REPEATED DOSE ORAL TOXICITY STUDY OF AN ANTIDIABETIC POLYHERBAL FORMULATION (*DIAPAL* TABLETS) IN RATS

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Summary

Diapal, a polyherbal antidiabetic tablet formulation, was evaluated for its safety at the therapeutic dose level by repeated dose oral toxicity study in rats. *Diapal* tablets contain ingredients of herbal origin viz, Neem, Turmeric, Fenugreek, Arjun, Triphala etc. The herbal formulation was administered orally to rats at the therapeutic dose of 250 mg/kg/day for 90 days. All animals were monitored daily for their health status for signs of abnormalities. The body weight, water consumption and food intake of the rats were measured once weekly. At the end of the study period, various hematological, biochemical parameters were measured and histopathological examination of selected organs were conducted. The study resulted from long term oral administration of herbal formulation (250 mg/kg) did not cause any relevance of serious signs and significant changes in physical, hematological and biochemical parameters as compared with control. Moreover, no pathological features were identified in treated group as monitored by histopathological analysis of the internal organs. The study established that *Diapal* tablets at the dose given in the present study did not induce any remarkable toxic effects and it is safer in the rats following oral administration for 90 consecutive days.

Key words: Repeated dose toxicity, Polyherbal formulation, Subchronic toxicity

Introduction

Herbal medicines are increasingly sought by patients as a source of prescription drugs in the developed and developing countries and it's proven with undeniable and tangible therapeutic benefits. Herbal formulations have attained wide recognition and popularity world wide as therapeutic agents in various ailments that impact quality of life. There is a need to look for more efficacious herbs and the most important goal is to assure the status of its safety. The WHO insists safety of herbal medicines which is a critical component in quality control. *Diapal* tablets, a polyherbal antidiabetic formulation containing fifteen ingredients of herbal origin which are used in traditional medicine to treat type II diabetes contains antidiabetic and antioxidant principles. The ingredients are extracts of *Syzygium cumini, Curcuma longa, Embelica officinalis, Momordica charantia, Terminalia arjuna, Gymnema sylvestre, Aegle marmelos, Ficus bengalensis, Azadirachta indica, Trigonella foenum graecum, Mukul, Triphala* etc. In the present study, the safety profile of *Diapal* tablets was investigated at the therapeutic dose level by sub chronic oral toxicity study (1).

Materials and Methods

Drugs

Diapal tablets, a polyherbal formulation containing crude extracts obtained from various herbs Viz, *Syzygium cumini,Curcuma longa, Embelica officinalis, Momordica charantia, Terminalia arjuna, Gymnema sylvestre, Aegle marmelos, Ficus bengalensis, Azadirachta indica, Trigonella foenum graecum, Mukul, Triphala etc.* Clinical chemistry kits for serum parameters were purchased from span diagnostics, Mumbai, India.

Animals

Female Albino Wistar rats were procured from Haffkine's research centre and housed in animal house of C.U.Shah College of Pharmacy. Animals were conditioned at room temperature and natural photoperiods. Each group of rats was separately housed in standard cages and had free access to water and standard pellet diet. The study protocol was approved by Institutional animal Ethical Committee of C.U.Shah College of Pharmacy (CUSCP/IAEC/10/09-10).

Dose selection

The rat therapeutic dose of *Diapal* tablets was selected as 250 mg/kg by calculating from the human clinical dose (2700 mg/ day/ 70 kg) using the conversion factor 0.018 based on the total body surface area of the rat (2). The animals were divided into two groups, each having five animals while the control group received 0.05 % suspension of CMC and the second group received tablet 250 mg/kg/day of *diapal*.The drug suspended in 0.05% CMC and the vehicle was administered in the volume of 5 ml/kg.

Repeated dose 90 days oral toxicity

For the 90 days sub chronic toxicity study, groups of 5 female albino wistar rats were randomly selected. The control animals received 0.05% CMC and the test animals received powdered *Diapal* tablets suspended in 0.05% CMC administered orally for 90 days at the calculated therapeutic dose levels of 250 mg/kg/day. All animals were monitored daily for their health status for signs of abnormalities. The animals were weighed weekly once. The water consumption and food intake of the rats were measured once weekly.

At the end of the experimental period, the rats were fasted overnight and blood samples were withdrawn from the retro orbital plexus. Blood and serum samples were used for the various hematological and biochemical estimations. A complete necropsy, including selected organ weights, was conducted on all rats. Histopathologic examination was conducted on selected organs preserved in 10% formalin solution.Tissues were processed, sectioned and stained with hematoxylin and eosin and were examined by a pathologist (3-7).

Statistical analysis

All statistic analyses were made using the software InStat for windows. Results were expressed as mean ± SEM. The values were considered statistically significant when $p<0.05$.

Results

Effect of *Diapal* tablets on body weight

Body weights of all the animals were measured every week through out the study and presented in Table 1. Body weight of the rats treated with therapeutic dose of tablet was not significantly different from control. Overall, the changes in body weight among the groups were not statistically significant.

Table 1 : Effect of *Diapal* tablets on body weight in rats treated for 90 days

Study week	Body weight (g)	
	Control	Diapal [a]
1	248.60 ± 12.98	246.40 ± 11.61
2	247.40 ± 10.89	244.20 ± 11.20
3	250.60 ± 11.35	249.80 ± 12.70
4	250.20 ± 11.11	247.00 ± 13.32
5	248.80 ± 10.74	247.40 ± 12.42
6	253.00 ± 10.73	250.80 ± 13.88
7	252.20 ± 12.36	249.60 ± 14.27
8	252.40 ± 11.15	248.20 ± 14.46
9	254.00 ± 12.42	246.40 ± 13.84
10	255.00 ± 12.69	248.00 ± 13.86
11	258.00 ± 13.54	251.60 ± 15.17
12	256.20 ± 13.47	252.80 ± 14.19
13	253.00 ± 13.85	254.40 ± 14.29

Values are expressed as mean ± SEM, n = 5

[a] Dose of 250 mg/kg *Diapal* tablets administered orally daily for 90 days.

Effects of *Diapal* tablets on food intake and water consumption

Food intake and water consumption of all the animals were measured once weekly through out the study and presented in Table 2. The food intake and water consumption of female rats treated with *Diapal* tablets at the given dose levels did not show any significance from their control.

Table 2 : Effects of *Diapal* tablets on food intake in rats treated for 90 days.

Study week	Food Intake (g)		Water intake (ml)	
	Control	Diapal [a]	Control	Diapal [a]
1	13.1 ± 0.3	12.5 ± 0.5	30.2 ± 0.7	30.8 ± 1.0
2	13.1 ± 0.3	11.5 ± 0.5	29.3 ± 0.7	30.2 ± 1.0
3	13.4 ± 0.5	11.9 ± 0.7	29.4 ± 0.7	29.8 ± 1.0
4	13.6 ± 0.4	12.7 ± 0.7	30.0 ± 0.8	29.4 ± 0.8
5	12.7 ± 0.6	10.8 ± 1.0	32.0 ± 1.2	30.0 ± 1.6
6	14.2 ± 1.0	13.9 ± 1.1	27.4 ± 2.0	31.4 ± 2.3
7	14.5 ± 1.0	12.7 ± 1.0	29.4 ± 1.2	29.0 ± 1.8
8	15.1 ± 0.5	14.1 ± 0.4	28.2 ± 1.1	26.5 ± 1.5
9	14.3 ± 0.5	13.0 ± 0.4	29.2 ± 1.0	29.8 ± 1.3
10	14.1 ± 0.8	12.8 ± 1.0	29.2 ± 0.4	28.0 ± 0.9
11	13.2 ± 1.3	12.9 ± 0.8	28.6 ± 1.3	30.6 ± 1.3
12	13.4 ± 1.2	13.3 ± 0.9	27.8 ± 1.2	31.6 ± 1.4
13	13.2 ± 0.9	12.3 ± 0.8	28.2 ± 1.0	30.8 ± 1.9

Values are expressed as mean ± SEM, n = 5

[a] Dose of 250 mg/kg *Diapal* tablets administered orally daily for 90 days.

Effect of *Diapal* tablets on gross morphology

At the end of the observation period all rats were sacrificed and autopsied. All major organs including heart, brain, lung, kidney, liver, spleen, ovary, thymus and pancreas were examined grossly. There were no detectable abnormalities in treated groups indicating that there were no remarkable gross differences among the treatment group when compared with control.

Effects of *Diapal* tablets on relative organ weight

The relative organ weights were taken after the animals being sacrificed (Table 3). No statistically significant changes in the organ weights and relative organ weights were seen in the treatment groups as compared with control. Even the internal organs did not exhibit any gross morphological lesions or histopathological features. Therefore, the results obtained suggest that *Diapal* tablets are fairly nontoxic.

Table 3 : Effect of *Diapal* tablets on relative organ weight in rats treated for 90 days

S. No	Organs	Relative Organ weights (kg)	
		Control	Diapal [a]
1	Liver	25.19 ± 1.43	25.10 ± 0.75
2	Kidney	6.37 ± 0.37	5.92 ± 0.47
3	Brain	6.24 ± 0.59	6.12 ± 0.49
4	Ovary	1.09 ± 0.18	0.73 ± 0.05
5	Uterus	0.33± 0.05	0.83 ± 0.25
6	Heart	4.23 ± 0.44	3.86 ± 0.42
7	Lung	7.90 ± 0.86	6.95 ± 0.77
8	Adrenals	0.72 ± 0.12	0.53 ± 0.04
9	Spleen	3.25 ± 0.29	3.40 ± 0.51

Values are expressed as mean ± SEM, n = 5

[a] Dose of 250 mg/kg *Diapal* tablets administered orally daily for 90 days.

Effect of *Diapal* tablets on Hematological parameters

The hematological profile of the control and treated groups are presented in Table 4. The repeated oral treatment for 90 days with *Diapal* did not cause any significant changes in all the hematological parameters among the control and treated groups. Therefore, no hematological changes were noted on treatment with *Diapal* tablets.

Table 4: Effect of *Diapal* tablets on hematological parameters in rats treated for 90 days

Parameter	Control	Diapal [a]
RBC(10x12/l)	8.01 ± 0.40	8.57 ± 0.49
WBC(10 x 9/l)	9.32 ± 1.48	7.60 ± 1.29
PLT(10 x 9/l)	1043.6 ± 146.61	793.60 ± 245.99
LYM%	74.3 ± 9.5	75.2 ± 8.5
MON%	0.71 ± 0.14	0.72 ± 0.04
GRA%	24.6 ± 9.6	24.1 ± 8.6
HGB(g/dl)	13.62 ± 0.66	12.32 ± 1.18
HCT%	53.34 ± 2.70	37.72 ± 10.23
MCV(fl)	67.0 ± 3.7	59.8 ± 3.4
MCH(pg)	17.08 ± 0.67	28.44 ± 8.19
MCHC(g/dl)	25.6 ± 0.7	47.4 ± 14.5
Clotting time(sec)	84.0 ± 11.2	78.0 ± 7.3

Values are expressed as mean ± SEM, n = 5

[a] Dose of 250 mg/kg *diapal* tablets administered orally daily for 90 days.

Table 5 : Effect of *Diapal* tablets on serum biochemical parameters in rats treated for 90 days

Parameter	Control	Diapal [a]
SGPT/ALT (IU/L)	49.9 ± 5.7	43.8 ± 4.6
SGOT/ AST (IU/L)	102.56 ± 6.10	100.02 ± 9.55
ALP (IU/L)	49.6 ± 10.9	45.2 ± 4.9
Total protein (g/dl)	9.9 ± 0.5	10.2 ± 0.5
Albumin (g/dl)	6.0 ± 0.2	6.8 ± 0.2
BUN (mg/dl)	14.1 ± 0.9	26.2 ± 4.0 *
Creatinine (mg/dl)	0.47 ± 0.02	0.45 ± 0.02
Glucose (mg/dl)	53.94 ± 7.31	59.39 ± 8.43
Cholesterol (mg/dl)	82.86 ± 6.61	75.72 ± 5.77
Triglyceride (mg/dl)	68.25 ± 10.47	70.73 ± 9.59

Values are expressed as mean ± SEM, n = 5

[a] Dose of 250 mg/kg *diapal* tablets administered orally daily for 90 days.

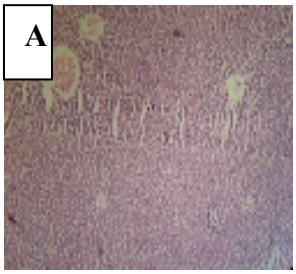
* Significantly different from Control, $p<0.05$

Effect of *Diapal* tablets on Biochemical parameters

The biochemical profile of the control and treated groups are presented in Table 5. The repeated oral treatment for 90 days did not cause significant changes in many of the biochemical parameters among the control and treated groups. But the statistically significant ($p<0.05$) increase in blood urea nitrogen (BUN) levels was seen in case of animals treated with *Diapal* tablets for 90 days. Nevertheless these changes were minimal, but statistically identified. But all values lie within the normal limits and the changes were considered to be of little significance. Despite these alterations, there were no gross or histopathological features on kidney related to treatment. The results are considered to be normal. The changes in other biochemical parameters were statistically insignificant.

Effects of *Diapal* tablets on histopathology of internal organs

The histopathological findings of internal organs of the control and treated groups are presented in Figure I. Histopathological examinations did reveal some non significant lesions in various internal organs of the female rats in control group as well as in rats treated with *Diapal* tablets (250 mg/kg). These pathological findings might be spontaneous or incidental in wistar rats. Overall histopathological observations were non significant and not related to test substance administered.



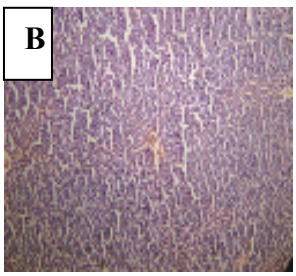
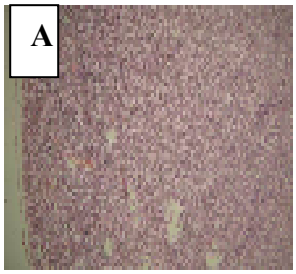


Fig 1 : Liver



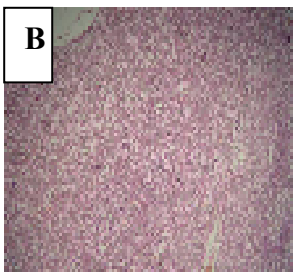
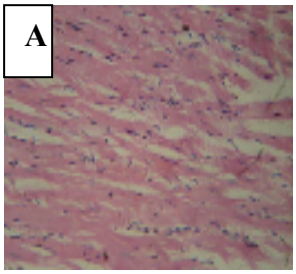


Fig 2: Kidney



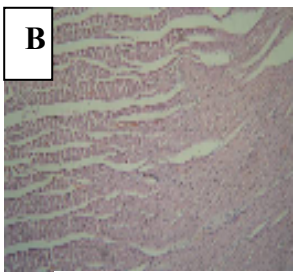
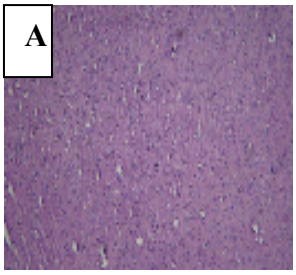
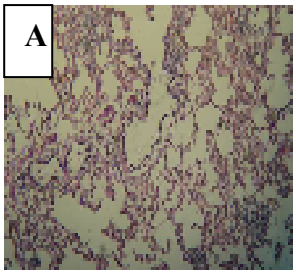
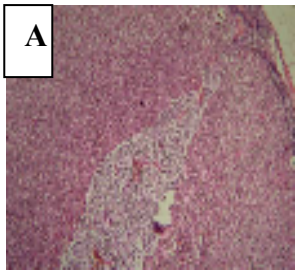


Fig 3 : Heart







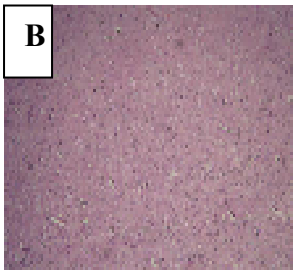


Fig 4: Brain

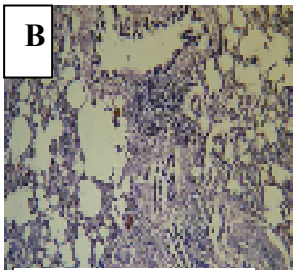


Fig 5: Lung

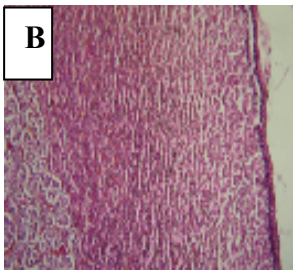
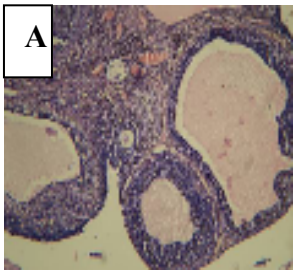
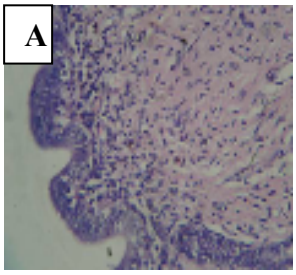
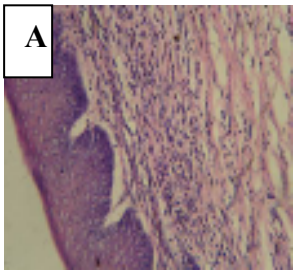


Fig 6 : Adrenal gland







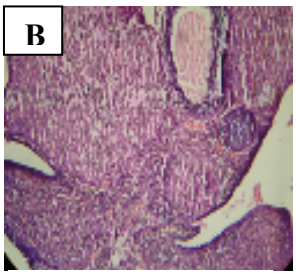


Fig7: Ovary

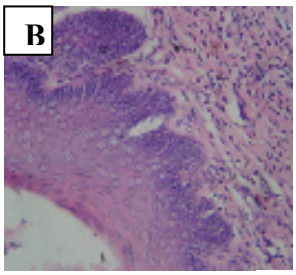


Fig 8 :Uterus

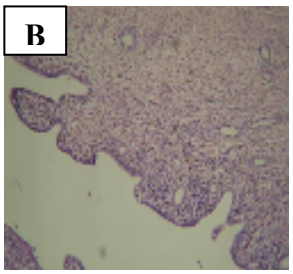


Fig 9: Cervix & Vagina

Figure: I Representative microscopic findings. Fig 1: Liver, Fig 2 Kidney, Fig 3: Heart, Fig 4: Brain, Fig 5: Lung, Fig 6: Adrenal gland, Fig 7: Ovary, Fig 8: Uterus and Fig 9: Cervix &vagina of female rats treated orally with control (A) and *Diapal* tablets (B) (250 mg/kg) for 90 days. (Hematoxylin-eosin stain, × 40).

Discussion

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders causing hyperglycemia, resulting from a defective or deficient insulin secretary response. To-date there are different groups of oral hypoglycemic drugs and insulin for clinical use, having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing the demand for complementary and alternative medicine with antidiabetic activity and fewer side effects. Numerous herbal preparations have been shown to affect blood glucose levels through various mechanisms, although the toxicity is limited there is a need for sufficient and systematic data on safety of herbal medicines since its been taken as medications for longer duration of time especially diseases like diabetes (8).

In the present study, results obtained for the safety profile of orally administered *Diapal* tablets, at the therapeutic dose of 250 mg/kg were elucidated and is compared with the control groups for body weight gain, food intake, water consumption, relative organ weight, morphological, hematological, biochemical and histopathological parameters.No mortality or abnormal behavior was seen in the animals treated with *Diapal* tablets at the therapeutic dose level. Based on the experimental result, no observed adverse effect level (NOAEL) of *Diapal* tablets was greater than 250 mg/ kg. Although many natural plant extracts used traditionally have passed the test of time in terms of toxicity or adverse effects, the safety of the active phytochemicals from these plants must precede their pharmaceutical use.

In conclusion, there was no relevance of serious signs and significant changes in physical,hematological,biochemical and histopathological parameters that resulted from the long term administration of *Diapal* tablets (250 mg/kg). Moreover, no pathological features were identified in both control and treated group as monitored by histopathological analysis of the internal organs.

It is therefore, concluded that *Diapal* tablets at the dose given in present study did not induce any remarkable toxic effects in the female rats treated for 90 days.

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