Original Article

Acute & Sub Acute Toxicity Study of The Ajaswagandhadhi Lehyam In Male Wistar Rats

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Abstract

Ajaswagandhadhi Lehyam is a Plant Preparation used in traditional Medicinal practice as a Nervine Tonic. Toxicity test has to be done to evaluate the toxic compounds present in Ajaswagandhadhi Lehyam. This study involves the acute and sub-acute toxicity study of Ajaswagandhadhi Lehyam in male Wistar rats. Ajaswagandhadhi Lehyam from Kottakal is preferred for the study. Haemotological, Biochemical and various other parameters were observed to find any evidence of acute and sub-acute toxicity of Ajaswagandhadhi Lehyam.

Keywords: Acute Toxicity, Sub-Acute Toxicity, Haematological Parameters and Biomedical Parameters.

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Introduction

Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity.

Acute Toxicity Study

Acute toxicity is involved in estimation of LD50 (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) (shetty akhila, et al., 2007).

Method

Acute oral toxicity of Ajaswagandhadhi lehyam is carried out as per the Organization of Economic Co-operation and Development (OECD) -423 guidelines after the animal ethical clearance from institutional animal ethics committee.

The albino mice are fasted over night and provided only water, after which the Ajaswagandhadhi Lehyam is administered by gastric intubations to the relevant group of animals orally at the dose of 50 mg.kg-1 body weight in tween-80. The animals are then observed for 14 days and maintained with normal food.

If a mortality rate of 2 or 3 animals in 14 days is recorded then the dose is said to be toxic dose. But when mortality of one animal is observed, then the same dose is repeated again for confirmation. However, if mortality is not observed, the procedure is repeated for further higher doses such as 300 and 2,000 mg.kg-1 body weight. Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality (shah ayub, 1997, bürger, 2005).

Cage side observations

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Special attention is directed for the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Body Weight, Food and Water Intake

Body weight, food and water intake are recorded at two-day intervals.

Pathology

Surviving animals are fasted overnight, weighed and humanely killed on the 15th day using anesthetic ether. All test animals are subjected to gross necropsy.

Sub Acute Toxicity Study

This experiment evaluates the sub acute toxicity potential of ajaswagandhadhi lehyam.

Method

Male and female wistar rats weighing 180 ± 10 g are used for the present study. The animals are divided into five groups of six animals each. The dose of the preparation is calculated based on the body weight of the animal. The animals in group I are administered with a single daily dose of 0.5 ml of tween 80 orally for 20 days. The animals in group II are administered with 50 mg.kg-1 of the Ajaswagandhadhi Lehyam orally once daily for 20 days. The animals in group III are administered with 100 mg.kg-1 of the Ajaswagandhadhi Lehyam orally once daily for 20 days.

The animals in group IV and V are administered once daily with 200 and 400 mg.kg-1 of the Ajaswagandhadhi Lehyam respectively for 20 days orally (pieme, et al 2006, joshi, et al 2007, mythilypriya, et al., 2007). The animals are then weighed every five days, from the start of the treatment, to record the weight variation

At the end of the treatment, blood samples are collected by puncturing retro orbital plexus after mild anesthesia for

biochemical analysis. The collected blood sample is centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which is analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels, LDL-cholesterol, plasma glucose, Alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea.

Results

Acute toxicity study with Ajaswagandhadhi lehyam

The acute toxicity of Ajaswagandhadhi Lehyam was evaluated using OECD- 423 guidelines. There was no mortality or morbidity observed in animals through the 15-days period following single oral administration at all selected dose levels of the ajaswagandhadhi lehyam (Table-1). The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such as fur, skin, eyes and nose appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self mutilation, walking backward and so forth were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal.

Table 1

	Dose (mg.kg-1)	*Sign of toxicity (st.nb-1)*	*Mortality (d.s-1)*
Group i	50	0/3	0/3
Group ii	300	0/3	0/3
Group iii	2000	0/3	0/3

The acute toxicity of Ajaswagandhadhi Lehyam on experimental mice was tested using OECD-423 guidelines, where St- sign of toxicity; nb- normal behaviour; d- died; s-survive. Values are expressed as number of animals (n=3).

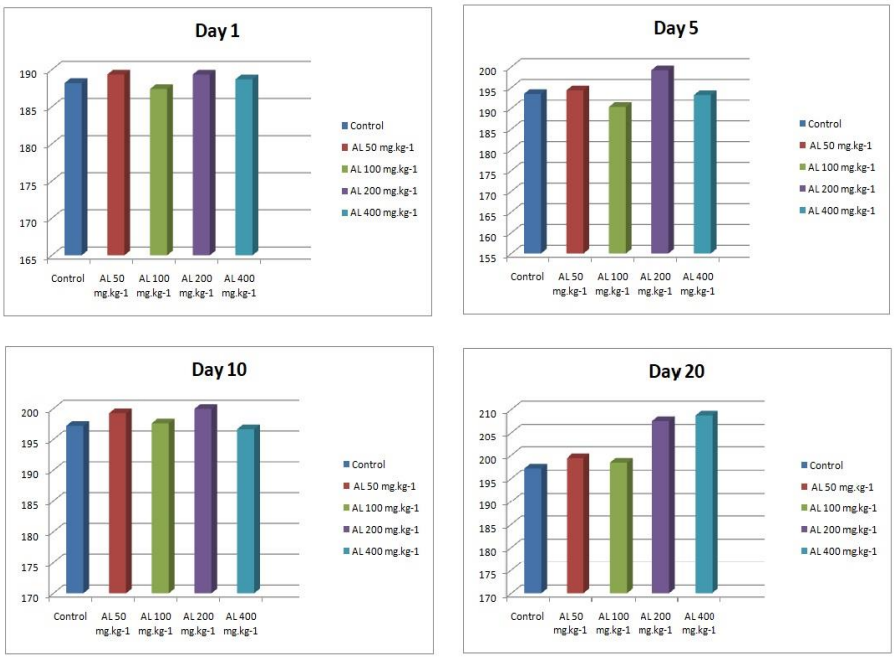
Effect of Ajaswagandhadhi lehyam in sub acute toxicity Ajaswagandhadhi lehyam were evaluated for sub acute toxicity.

Effect of Ajaswagandhadhi lehyam on body weight of rats

The effect of Ajaswagandhadhi Lehyam on the body weight of rats was observed, from the study there was significant increase ($p<0.05$) in body weight of all the animals were observed. The results are shown in table 2.

Table 2

Treatment	*Day 1*	*Day 5*	*Day 10*	*Day 20*
Control	188.20±6.10	193.50 ±6.22	197.20 ±6.37	197.12±6.61
Ajaswagandhadhi lehyam 50 mg.kg-1	189.35 ±6.6	194.35 ±6.32	199.23±6.72	199.35±6.74
Ajaswagandhadhi lehyam 100 mg.kg-1	187.40 ±5.9	190.35 ±6.42	197.60 ±7.12	198.41±6.33
Ajaswagandhadhi lehyam 200 mg.kg-1	189.35 ±6.6	199.20±6.52	199.95 ±7.22	207.50±7.29
Ajaswagandhadhi lehyam 400 mg.kg-1	188.70 ±6.25	193.20 ±5.62	196.65 ±6.37	208.71±7.41



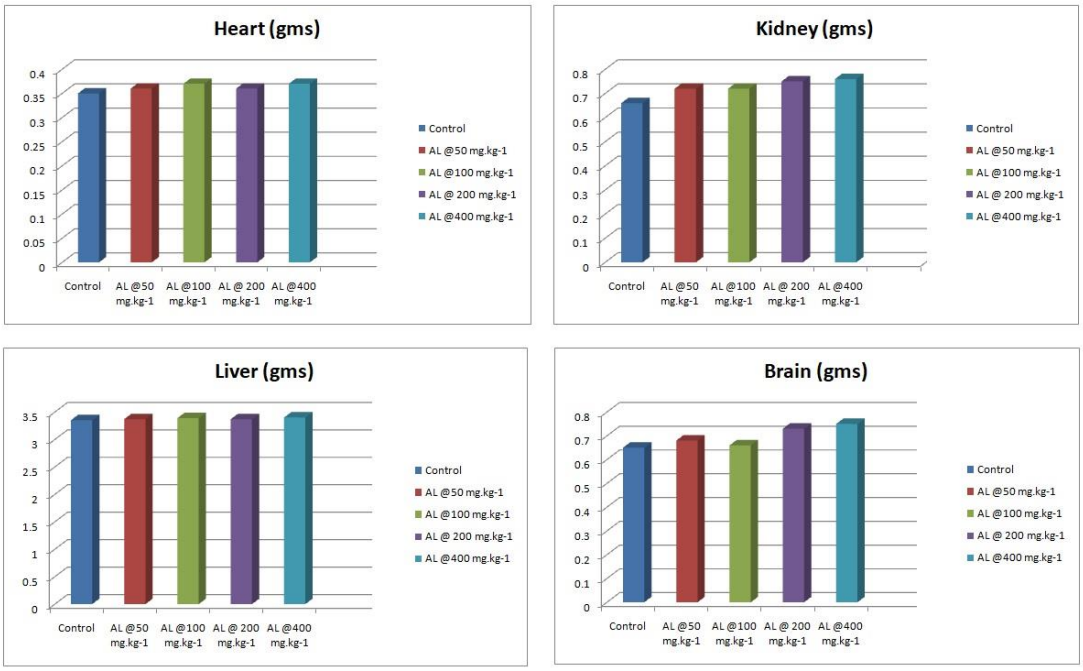
The effects of Ajaswagandhadhi lehyam on body weight changes in rats. A study on the effects of Ajaswagandhadhi lehyam on body weight changes in rats was carried out. Where, group I animals (gpi) were treated with normal saline (5 ml.kg-1), group II animals (gpii) with 50 mg.kg-1 of Ajaswagandhadhi lehyam, group III animals (gpiii) with 100 mg.kg-1 of Ajaswagandhadhi lehyam, group iv animals (gpiv) with 200 mg.kg-1 of Ajaswagandhadhi lehyam, group V animals (gpv) with 400 mg.kg-1 Ajaswagandhadhi lehyam. The values are expressed as Mean ± SEM. N=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way anova method, where **p<0.01 *p<0.05.

Effect of Ajaswagandhadhi lehyam on kidney, heart, liver and brain in rats

The effects of Ajaswagandhadhi lehyam on kidney, heart, liver and brain of the rats were observed. From the study it was clear that, there was significant ($p<0.01$) changes in the weights of various organs of the animals. The results are shown in table 3.

Table 3

Treatment	*Heart (gms)*	*Kidney (gms)*	*Liver (gms)*	*Brain (gms)*
Control	0.35 ± 0.04	0.66± 0.04	3.35± 0.07	0.65± 0.07
Ajaswagandhadhi lehyam @50 mg.kg-1	0.36± 0.03	0.72± 0.04	3.37± 0.05	0.68± 0.5
Ajaswagandhadhi lehyam @100 mg.kg-1	0.37± 0.07	0.72± 0.05	3.39±0.04	0.66± 0.4
Ajaswagandhadhi lehyam @ 200 mg.kg-1	0.36± 0.05	0.75± 0.03	3.37± 0.04	0.73± 0.08
Ajaswagandhadhi lehyam @400 mg.kg-1	0.37± 0.04	0.76± 0.04	3.40± 0.05	0.75± 0.06



A study on the effects of ajaswagandhadhi lehyam on kidney, heart, liver and brain of the rats was tested. Where, group i animals (gpi) treated with normal saline (5 ml.kg-1), group ii animals (gpii) with 50 mg.kg-1 of ajaswagandhadhi lehyam, group iii animals (gpiii) with 100 mg.kg-1 of ajaswagandhadhi lehyam, group iv animals (gpiv) with 200 mg.kg-1 of ajaswagandhadhi lehyam, group v animals (gpv) with 400 mg.kg-1 ajaswagandhadhi lehyam. The values are expressed as Mean ± S.E.M N=6. The results of group i were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way anova method, where **p<0.01.

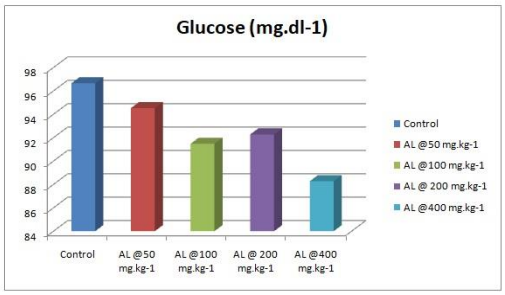
Effect of Ajaswagandhadhi lehyam on biochemical profiles of rats

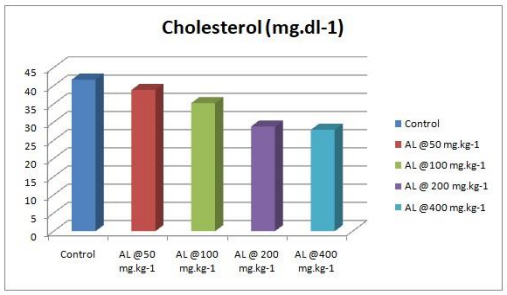
The effect of ajaswagandhadhi lehyam on various biochemical parameters of the experimental animal 'rats' were tested. From the study it was evident that, there was significant decrease (p<0.05) in the plasma glucose level in treated rats especially at higher dose (400 mg.kg-1) compared with control rats. The control rats were administered only with 5 ml of normal saline. Significant decrease (p<0.05) in the plasma total cholesterol (tc), triglyceride (tg) and ldl-cholesterol levels were observed. But a significant increase (p<0.05) in hdl-cholesterol levels were observed in all the treated animals compared with the control animals. Ast, alt and alp levels were also normal in the ajaswagandhadhi lehyam treated animals. From the results of biochemical study there was no evidence of severe toxicity associated with the administration of higher concentration of ajaswagandhadhi lehyam. The results are

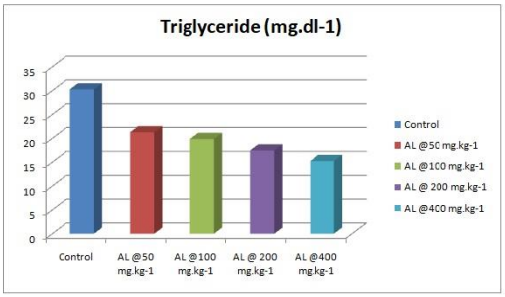
shown in table 4.

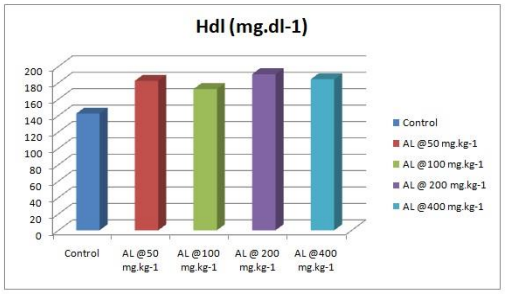
Table 4

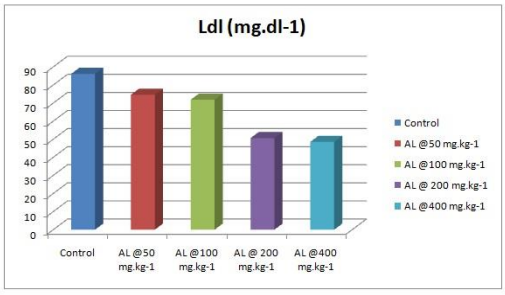
Treatment	*Glucose (mg.dl-1)*	*Cholesterol (mg.dl-1)*	*Triglyceride (mg.dl-1)*	*Hdl (mg.dl-1)*	*Ldl (mg.dl-1)*
Control	96.65± 0.65	41.66± 0.60	30.28± 0.48	142.29± 0.9	86.17±1.79
Ajaswagandhadhi lehyam @ 50 mg.kg-1	94.53± 0.59	38.85±0.37	21.28± 0.34	172.32± 0.69	74.64±1.33
Ajaswagandhadhi lehyam @ 100 mg.kg-1	91.48± 0.50	35.22± 0.34	19.86± 0.42	172.23±0.62	71.88±1.18
Ajaswagandhadhi lehyam @ 200 mg.kg-1	92.28± 0.58	28.78± 0.30	17.46± 0.32	180.39± 0.84	50.64±1.35
Ajaswagandhadhi lehyam @ 400 mg.kg-1	88.28± 0.48	27.89± 0.29	15.24± 0.28	182.20± 0.88	48.57±0.86











The effect of ajaswagandhadhi lehyam on biochemical parameters such as glucose, cholesterol, triglyceride, hdl and ldl. A study on the effect of ajaswagandhadhi lehyam on biochemical parameters such as glucose, cholesterol, triglyceride, hdl and ldl in rats was tested. where, group i animals (gpi) treated with normal saline (5 ml.kg-1), group ii animals (gpii) with 50 mg.kg-1 of ajaswagandhadhi lehyam, group iii animals (gpiii) with 100 mg.kg-1 of ajaswagandhadhi lehyam, group iv animals (gpiv) with 200 mg.kg-1 of, group v animals (gpv) with 400 mg.kg-1

ajaswagandhadhi lehyam. The values are expressed as Mean ± S.E.M. N=6. The results of group i were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way anova method, where **p<0.01 *p<0.05.

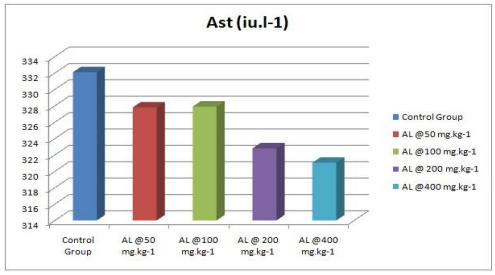
Effect of Ajaswagandhadhi Lehyam on bio chemical parameters in rats

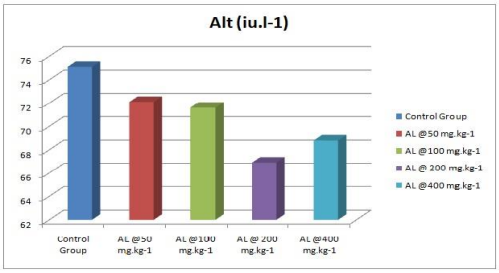
The effects of ajaswagandhadhi lehyam were observed for its effect on bio chemical parameters on the experimental rats.

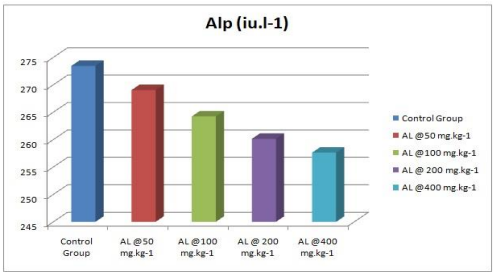
Table 5

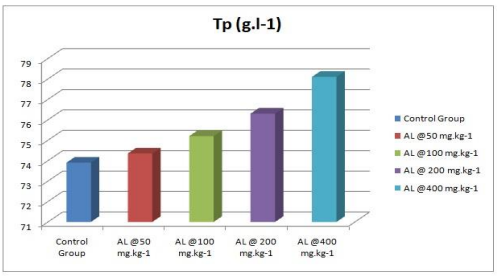
Treatment	***Ast (iu.l-1)***	***Alt (iu.l-1)***	***Alp (iu.l-1)***	***Tp (g.l-1)***	***Albumin (g.l-1)***
Control	332.10±12.45	75.10± 3.23	273.45± 4.45	73.90± 3.37	43.20±2.40
Ajaswagandhadhi lehyam@50 mg.kg-1	327.8± 8.25	72.10± 2.25	269.05± 5.25	74.35± 2.37	43.35±2.70
Ajaswagandhadhi lehyam @ 100 mg.kg-1	327.9±8.0	71.6± 3.20	264.23± 6.75	75.20± 2.87	44.35±3.10
Ajaswagandhadhi lehyam @ 200 mg.kg-1	322.8±7.25	66.9± 2.95	260.15± 2.80	74.90± 2.80	44.25±2.80
Ajaswagandhadhi lehyam @ 400 mg.kg-1	321.05±9.55	68.8± 3.57	257.63± 8.85	75.45± 2.98	44.53±2.85

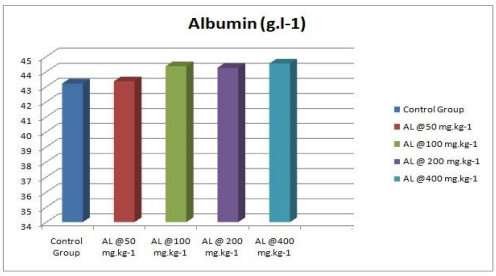
A study on the effects of ajaswagandhadhi lehyam on biochemical parameters such as ast, alt, alp, tp and albumin in rats was tested. Where, group i animals (gpi) were treated with normal saline (5ml.kg-1), group ii animals (gpii) with 50 mg.kg-1 of ajaswagandhadhi lehyam group iii animals (gpiii) with 100 mg.kg-1 of ajaswagandhadhi lehyam, group iv animals (gpiv) with 200 mg.kg-1 of ajaswagandhadhi lehyam, and group v animals (gpv) with 400 mg.kg-1 ajaswagandhadhi lehyam the values are expressed as Mean ± S.E.M. N=6. The results of group i were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way anova method, where **p<0.01 *p<0.05.









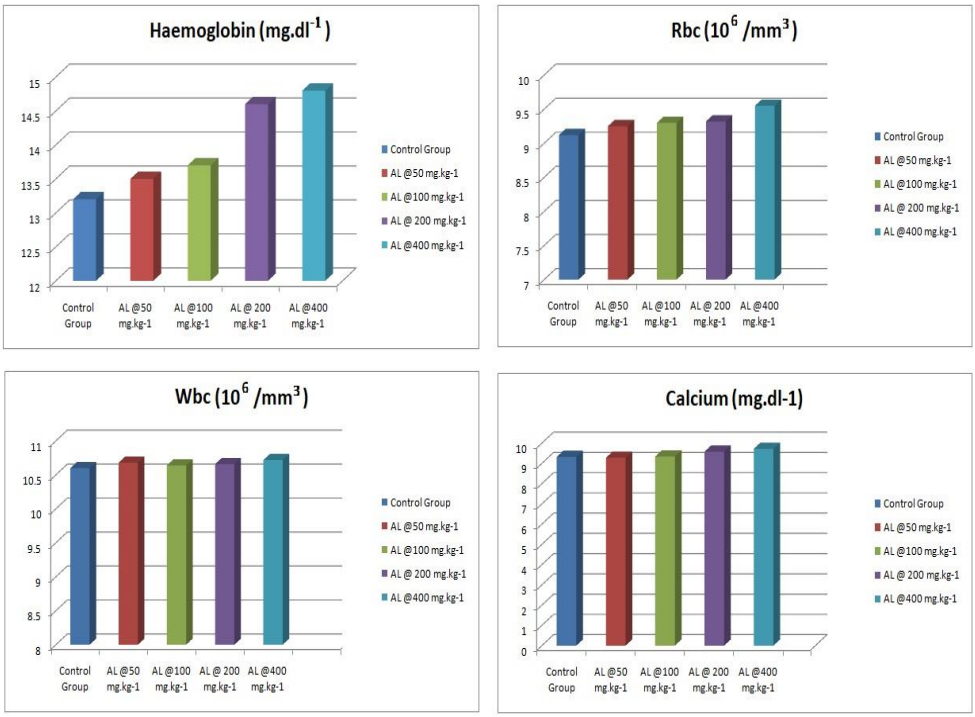


Effect of Ajaswagandhadhi Lehyam on haematological parameters in rats

The effects of ajaswagandhadhi lehyam were observed for its effect on haematological parameters on the experimental rats. From the study it was evident that, a significant increase ($p<0.01$) were observed in the haemoglobin and Rbc of the treated animals compared with the control. There was no significant change in the Wbc and calcium level in all the treated animals compared to the control.

Table 6

Treatment	***Haemoglobin (mg.dl-1)***	***Rbc (106 /mm3)***	***Wbc (106 /mm3)***	***Calcium (mg.dl-1)***
Control	13.2± 0.23	9.12± 0.02	10.60± 0.05	9.32 ±0.04
Ajaswagandhadhi lehyam @ 50 mg.kg-1	13.5± 0.25	9.25± 0.02	10.68± 0.04	9.28 ±0.04
Ajaswagandhadhi lehyam @ 100 mg.kg-1	13.7± 0.37	9.30± 0.02	10.64± 0.02	9.34 ±0.12
Ajaswagandhadhi lehyam @ 200 mg.kg-1	14.6± 0.15	9.32± 0.12	10.66± 0.03	9.58 ±0.10
Ajaswagandhadhi lehyam @ 400 mg.kg-1	14.8± 0.26	9.55± 0.27	10.72± 0.05	9.72 ±0.04



The effect of ajaswagandhadhi lehyam on haematological parameters such as hb, calcium, rbc and wbc in rats. A study on the effect of ajaswagandhadhi lehyam on haematological parameters such as hb, rbc, wbc, calcium in rats was tested. Where, group i animals (gpi) treated with normal saline (5 ml.kg1), group ii animals (gpii) with 50 mg.kg-1 of ajaswagandhadhi lehyam, group iii animals (gpiii) with 100 mg.kg-1 of ajaswagandhadhi lehyam, group iv animals

(gpiv) with 200 mg.kg-1 of ajaswagandhadhi lehyam, and group v animals (gpv) with 400 mg.kg-1 ajaswagandhadhi lehyam. The values are expressed as Mean ± S.E.M. N=6. The results of group i were compared with other groups such as II, III, IV and V. The statistical analysis was carried out using one way anova method, where *p<0.05.

Discussion

The evaluation of sub-acute and chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the plant preparation. The highest overall concordance of toxicity in animals in comparison with humans is with hematological, gastrointestinal, and cardiovascular adverse effects whiles certain adverse effects in humans, especially hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals (olson, et al., 2000).(7)

In the present study, where the acute toxicity study of ajaswagandhadhi lehyam was carried out as per oecd-423 guidelines, no mortality was observed in both the animals of control group as well as animals treated with a maximum dose of 2000 mg.kg-1. Hence, 1/10th of 2000 mg.kg-1 i.e. 200 mg.kg-1 of dose was selected as a minimum dose for sub-acute toxicity study (abu taha nael, et al., 2008).(8)

The results of sub-acute toxicity study show that there was no significant change in animal behaviour due to the absence of toxicity. The animals treated with ajaswagandhadhi lehyam showed normal growth pattern and body weight compared with control rats treated with normal saline. So the changes in body weight can be used as an indicator of adverse effects of drugs and chemicals (tofovic and jackson, 1999; raza, et al., 2002; teo, 2002).(9,10,11)

The changes in enzymes like alp, ast and alt levels show liver impairment, due to toxicity (hayes, 1989).(12) serum cholesterol and proteins mainly regulated via synthesis in the liver and increase or decrease in serum concentrations of constituents suggest liver toxicity. The results of the present study were assessed after 28 days of administration of ajaswagandhadhi lehyam, and it was found that ajaswagandhadhi lehyam at all concentrations do not produce liver damage.

There was a slight decrease in plasma glucose level, when higher doses of ajaswagandhadhi lehyam (400 mg.kg-1) were administered in the treated rats.

Analysis of blood parameters is likely to risk evaluation as the change in hematological system has a higher predictive value for human toxicity, when data are translated from animal studies (olson, et al., 2000).(7) after 28 days of treatment, there were no significant changes in the haematological parameters between control and treated groups. No significant changes in the levels of wbc, rbc were observed between control and test groups following repeated

administration of ajaswagandhadhi lehyam. Interestingly, significant increase in the levels of hemoglobin was found in treatment with ajaswagandhadhi lehyam with a higher dose of 400mg.kg-1. The possible reason could be that one of the constituent of ajaswagandhadhi lehyam may increase absorption of iron.

The overall results suggest that ajaswagandhadhi lehyam are non toxic to the haaematopoietic and leucopoietic system.

The haematopoietic and leucopoietic systems are the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (adeneye, et al., 2006).(13) therefore, it is possible to assume that the ajaswagandhadhi lehyam is non haematotoxic.

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