03	0.6±0.04	0.7±0.03	0.6±0.05	0.6±0.03	0.5±0.03	0.6±0.02	0.6±0.02	0.6±00.8	0.6±0.03
8	Duodenum	0.4±0.02	0.5±00.4	0.6±0.05	0.6±0.07	0.6±0.03	0.6±00.3	0.6±0.06	0.5±0.05	0.6±0.04	0.3±0.06	0.3±0.03	0.6±0.02
9	Ileum	0.5±0.03	0.5±0.04	0.6±0.06	0.6±0.03	0.6±0.03	0.6±0.02	0.6±0.01	0.4±0.06	0.5±0.07	0.5±0.04	0.4±0.03	0.7±0.06
10	Thyroid	0.4±0.02	0.4±0.06	0.5±0.04	0.4±0.02	0.4±0.03	0.4±0.05	0.4±0.06	0.3±0.02	0.2±0.04	0.2±0.02	0.2±0.04	0.2±0.03
11	Brain	1.5±0.3	1.5±0.4	1.4±0.6	1.5±0.7	1.6±0.5	1.6±0.8	1.6±0.6	1.5±0.5	1.6±0.8	1.6±0.3	2.0±0.7	1.6±0.4
12	Pancreas	0.3±0.02	0.4±0.03	0.3±0.04	0.4±0.07	0.2±0.08	0.2±0.03	0.3±0.04	0.3±0.04	0.2±0.07	0.2±0.05	0.4±0.02	0.3±0.05
13	Pituitary	0.2±0.01	0.1±0.0.01	0.2±0.03	0.2±0.01	0.1±0.03	0.3±0.01	0.1±0.02	0.2±0.03	0.1±0.01	0.1±0.04	0.1±0.1	0.2±0.01
14	Adrenal	0.2±0.04	0.3±0.05	0.4±0.06	0.4±0.05	0.4±0.03	0.4±0.03	0.3±0.05	0.4±0.03	0.4±0.05	0.3±0.03	0.4±0.06	0.4±0.03
15	Spleen	0.4±0.02	0.4±0.05	0.5±0.04	0.6±0.05	0.8±0.04	0.8±0.05	0.6±0.05	0.6±0.04	0.3±0.06	0.3±0.04	0.6±0.03	0.6±0.07

Value are expressed as mean ± SEM , significance with Newman-keuls Multiple comparison test following one way anova is evaluated as P* < 0.05

Relative Organs Weight: In repeated dose toxicity organs weight finding did not reveal change in any of the organ examined. The relative organ weight dose 250, 500, 1000 and 2000 mg/kg b.w. body wt. recorded at the end of study did not show any significant difference **Table 8** and **9**.

TABLE 9: REPEATED DOSE TOXICITY STUDY: RELATIVE WEIGHT OF DIFFERENT ORGANS AT DIFFERENT TIME INTERVALS AFTER REPEATED ORAL DOSING WITH PTWE FOR 28 DAYS (n=6), UNIT GRAM

S. no.	Organ wt in gram (n=5)	1000mg/kg b.w.				2000mg/kg b.w.			
	Days	7	14	21	28	7	14	21	28
1	Bw	155±5.6	155±4.8	160±5.5	160±5.5	155±4.6	150±5.5	140±4.7	-
2	Liver	5.2±0.4	5.8±0.6	5.8±0.7	5.5±0.3	4.6±0.8	4.97±0.6	5.1±0.7	-
3	Kidney L	1.21±0.3	0.9±0.5	1.41±0.6	1.1±0.2	1.23±0.4	1.57±0.6	1.6±0.5	-
4	R	1.1±0.4	1.0±0.6	1.1±0.3	1.2±0.4	1.12±0.7	1.12±0.3	1.2±0.2	-
5	Heart	1.0±0.3	1.0±0.5	1.0±0.3	0.9±0.04	0.9±0.03	0.9±0.02	0.9±0.07	-
6	Testis	1.14±0.5	0.9±0.06	1.38±0.8	1.0±0.5	1.12±0.6	1.21±0.4	1.21±0.3	-
7	Jejunum	0.6±0.02	0.6±0.05	0.6±0.04	0.82±0.06	0.7±0.03	0.5±0.02	0.3±0.08	-
8	Duodenum	0.7±0.04	0.45±0.03	0.41±0.06	0.41±0.04	0.6±0.03	0.3±0.04	0.4±0.04	-
9	Ileum	0.5±0.02	0.53±0.04	0.52±0.03	0.82±0.06	0.6±0.02	0.62±0.04	0.62±0.03	-
10	Thyroid	0.2±0.01	0.4±0.04	0.2±0.01	0.5±0.02	0.3±0.02	0.3±0.04	0.5±0.03	-
11	Brain	1.64±0.3	1.61±0.5	1.82±0.6	1.16±0.4	1.56±0.6	1.65±0.8	1.80±0.5	-
12	Pancreas	0.24±0.02	0.24±0.04	0.71±0.05	0.44±0.06	0.25±0.07	0.29±0.04	0.29±0.03	-
13	Pituitary	0.1±0.03	0.2±0.01	0.2±0.02	0.2±0.02	0.1±0.01	0.1±0.01	0.1±0.03	-
14	Adrenal	0.4±0.02	0.5±0.03	0.4±0.04	0.4±0.03	0.3±0.06	0.4±0.06	0.4±0.03	-
15	Spleen	0.6±0.03	0.52±0.04	0.63±0.05	0.63±0.06	0.8±0.04	0.7±0.05	0.61±0.04	-

Value are expressed as mean ± SEM , significance with Newman-keuls Multiple comparison test following ONE WAY ANOVA is evaluated as P* < 0.05

Histology: (Repeated dose toxicity study)

Liver: Section of liver tissue at the doses of 250 and 500 mg/kg bw of PTWE was normal. Mild infiltration was found in mononuclear cell in hepatic parenchyma without hepatocyte necrosis. At the dose 1000 mg/kg bw of PTWE did not show any adverse effect in 7th day but latter on it show slight necrosis which gradually increased up to 28 days. Where these necrosis was in range of +2. 2000 mg /kg b.w.- at dose 2000 mg/kg bw of PTWE liver damage was observed in 7th day, all those same recovery was observed on 14th day but the overall physiology of rat was not good. And the animal died on 21 days. The liver section of these dead animal showed about 60% (+ 2) necrosis and other observations are given in **Table 6, 7, 8, 9, 10, Fig. 1**.

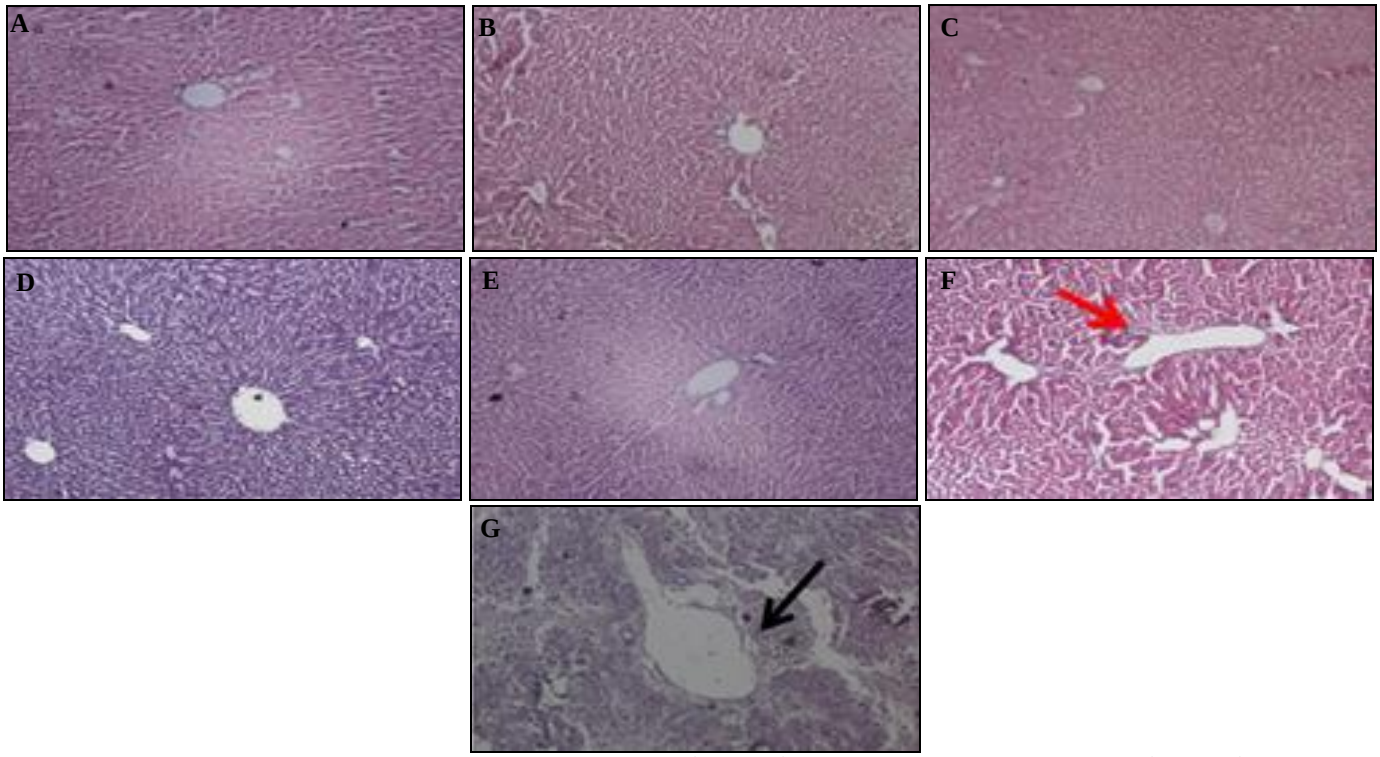

FIG. 1: LIVER HISTOLOGY- Normal control (a), Treated 2000mg/kg bw of PTWE of single dose (b), Treated 5000mg/kg bw of PTWE of single dose (c), Treated 250mg/kg bw of PTWE of Repeated dose (d), Treated 500mg/kg bw of PTWE of Repeated dose(e), Treated 1000mg/kg bw of PTWE of Repeated dose show mild necrosis (red arrow) in hepatocytes (f), Treated 2000mg/kg bw of PTWE of Repeated dose show 21 days high necrosis, hyperplasia (black arrow) in liver section (g).The image was taken at 10 X magnification

Kidney: 1000 mg/kg bw has been found to be safe upto 28 days of PTWE dosing, while 2000 mg/kg bw is safe upto 21 days with non significant and mild glomeruler dilation, in repeated dose toxicity study **Fig. 2**.

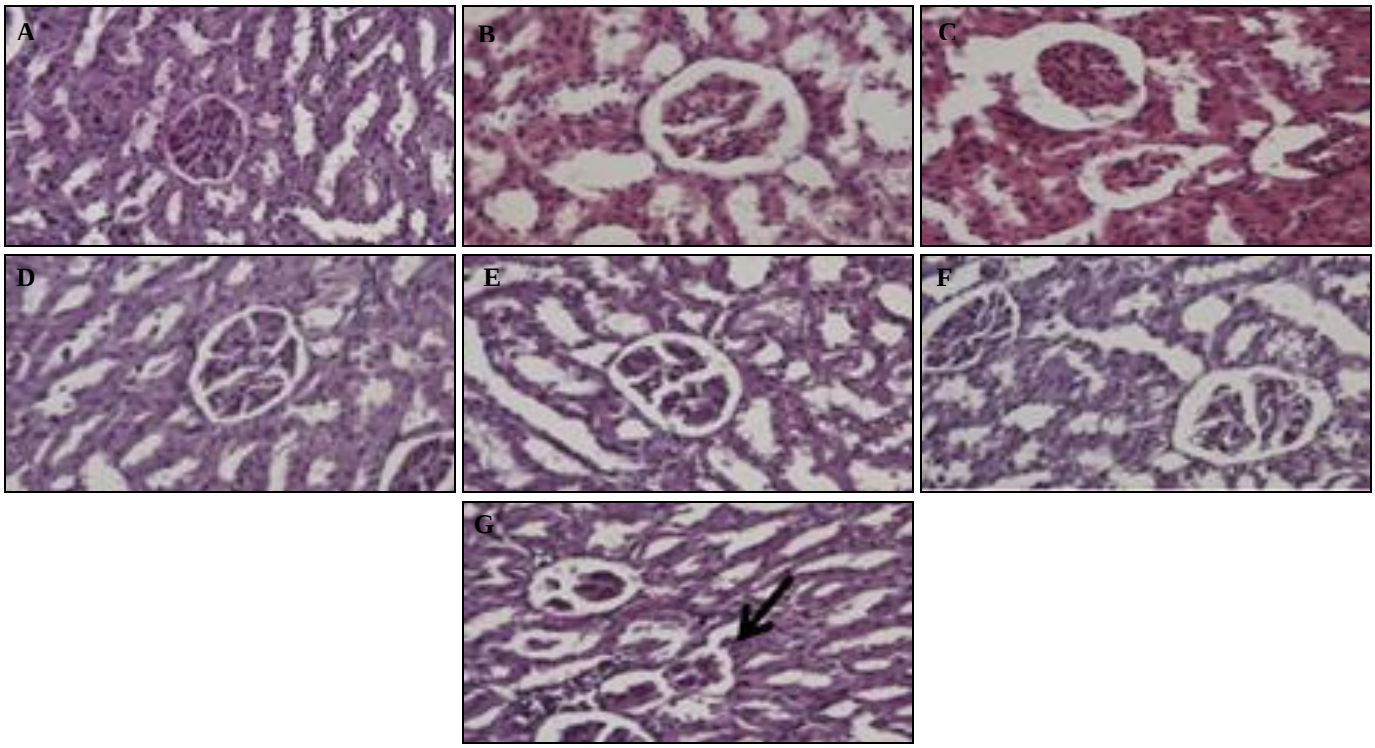

FIG. 2: KIDNEY HISTOLOGY: Normal control (A), Treated 2000mg/kg b.w. of PTWE of single dose (B), Treated 5000mg/kg b.w. of PTWE of single dose (C), Treated 250mg/kg b.w. of PTWE of repeated dose (D), Treated 500mg/kg b.w. of PTWE of Repeated dose (E),Treated 1000mg/kg b.w. of PTWE of Repeated dose (F), Treated 2000mg/kg b.w. of PTWE of Repeated dose show 21 days show mild glomerulus dilation and tubuler necrosis (black arrow) in kidney section (G) The image was taken at 40 X magnification

DISCUSSION: In developing country, herbal product prepared from medicinal plant have become famous in health care and some have been falsely considered as safe as they are obtained from natural sources. However there is a lack of data on the toxicological profile and adverse effect of many herbal product, which is limiting its use by common masses. Organ weight also is an important indicator of pathophysiological study of animal. The relative organ weight is primarily to confirmed for toxicity created or not. The heart, liver, kidney, lungs, spleen are primary organs effected by reaction caused by toxicant.

A major influence on these developments has been the test guidelines programmed of the organisation for Economic cooperation and development, which has developed slandered method of testing that are acceptable in principle by all 30 OECD member country [16]. Through on argument on the mutual acceptance of data [17]. Different test method have been devised by OECD for acute and repeated toxicity evaluation. *Pueraria tuberosa* (PTWE) was tested an acute oral oral exposure as per OECD guideline 425 and repeated dose as per OECD 407 stepwise dose procedure that utilized minimal animal to determine the toxicity of test material.

Determination of the food consumption were used in the study of safety of product with therapeutic purpose intake of nutrients is essential to the physiological states of the animals.

Acute toxicity data of limited clinical significant since cumulative toxic effect to occurs on the low dose. Hence multiple dose study are usually helpful in evaluating the safety study for phytomedicines. Body wt. changes are an indicator of adverse side effect of the animal. That survival cannot lose more than 10% of initial body wt. The result obtained from the acute toxicity study showed that the water extract of *Pueraria tuberosa* (PTWE). *Pueraria tuberosa* (PTWE) demonstrated high safety margin for the animal tolerated upto 5000 mg/kg b.w. of the extract taken orally and the LD_{50}> 5000 mg/kg b.w.

The concentration of LD_{50} is used to determined the threupatic dose of a drug. We have based on the result it was decided to used the doses 2000 and 5000mg/kg bw for single dose and the multiple doses are 250, 500, 1000, 2000mg/kg b.w.

The repeated dose toxicity studied is conducted to evaluate the adverse effect of test material *Pueraria tuberosa* (PTWE) and is carried out to provide information about the possible health hazards likely to arise from repeated exposure over a relatively limited period. The possibility of cumulative effect and the estimate of the dose at which is no observed adverse effect. The histopathological studied provided supportive evidence for biochemical and haematological observation. In the reappeared dose we found no change upto dose of 1000 mg/kg bw given for 21 days. However, on higher dose show toxicity reported as evident by body change, organ wt change and survival. In biochemical analysis for kidney no creatinine level was found in above as compared nojkrmal level, the creatinine lelvel increased those indication of renal failure [18].

Here, we have done histopathology on both liver and kidney. Liver plays wide role in drug metabolism. The specific histopathological changes found in liver usually occur after herbal drug poisoning are megalocytosis, in trahepatic blood vessel fibrosis, bile duct hyperplasia, connective tissue growth, mild steatosis [19, 20, 21,22, 23]. Similar finding have also been reported with reference to other herbal product [12, 7]. So, these dose was chosen for further studies. The toxicity at higher dose could be attribute to the presence of various phytochemical.

Many phytoestrogen stigmasterol, daidzein, genistien, [24] which are found in extract of *Pueraria tuberosa* (PTWE). The phytochemical are reported to the toxic at higher dose besides there are may have phytochemical like peurarin usually to the flavenoides are also reported to the toxic at higher dose 738 mg/kg in rodent rats [25]. *Pueraria tuberosa* (PTWE) extract mainly show hepatic toxicity of higher dose. The significance toxicity by the *Pueraria tuberosa* (PTWE) extract in the liver on the multiple dose experiments. Even at 1000mg/kg b.w. given for longer time 21 days could be presence of phytochemicals specially activity various enzyme of liver. Similar finding has been reported in drug ciprofibrate which have PPAER α agonist activity [26] the need further studies. Based on the research there was no observed adverse effect level (NOAEL) of the extract of PTWE up to 1000 mg/kg bw. The herbal tablets of PTWE was already prepared in our lab [33].

CONCLUSION: When compared the organ specific toxicity of *Pueraria tuberosa* (PTWE) it was observed the *Pueraria tuberosa* (PTWE) is more hepatotoxic and safe to the kidney at the therapeutic dose b/w hepatotoxicity was observed at 500 mg/kg bw dose beyond 14 days. But no toxicity was found in the kidney at the dose of 1000 mg/kg bw upto 28 days.

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