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**TOXICOLOGICAL STUDIES ON HERBAL FORMULATION USED FOR  
TREATMENT OF INFERTILITY**

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Received 12<sup>th</sup> Nov. 2016; Revised 10<sup>th</sup> Jan. 2017; Accepted 4<sup>th</sup> April 2017; Available online 1<sup>st</sup> Sept. 2017

**ABSTRACT**

Infertility is inability of human, animals to breed by natural means. In Quetta Baluchistan, many herbal practitioners use the formulation (Infertalan) which consists of *Chlorophytum borivilianum*, *Curculigo orchioides*, *Butea frondosa*, *Dactylorhiza hatagirea*, *Asteracantha longifolia*, *Plantago ovata* husk and *Orchis laxiflora*. Current study was carried out to determine the toxicological effects of formulation. Rabbits were used as test animal and 300mg/kg oral dose of formulation was administered for ninety (90) days. Long term toxicity was determined by biochemical parameters i.e. Kidney function test, blood glucose, cardiac enzymes, hematological parameters, urea, liver function test, serum calcium, and lipid profile. Results of this study showed that administration of formulation results in significant decrease ( $P < 0.05$ ) in serum creatinine and urea level. Nominal increase was observed in blood glucose level, in cardiac enzymes the level of LDH was notably ( $P < 0.05$ ) increased. In liver function test there was non-

substantial change in Total Bilirubin, Direct Bilirubin, Alkaline Phosphatase, SGOT, Total proteins, globulin, albumin and A/G ratio. In lipid profile test there was non-significant change in Cholesterol level and Triglycerides. There was non-significant change in the level hematological parameters and there was noteworthy ( $P < 0.05$ ) increase in platelets count. Results of current reveals that formulation did not show significant toxicity on kidneys, heart, liver, and Blood.

**Keywords: Balochistan, Chronic Toxicity, infertility**

## INTRODUCTION

Infertility is a pathological condition in which individuals, male or female, fail to conceive and produce offspring [1]. However, it has been set as a global trend not to bear child due to postponement of marriage, educational achievements, and professional pursuits [2]. Infertility is a very common with financial, medical, and psychological causes. There has always been a uncertainty among such patients about the effectiveness of the treatment plans for the infertility [2]. It is a feared condition that is related with damaging psychosocial consequences [3-4]. The child-bearing is greatly valued, infertile couples are faced with many problems ranging from overtvert or divorce which may cause isolation and mental distress in most of the countries [5, 6-8]. Due to lethal side effects of allopathic medicines and patient noncompliance, herbal medicines are becoming more and more popular as an alternative treatment for the cure of infertility [9].

Patients using herbal treatment had 1.74 times greater probability of being pregnant as compare to using western medicines as per systemic review [2]. Balochistan is largest province of the country [10, 11] and many medical plants are found in Balochistan [12]. In Quetta Baluchistan many herbal practioners use the formulation (Infertalan) which consist of *Chlorophytum borivilianum*, *Curculigo orchioides*, *Butea frondosa*, *Dactylorhiza hatagirea*, *Asteracantha longifolia*, *Plantago ovata husk*, *Orchis laxiflora*. No Data was available on the toxicity of the formulation; in this regard current study was aimed to investigate the toxicological effects of the formulation on rabbits by using chronic toxicity tests.

## MATERIAL AND METHOD

### Plant Material /Formulation

Formulation was consist of *Chlorophytum borivilianum* (18 gm) *Curculigo orchioides* (18 gm) *Butea frondosa* (31.25gm) *Astera cantha longifolia* (31.25gm) *Dactylo*

*rhizahatagirea* (18 gm) *Plantago ovata* husk (31.25gm) *Orchis laxiflora* (18 gm), plant material of formulation was collected from local market, converted into fine powder form.

### Animals

For chronic toxicity studies, rabbits (male and female) weighing about 1000 -1400 g were used. All the rabbits were provided by Dow University of Health Sciences (DUHS). Temperature and humidity (Standard conditions) were maintained for 12-hour light / dark cycle [13]. Standard protocols (Good Laboratory Practice) were followed [14].

### Dose Administration

Suspension of fine powder of formulation was made with distil water and administered at 300 mg/kg oral dose orally for 90 days.

### Biochemical test

Blood sample was drawn by using sterile needle of 22 gauge by cardiac puncture technique. The blood was drawn in test tube and allowed to coagulate. Serum was separated by using centrifuge. For Biological test, method described by Ahamefule [15] was used. All tests were performed to find out the Total proteins such as, Globulin, Albumin, Lipid Profile i.e., Cholesterol, Triglycerides. Liver function test i.e. Alkaline Phosphatase, Direct Bilirubin, Total

Bilirubin, and SGOT. Cardiac enzymes i.e. LDH, CK-MB and SGOT. Renal profile i.e. Urea and Creatinine and Blood Glucose, [15] were determined by using serum chemistry analyzer [16].

### Analysis of Hematological Profile

By using sterile needle Blood sample was drawn by cardiac puncture technique. Sample was collected in test tubes containing EDTA (anticoagulants) to prevent clotting [13]. Parameter were RBC count, WBC platelet count, hemoglobin (HB), mean corpuscular hemoglobin (MCH), Hematocrit (HCT) / Pack cell volume( PVC) count, MCV ( mean corpuscular volume), MCHC, Total WBC Count, Platelet Count is determined [16].

### STATISTICAL ANALYSIS

Data calculated as mean  $\pm$  SEM. Whereas ANOVA ( one way ) was used for analysis of biochemical parameter followed by Dunnett's t test, extract treated group were compared with control group and  $P < 0.05$  values was considered as significant, while  $P < 0.01$  value considered as highly significant [16, 23].

### RESULTS

#### Kidney function test

Level of Urea (mg/dL) was  $77 \pm 2.923$  for control and  $70 \pm 3.409$  for drug treated rabbit. And Level of Creatinine was  $1.2 \pm 0.190$  for

control and  $0.6 \pm 0.034$  for drug treated rabbit (Table 1).

#### **Blood Glucose (random)**

Blood Glucose (Random) was  $100 \pm 9.454$  for control and  $104.9 \pm 1.456$  for drug treated rabbit (Table 2)

#### **Cardiac Enzymes**

Level of LDH (U/L) was  $720.2 \pm 98.315$  for control and  $730 \pm 19.290$  for drug treated rabbit. CK- MB (U/L) level was  $30.2 \pm 0.802$  for control, and  $24.4 \pm 0.821$  for drug treated rabbit. SGOT (U/L) level was  $94.2 \pm 3.797$  for control, and  $95.8 \pm 3.550$  for drug treated rabbit (Table 3).

#### **Serum Calcium and Uric acid**

Calcium-Serum (mg/dL) was  $13.3 \pm 0.512$  for control, and  $12.9 \pm 0.278$  for drug treated rabbit. Uric acid (mg/dL) level was  $0.2 \pm 0.054$  for control and  $0.1 \pm 0.008$  for drug treated rabbit (Table 4).

#### **Hematological profile**

Hb (g/dl) was  $12.22 \pm 0.571$  for control, and  $11.64 \pm 0.150$  for drug treated rabbit. RBC Count (million/ul) was  $5.95 \pm 0.333$  for control and  $5.72 \pm 0.102$  for drug treated rabbit. Hematocrit (HCT/PCV) % was  $40.74 \pm 2.292$  for control, and  $39 \pm 1.143$  for drug treated rabbit. MCV (fl) was  $67.9 \pm 0.878$  for control and  $63.3 \pm 0.660$  for drug treated rabbit. MCH (pg) was  $20.46 \pm 0.386$  for control, and  $20.44 \pm 0.238$  for drug treated

rabbit. MCHC (g/l) was  $30.14 \pm 0.692$  for control and  $29.8 \pm 0.965$  for drug treated rabbit. Total WBC Count ( $\times 10^9/L$ ) was  $9.076 \pm 0.773$  for control, and  $8.32 \pm 0.193$  for drug treated rabbit. Platelet Count ( $\times 10^9/L$ ) was  $324.4 \pm 34.223$  for control and  $471.2 \pm 4.488$  for drug treated rabbit (Table 5).

#### **Liver function test**

Total Bilirubin (mg/dL) was  $0.234 \pm 0.020$  for control, and  $0.216 \pm 0.008$  for drug treated rabbit. Direct Bilirubin (mg/dL) was  $0.072 \pm 0.013$  for control and  $0.07 \pm 0.003$  for drug treated rabbit. Alkaline Phosphatase (U/L) was  $51.8 \pm 3.421$  for control, and  $52.4 \pm 1.754$  for drug treated rabbit. SGOT (U/L) was  $80.2 \pm 3.797$  for control and  $81.8 \pm 3.550$  for drug treated rabbit (Table 6).

#### **Total protein**

Level of Total proteins (g/dL) was  $6.64 \pm 0.116$  for control, and  $6.2 \pm 0.070$  for drug treated rabbit. Globulin (g/dL) level was  $2.54 \pm 0.129$  for control and  $2.72 \pm 0.107$  for drug treated rabbit. Albumin (g/dL) level was  $4.1 \pm 0.138$  for control, and  $4.2 \pm 0.083$  for drug treated rabbit. A/G ratio was  $1.634 \pm 0.127$  for control, and  $1.66 \pm 0.024$  for drug treated rabbit (Table 7).

#### **Lipid profile**

Cholesterol (mg/dL) was  $34.2 \pm 4.028$  for control, and  $35.2 \pm 1.360$  for drug treated rabbit. Triglycerides (mg/dL) were

49.2±4.621 for control and 51.6±1.079 for drug treated rabbit (Table 8).

## **DISCUSSION**

Results of this study showed that after administration of formulation, serum creatinine and urea concentration was decreased significantly probably due to the extract effect on liver function [18]. This protective effect may be due to high level of total antioxidant contents in this plant [19]. Finding of the current study shows that formulation has non toxic profile on rabbit kidneys. Administration of formulation results in non significant increase in blood glucose level that may be carbohydrate content of the formulation. It is well known that diagnosis of cardiac enzymes is important. Serum CK activity is a more sensitive indicator in early stage of myocardial ischemia, while peak rises in LD is roughly proportional to the extent of injury to the myocardial tissue [20]. Administration of formulation results in non significant change in the level of LDH and SGOT. Level of CKMB was decreased significantly. The significantly lowered activities CKMB signifies that the potential of reducing the factors that produce infarction in the myocardium [21]. So the results of the current study suggest that formulation have

protective effect on heart. Administration of formulation results in non significant change in the level serum calcium and uric acid. In Hematological profile administration of formulation results in non significant change in the level of Hb, RBC Count, Hematocrit (HCT/PCV) %, MC, MCH, MCHC, WBCs count. There was significant increase in the level of platelets. Literature reveals that reulation of the ALOX 12 and PTAFR gene leads to an increased production of megakaryocytes and its conversion into platelets [22] evidence of current study shows that formulation may increases ALOX 12 activity and PTFAR activity which is responsible for increased platelet production [22]. In liver function test administration of formulation results in non significant change in the level of Total Bilirubin, Direct Bilirubin, Alkaline Phospatase, SGOT., Total proteins , Albumin, A/G ratio. Results f current study shows that the formulation does not any toxicity in liver function test. Administration of formulation results in non significant change the level of cholesterol and triglycerides, results reveals that the formulation have non toxic effect on lipid profile.

Table 1: Effect of Herbal Formulation on Kidney function test on rabbit

S. No.	Test	Control (Mean± SEM)	Drug treated (Mean ±SEM)
1	Urea (mg/dL)	77±2.923	70±3.409
2	Creatinine	1.2±0.190	0.6±0.034

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

Table 2: Effect of Herbal Formulation on Blood Glucose (random) of rabbits

S. No.	Test	Control (Mean ± SEM)	Drug treated (Mean ±SEM)
1	Blood Glucose Random	100± 9.454	104.9±1.456

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

Table 3: Effect of Herbal Formulation on Cardiac Enzymes of male rabbit

S. No.	Test	Control (Mean± SEM)	Drug treated (Mean ±SEM)
1	LDH (U/L)	720.2±98.315	730±19.290
3	CK-MB (U/L)	30.2±0.802	24.4±0.821
4	SGOT (U/L)	94.2±3.797	95.8±3.550

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

Table 4: Effect of Herbal Formulation on Serum Calcium and Uric acid

S. No.	Test	Control (Mean ± SEM)	Drug treated (Mean ±SEM)
1	Calcium-Serum (mg/dL)	13.3±0.512	12.9±0.278
3	Uric acid (mg/dL)	0.2±0.054	0.1±0.008

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

Table 5: Effect of Herbal Formulation on Blood of male rabbits

S. No.	Test	Control (Mean± SEM)	Drug treated (Mean ±SEM)
1	Hb (g/dl)	11.22±0.571	11.64±0.150
2	RBC Count (million/ul)	5.95±0.333	5.72±0.102
3	Hematocrit (HCT/PCV) %	40.74±2.292	39±1.143
4	MCV (fl)	67.9±0.878	63.3±0.660
5	MCH (pg)	20.46±0.386	20.44±0.238
6	MCHC (g/l)	30.14±0.692	29.8±0.965
7	Total WBC Count ( $\times 10^9/L$ )	9.076±0.773	8.32±0.193
8	Platelet Count ( $\times 10^9/L$ )	324.4± 34.223	471.2±4.488

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

Table 6: Effect of Herbal Formulation on Liver function test of rabbit.

S. No.	Test	Control (Mean± SEM)	Drug treated (Mean ±SEM)
1	Total, Bilirubin (mg/dL)	0.234±0.020	0.216±0.008
2	Direct Bilirubin (mg/dL)	0.072±0.013	0.07±0.003
4	Alkaline Phospatase(U/L)	51.8±3.421	52.4±1.754
6	SGOT (U/L)	80.2±3.797	81.8±3.550

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

Table 7: Effect of Herbal Formulation on Total Protein test of male rabbits

S. No.	Test	Control (Mean± SEM)	Drug treated (Mean ±SEM)
1	Total proteins (g/dL)	6.64±0.116	7.2±0.070
2	Albumin (g/dL)	4.1±0.138	4.2±0.083
3	Globulin (g/dL)	2.54±0.129	2.72±0.107
4	A/G ratio	1.634±0.127	1.66±0.024

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

Table 8: Effect of Herbal Formulation on Lipid profile of rabbit

S. No.	Test	Control (Mean± SEM)	Drug treated (Mean ±SEM)
1	Cholesterol (mg/dL)	34.2±4.028	35.2±1.360
2	Triglycerides (mg/dL)	49.2±4.621	51.6±1.079

All values are mean ± SEM; n=5; \* = Significant ( $P<0.05$ ), \*\* = highly significant ( $P<0.01$ ).

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