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Research Article

ACUTE AND SUB ACUTE TOXICITY STUDIES OF STANDARDIZED EXTRACT BASED TAB PCOSBLESS IN RATS

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ABSTRACT

The present study investigated the acute and sub acute toxicity of Tab Pcosbless a standardized extract formulation developed for the management of Poly cystic ovarian syndrome (PCOS) in experimental animals. The raw materials were standardized by gravimetric, HPTLC, pH (1.0 % Solution), Loss on drying and Water soluble extractive methods for their respective bioactive marker compounds. In acute toxicity study, Tab Pcosbless was administered orally at doses 2000 mg/kg and the animals were observed for any toxic symptoms up to the period of 14 days. The results indicated there were no toxic symptoms seen at the dose level of 2000 mg/kg. In sub acute toxicity study, Tab Pcosbless was tested at the doses 500 mg/kg, 1000 mg/kg and 2000 mg/kg once daily for 28 days. The animals were sacrificed on the 29th day and various blood biochemical parameters were measured. The liver, kidney, heart, spleen, testis (male), ovary (female), and lungs were processed for histopathological study. The results of the sub acute toxicity study did not show evidence of any abnormal changes in body weight, food intake, water intake, haematological parameters, liver function test (LFT), and renal function test (RFT) when compared with the normal animals. The female animals treated with formulation had prominent follicles developing in ovaries as observed in gross necropsy and histopathology. The vital organs of animals treated with Tab Pcosbless for 28 days did not show any histopathological evidence of pathological lesions. From the results it was concluded that Tab Pcosbless at the dose of 2000 mg/kg is safe for treatment in Poly cystic ovarian syndrome.

Keywords: Ayurveda, Acute and Sub acute toxicity, Tab Pcosbless.

INTRODUCTION

Poly Cystic Ovarian Syndrome (PCOS) is the most common endocrinologic disorder affecting women during reproductive years.[1] The rationale of present research work is to evaluate pre clinical safety profile of standardized extract based "Tab Pcosbless-" in rats as per OECD guidelines. The growing use of plant based medicines urges scientific data for safety of herbals. Tab Pcosbless, *Ayurvedic* formulation contains dried aqueous extracts ten ingredients of plants origin to treat PCOS. Maximum ingredients of "Tab Pcosbless" are *katu tikta rasatmak, laghu ruksha snigdha gunatmak, ushna viryatmak,* and *tridosha shamak*. According to Sushruta *agneya dravyas* act as a antidote for *vata* and *kapha*. They are said to be *pittakara*, too. Administration of such *dravyas* in cases of amenorrhea is advised as these drugs are *vata-kaphaghna* and due to *ushna guna*, removes *srotorodha, laghu, ruksha* and decreases *medodhatu* and improves *dhatu* metabolism by removing *ama*. In PCOS considering the *doshic* involvement, the treatment should be aimed at pacifying the vitiated *kapha*, making the *vata anuloma* and increasing the *agneyaguna* of *pitta*[2].

In present study, the acute and sub acute toxicity of Tab Pcosbless a new Ayurveda formulation, were investigated to assess its safety and tolerability profile in long term treatment.

MATERIALS AND METHODS

Drugs

All ingredients dry extract was collected from pharmacy Vadodara.

List of ingredient of Tab Pcosbless mention in (Table 1); In Batch No. VRC/001date of manufacturing 11/01/2018 in Vasu Research centre Vadodara Gujarat. In this Batch total 1000 Tablets were prepared. In each tab 500 mg active ingredients was present. After mixing of binders diluents and lubricants total weight of each tablet was around 750 mg.

Pharmacognostical study

Identification by TLC To comply with standard sample; Chemical Analysis of Tab Pcosbless mentioned in (Table 2 -3)

Approval

Experimental protocol was submitted to the animal ethical committee of the institute and approval was obtained for conducting the experimental study.

(Approval Number - CPCSEA REG NO: 921/PO/EReBi /S/05/CPCSEA, PIPH 05/17).

Animals

Wistar Albino Rats of both sexes were used. All selected animal was housed in polypropylene rat cages with 3 animals per cage. The dry rice husk was used as the bedding material and was changed every other day. Diets of Laboratory Rat pellet feed and pure drinking water was supplied *ad libitum.* Animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 30-70% and the ambient temperature during the period of experimentation was 22 ± 3˚c.

Acute Toxicity Study

Acute toxicity study was conducted as per OECD guidelines No. 423 (Acute Toxic class Method)[3]. Overnight fasted Wistar albino rats of either sex weighing 200-300 gm were administered the test formulation at 4th group dose of 2000 mg/kg body weight. The animals were closely observed for the first 12 h for any toxic symptoms and for 14 days for morbidity or mortality.

Sub acute Toxicity Study

Sub acute toxicity study was conducted as per OECD guidelines No. 407. [4] Wistar albino rats of either sex weighing 200-300 gm were randomly distributed to 4 groups, 48 adult Wistar rats (24 males + 24 females) (Table 5) were taken for the experiment. Animals were acclimatized in Standard Animal House environmental conditions for 15 days before the start of experiment. A1 served as vehicle control group treated in a similar manner as treatment group except the administration of test item. A2, A3 and A4 served as high, intermediate and low dose groups, treated with 2000, 1000 and 500 mg/kg/day of test item respectively given orally. All test group animals were doused with the freshly prepared suspension of formulation daily for a period of 28 days. Freshly prepared suspension of the formulation was administered by oral route between 11:00 to12:00 PM. The concentration of suspension was adjusted to limit dose volume. The dose was given till 28th day, and weight of animals was included in table of body weight.

Weight of the rats

The weight of the Male and Female rats was noted and recorded (Before treatment – After treatment). (Table 6-7)

The animals were sacrificed by cervical dislocation on 29thday and blood samples were collected for hematological parameters like Hb, RBC, WBC, ESR, DC, Neurophils, Eosinophils, Basophils, Lymphocytes, Hematocrit (PCV), MCV, MCH, MCHC, RDW-CV Platelet count, MPV, PCT, PDW and biochemical parameters like S. Cholesterol, S.G.O.T, S.G.P.T., S. urea, S. Creatinine after dissection specimens of heart, liver, kidney, spleen, testis (male), ovary (female) and lungs was collected.

Statistical Analysis

The data were presented as the mean ± S.EM Results were analyzed statistically using one way Analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The minimum level of significance was set at $P < 0.05$.

RESULT AND DISCUSSION

Laboratory investigation and Biochemistry of rats in Sub acute Toxicity Study mentioned in (Table 8-11) There were no signs of toxicity or mortality observed in any of the animals. Similarly other three animals which were administered the dose in second step also didn't show any signs of toxicity or mortality. From the observation we can conclude that the formulation Tab. Pcosbless does not produce any toxic effect at the dose of 2000 mg/kg. Tab Pcosbless did not show any significant changes in body weight increment at weekly intervals in all groups, indicating that it did not have any adverse effect on body weight, which is used to assess the response to therapy of drug and to indicate no adverse effect of the drug. The organ (liver, kidney, spleen, testis of male, ovary of female, lungs, heart etc) weights in the test drug treated groups remained normal, indicating that Tab Pcosbless was not toxic in these vital organs. Sections from all organs reveal normal histology except liver only one male rat of high dose group shows hemorrhagic necrosis of the hepatocytes with portal tract inflammation and congestion in spleen. Ovaries show prominent developing follicles, which is in accordance with its expected therapeutic effect. There were no significant changes in treated animals liver function parameters, such as SGPT, SGOT, S. Cholesterol. The normal levels of blood urea and S. Creatinine indicate that Tab Pcosbless did not interfere with renal function and renal integrity was preserved. Also, there were no significant changes in various hematological parameters such as Hb%, RBC, WBC, ESR, and differential count so that Tab Pcosbless does not give any toxic affect in circulating red cells, hematopoiesis, or leukopoiesis. There were no significant changes in general motor activity of animal as assessed by photoactometor.

CONCLUSION

The present findings suggest that Tab Pcosbless is nontoxic up to the dose of 2000 mg/kg, since no marked changes in haematological, biochemical, and histopathological parameters were observed. Tab Pcosbless brings about favourable changes in ovaries. Thus, at normal therapeutic doses, Tab Pcosbless is considered to be safe for long – term use.

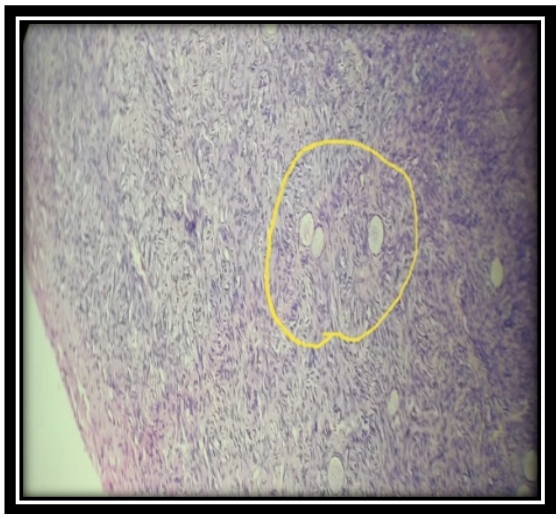

Figure 1: Ovary

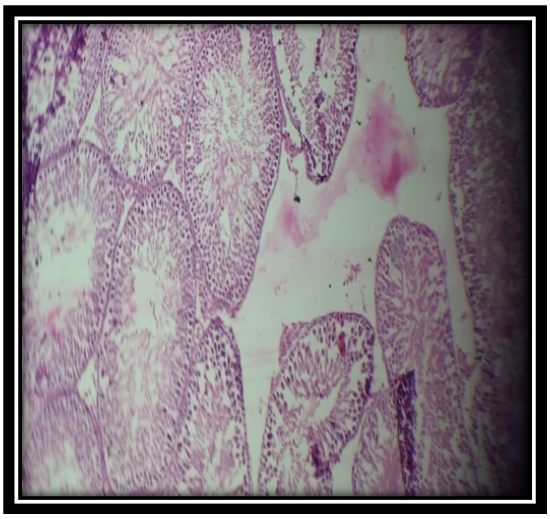

Figure 2: Testis

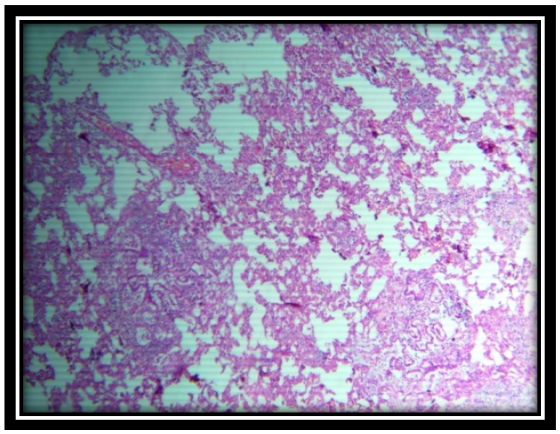

Figure 3: Lung

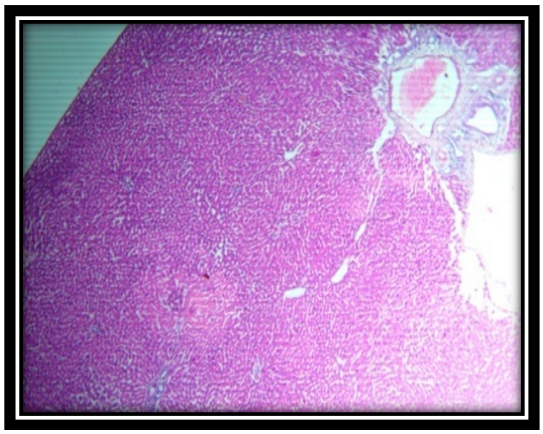

Figure 4: Liver

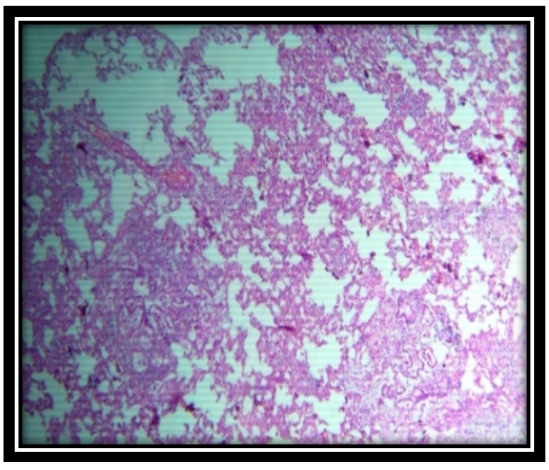

Figure 5: Spleen

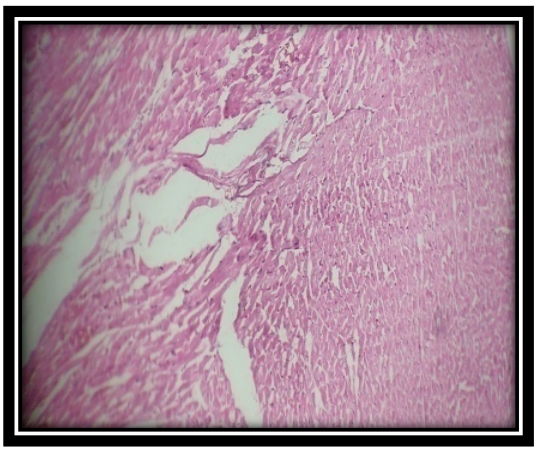

Figure 6: Heart

Table 1: List of ingredient of Tab Pcosbless

Sr.No.	Ingredients	Partused	Mg/Tab	%
1.	Latarakanj *(Caesalpinia crista)*[5] Bitter 1%	Seed	100.00	13.3
2.	Patha *(Cissampelos pareira)*[6]	Mool	50.00	6.66
3.	Guduchi *(Tinospora cordifolia)*[7]Bitter 3%	Stem	100.00	13.3
4.	Shatavari *(Asparagus racemosus)*[8]Saponin 20%	Root tuber	100.00	13.3
5.	Sathapushpa *(Anethum sowa)*[9]	Fruit	20.00	2.66
6.	Shunthi *(Zingiber officinale)*[10]	Rhizome	10.00	1.33
7.	Marich *(Piper nigrum)*Piperine[11] 10 %	Fruit	10.00	1.33
8.	Pippali (*Piper longum*)Piperine[12]10%	Fruit	10.00	10.6
9.	Kutaj (*Holarrhena antidysenterica)*[13] Alkaloid 2 %	Bark	80.00	2.66
10.	Krishna Jirak *(Carum carvi)*[14] Bitter 1.5%	Fruit	20.00	

Table 2: Chemical Analysis of Tab Pcosbless

S. No.	Name of the Test	Value
1.	LOD by Karl Fischer	01.25 %
2.	Bulk density of dried granules	0.58 gm/ml
3.	Tapped density of dried granules	0.61 gm/ml
4.	Bulk density of lubricated granules	0.62 gm/ml
5.	Tapped density of lubricated granules	0.62 gm/ ml

Table 3: Compression parameters of Tab Pcosbless

S. No.	Name of the Test	Value
1.	DT	20 mins- 22 mins
2.	Hardness	55 hg/cm^2 – 6.0 hg/cm^2
3.	Friability- Initial act	7.50gm
4.	After 100 RPM	7.49 gm 7.50 – 7.49 ÷ 7.50 × 100 = 0.13ss
5.	Thickness	6.00 ± 0.2mm
6.	Weight variation	750 mg ± 3 %
7.	Min weight	728 mg
8.	Max weight	772 mg

Table 4: Acute Toxicity Study

Study plan (Acute toxicity study)				
Group	**I**	**II**	**III**	**IV**
Dose (mg/kg/day)	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Number of animals	6 Rats F	6 Rats F	6 Rats F	6 Rats F
Duration of treatment	1 day	1 day	1 day	1 day
Route of administration	Oral	Oral	Oral	Oral

Table 5: Sub acute Toxicity Study

Group	Treatment	Dose (mg/kg bw/day)	Route of administration	No. of animal / group
A1	Control	-	Oral	6 male + 6 female
A2	Low dose	500		6 male + 6 female
A3	Medium dose	1000		6 male + 6 female
A4	High dose	2000		6 male + 6 female

Table 6: Every 15th day the weight of the Male rats was noted and recorded

Animals Male Rat	A4Wt difference B.T to A.T	A3 Wt difference B.T to A.T.	A2 Wt difference B.T to A.T
Head	310 gm- 400 gm	260 gm-350gm	260 gm-390gm
Body	330 gm- 400 gm	250 gm-300gm	200 gm-280gm
Tail	260 gm-350gm	260 gm-260gm	330 gm-410gm
Head body	270 gm-340gm	240 gm-350gm	250 gm-330gm
Head tail	260 gm-350gm	330 gm-390gm	250 gm-350gm
No marking	340 gm-400gm	270 gm-340gm	350 gm-400gm

Table 7: Every 15th day the weight of the Male rats was noted and recorded

Animals Female Rat	A4Wt difference B.T to A.T	A3Wt difference B.T to A.T.	A2 Wt difference B.T to A.T
Head	360 gm-400gm	420 gm-400gm	450 gm-440gm
Body	370 gm-400gm	450 gm-460gm	390 gm-400gm
Tail	310 gm-350gm	400 gm-430gm	390 gm-410gm
Head body	240 gm-330gm	250 gm-360gm	330 gm-410gm
Head tail	350 gm-390gm	260 gm-350gm	270 gm-380gm
No marking	220 gm-310gm	310 gm-340gm	260 gm-340gm

Table 8: Laboratory investigation of female rats in Subacute Toxicity Study

FEMALE RATS									
Laboratory investigation	**CG**		**HD**		**MD**		**LD**		
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Remarks**
Haemoglobin	15.9	0.569	14.450	0.527	15.800	0.545	13.68	0.510	NS
WBC	4388.33	361.7	5662.78	463.2	6210.9	356.67	4845.6	376.6	NS
Neurophils	72.66	2.87	74.74	3.76	73.54	2.95	72.65	2.79	NS
Eosinophils	2	0	2	0	1	0	2	0	NS
Basophils	0	0	0	0	0	0	0	0	NS
Lymphocytes	22.33	2.55	23.5	2.78	22.45	2.46	24.87	3.1	NS
Monocytes	2	0	2	0	3	0	2	0	NS
RBC count	8.82	0.29	9.20	0.31	8.50	0.26	7.98	0.24	NS
Hematocrit (PCV)	47.31	1.01	48.43	1.02	43.78	0.9	45.78	0.89	NS
MCV	51.4	0.581	52.4	0.59	50.9	0.56	50.7	0.55	NS
MCH	17.38	0.317	17.89	0.42	16.98	0.29	17.78	0.31	NS
MCHC	32.05	0.93	31.09	0.96	32.68	0.92	30.08	0.89	NS
RDW-CV	14.58	0.64	14.87	0.67	13.98	0.62	14.93	0.61	NS
Platelet count	774833	89339	765468	88348	785078	89872	785672	90678	NS
PCT	0.526	0.012	0.543	0.022	0.495	0.021	0.463	0.019	NS
PDW	14.68	0.166	14.27	0.17	15.11	0.18	15.42	0.19	NS

Table 9: Biochemistry of female rats in Subacute Toxicity Study

Female Rats									
Biochemistry	**CG**		**HD**		**MD**		**LD**		**Remarks**
S. Cholesterol	65.83	6.52	66.73	6.9	64.87	6.1	67.72	7.4	NS
S.G.O.T	133.8	26.5	129.2	24.3	145.1	28.5	134.4	26.1	NS
S.G.P.T.	45.8	2.58	44.7	2.24	46.8	1.91	45.7	2.57	NS
S.Urea	51.5	3.20	51.9	3.6	55.7	3.7	50.9	3.6	NS
S. Creatinine	0.80	0.036	0.79	0.032	0.88	0.042	0.81	0.038	NS

Table 10: Laboratory investigation of male rats in Sub acute Toxicity

Male Rats									
	CG		**HD**		**MD**		**LD**		
Laboratory investigation	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Remarks**
Haemoglobin	16.1	0.569	14.55	0.527	15.700	0.315	14.68	0.510	NS
Twc	4845.65	376.6	5662.78	463.2	6210.9	356.6	4388.3	361.7	NS
Neurophils	72.66	2.87	74.74	3.76	73.54	2.95	72.65	2.79	NS
Eosinophils	2	0	2	0	2	0	2	0	NS
Basophils	0	0	0	0	0	0	0	0	NS
Lymphocytes	22.33	2.55	23.5	2.78	22.45	2.46	24.87	3.1	NS
Monocytes	2	0	2	0	2	0	2	0	NS
RBC count	8.82	0.29	9.20	0.31	8.50	0.26	7.98	0.24	NS
Hematocrit (PCV)	47.31	1.01	48.43	1.02	43.78	0.9	45.78	0.89	NS
MCV	51.4	0.581	52.4	0.59	50.9	0.56	51.7	0.55	NS
MCH	17.38	0.317	17.89	0.42	16.98	0.29	17.78	0.31	NS
MCHC	32.05	0.93	31.09	0.96	32.68	0.92	30.08	0.89	NS
RDW-CV	14.58	0.64	14.87	0.67	13.98	0.62	14.93	0.61	NS
Platelet count	785672	89339	765468	88348	785078	89872	774833	89678	NS
PCT	0.526	0.012	0.543	0.022	0.495	0.021	0.463	0.019	NS
PDW	14.68	0.166	14.27	0.17	15.11	0.18	15.12	0.17	NS

Table 11: Biochemistry of male rats in Subacute Toxicity Study

Male Rats									
Biochemistry	**CG**		**HD**		**MD**		**LD**		**Remark**
S. Cholesterol	64.83	6.52	66.73	6.9	66.17	6.8	64.72	6.4	NS
S.G.O.T	134.8	26.8	128.2	25.3	145.1	28.5	134.4	26.1	NS
S.G.P.T.	45.8	2.58	44.7	2.24	46.8	1.91	45.7	2.57	NS
S.Urea	51.5	3.20	51.9	3.6	55.7	3.7	50.9	3.6	NS
S. Creatinine	.79	.032	.80	.036	.88	.042	.81	.038	NS

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