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SUB ACUTE TOXICITY STUDY OF *Daikon* (VEGETABLE) EXTRACT ON ALBINO RATS

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ABSTRACT

The present study was to evaluate phytochemical composition and effect of various concentration of methanolic extract of *Daikon* (vegetable) on haematolgical parameters in male albino rats. The phytochemical screening were done with methanol, ethanol, chloroform, petroleum ether and water. The result showed the presence of alkaloids, flavonoids, tannins, steroids, terpenoids, and glycosides. The best results were found in methanolic extract, hence it is used for further study. In toxicity studies they were given 150,250,350,450,550mg/kg doses of the methanolic extract for 90 days. The rats were sacrificed and the blood was collected by cardiac puncture then used for haematolgical and biochemical parameters. Results from the present study have elucidated that there was no significant signs of toxicity at any dose level used in the study. Physical, biochemical as well as hematological parameters was unaltered throughout the study.

KEYWORDS: Haematological and biochemical parameters, *Daikon*, phytochemical screening.

INTRODUCTION

India has rich dietary resources and also famous for a combination of different food items that can provide adequate quantity of nutrients and medicinal values in sustainable way. Some familiar crops have a vital role in playing an economic value beyond dispute. These crops generally possess rich source of many active metabolites and other nutrients and can provide a solution for many problem. (Balasankar *et.,al*, 2013).

Intake of alternative and complementary medicine, including medicinal plants, is increasing and is common among patients caused by many infectious disease. Generally medicinal plants have a history of low side effects. The plants can be used as a source for preparation of new drugs. Many plants show resistance towards growth of bacteria, fungi and viruses in lab conditions. (Hamid Nasri *et al*., 2015).

Many diseases can be cured by nevertheless many prescriptions for dealing with symptoms of certain diseases. It is possible to maintain our health and prevent diseases by improving our lifestyle by enhancing our natural medicines. (Fumio Ikegami *et.al*, 2012).

Daikon contain substances that can protect or treat hepatic injury and several agents with the ability to protect liver toxicity have been isolated from plants. Parts of radish including roots, seeds and leaves are mainly used for medicinal purposes. Radish has been used ethnically as a laxative, stimulant, digestive aid, and in the treatment of stomach disorder. The main constituents of radish include 4-(methylthio)-3-butenyl isothiocyanate, allyl isothiocayanate, benzyl isothiocyanate and phenethyl isothiocyanate. It also contains flavonoids such as kaempherol glycosides, peroxidases and antioxidants. Studies reported that the crude extract of radish showed antimicrobial, antimutagenic and anticarcinogenic effects. (Sang Wha Lee et al., 2012).

In all kind of traditional medicines, *Daikon* is used to treat or protect from disease such as jaundice, gallstone, liver disease, rectal prolapse, indigestion etc. In general, *Daikon* consists of carbohydrates, sugars, dietary fibers, protein, fat, fluoride and water soluble vitamins (B1,B2,B3,B5,B6,B9 and C) and minerals such as calcium, iron, magnesium, magnesium, manganese zinc, potassium and phosphorous. (Saleem Ali *et al.*, (2017).

MATERIALS AND METHODS

Experimental animals

Healthy adult male albino rats weighing about 150 to 200 g were purchased from Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. Paddy husk was used and changed twice a week. The animals were maintained in 12 hrs, light and dark cycle at 28°C ±2° C in a well-ventilated animal house under natural conditions and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with

standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India. All animal experiments were performed according to the ethical guidelines.

Preparation of Vegetable extract

The *Daikon* vegetable was rinsed with distilled water. It was sliced and incubated for 4 days and grain with electric motor. The phytochemical screenings was carried with methanol, ethanol, petroleum ether, chloroform and aqueous extracts of *Daikon* vegetable were done by modern method of Peach and Traycey.

Experimental protocol

The rats (120-150g) were randomly assigned into six groups. Group one is treated as control group with normal diet while the test groups were given 150,250,350,450 and 550 mg/kg, b.w of the methanolic extract for 90 days. The weight of the rats in each group was determined and documented.

Collection of blood sample

Rats were sacrificed using ether anesthesia. Blood sample were collected by cardiac puncture into ETDA capped bottles with the aid of 2ml syringe. The blood sample were then used for the experiments.

Determination of haematological parameters

There was an increase in rat RBC, PCV, Hb and WBC counts at all concentration of methanolic extract of *Daikon* when compared with the control decrease in MCH and MCHC. The levels of MCV were not significantly altered at all extract concentration.

RESULT AND DISCUSION

The qualitative analysis obtained under the standard conditions is useful for quality control of phytochemicals. The phytochemical screening with water, ethanol, methanol, petroleum ether and chloroform of *Daikon* (vegetable) show to possess secondary metabolite which are shown in table 1.

Table 1: Qualitative analysis of phytochemicals of vegetable extract of *Daikon*.

S. No	Test	Water	Petolium ether	Methnol	Ethanol	Chloroform
1	ALKALOIDS	++	-	+++	+	-
2	FLAVONOIDS	+	-	+	-	-
3	TANNIS	+	-	++	+	-
4	SAPONINS	-	-	+++	++	-
5	STEROIDS	-	-	+++	++	+
6	TERPINOIDS	-	+	++	+	+
7	RESINS	-	-	-	-	-
8	GLYCOSIDES	-	-	+	+	-
9	PHENOL	-	-	-	-	-

The phytochemical screening result shows the presence of alkaloids, tannins, saponins, steroids, terpenoids, and glycosides, here the methanolic extract showed the good results, compared to others and used for the further study.

Table 2: Body weight of experimental rats in the final systemic toxicity studies the due to methanol vegetable extract of *Daikon*

Days / Dose mg/kg b.w	BODY WIGHT (g)		
	0^{th} days	30^{th} days	30^{th} days
Control (Group I)	165.47±0.19	170.54±0.19	174.56±0.17
150 (Group II)	$166.50±0.15a^{ns}$	$172.57±0.69a^{ns}$	$174.70±0.18\ a^{ns}$
250 (Group III)	$166.54±0.27b^{ns}$	$171.57±0.16b^{ns}$	$174.60±0.12b^{ns}$
350 (Group IV)	$164.50±0.25c^{ns}$	$173.08±0.014c^{ns}$	$173.62±0.15c^{ns}$
450 (Group V)	$164.05±0.01d^{ns}$	$172.65±0.013d^{ns}$	$174.68±0.17d^{ns}$
550 (Group VI)	$164.05±0.02e^{ns}$	$172.62±0.24e^{ns}$	$174.67±0.14e^{ns}$

Statistical comparisons

a- Group II is compared with group I b- Group III is compared with group I

c- Group IV is compared with group I d- Group V is compared with group I

e- Group VI is compared with group I ns- non significant

Mean body weight of rats treated with methanolic fruit extract of *Daikon* for 90days were recorded in table 2 the control also gain body weight throughout the duration of treatment. There was no statistically significant weight gain or loss in experimental rats when compare to control rats for the doses tested.

Table 3: Organ weight of rats treated with methanolic extract of *Daikon*.

Organ weight	Dose (mg/kg b.w.)/ Groups					
	Control mg/kg Group I	150mg/kg Group II	250mg/kg Group III	350mg/kg Group IV	450mg/kg Group V	550mg/kg Group VI
Heart	1.63±0.01	1.21±0.02a^{ns}	1.15±0.02b^{ns}	1.26±0.05c^{ns}	1.19±0.02d^{ns}	1.16±0.01e^{ns}
Kidney	1.61±0.03	1.65±0.02a^{ns}	1.57±0.01b^{ns}	1.55±0.01c^{ns}	1.54±0.01d^{ns}	1.64±0.02e^{ns}
Liver	5.57±0.16	5.60±0.06a^{ns}	5.55±0.04b^{ns}	5.44±0.02c^{ns}	5.43±0.01d^{ns}	5.44±0.03e^{ns}
Spleen	0.56±0.01	0.65±0.01a^{ns}	0.63±0.01b^{ns}	0.54±0.01c^{ns}	0.54±0.01d^{ns}	0.54±0.01e^{ns}
Brain	1.58±0.05	1.44±0.02a^{ns}	1.55±0.01b^{ns}	1.46±0.01c^{ns}	1.44±0.01d^{ns}	1.42±0.02e^{ns}

Values are expressed as mean ±S.D of six animals

Statistical comparisons

a- Group II is compared with group I b-group III is compared with group I

b- Group IV is compared with group 1 d-Group V is compared with group I

e - Group VI compared with group I ns – non significant

Table 4: Haematological values of the rats treated with *Daikon* (vegetable) methanolic extract for 30 days.

Hematological parameters	Dose(mg/kg b.w.)/ Groups					
	Control mg/kg Group I	150mg/kg Group II	250mg/kg Group III	350mg/kg Group IV	450mg/kg Group V	550mg/kg Group VI
RBC (*10^{12}/L)	9.85±0.03	9.34±0.02a^{ns}	9.15±0.02b^{ns}	9.45±0.04c^{ns}	9.63±0.01d^{ns}	9.45±0.01e^{ns}
Hb(g/dl)	14.65±0.03	15.12±0.02a^{ns}	15.89±0.02b^{ns}	16.28±0.09c^{ns}	16.83±0.02d^{ns}	15.15±0.04e^{ns}
WBC(10^{9}/L)	6.55±0.01	6.68±0.14a^{ns}	6.74±0.01b^{ns}	6.83±0.03c^{ns}	6.66±0.01d^{ns}	6.80±0.07e^{ns}
MCV(µM^{6})	75.43±0.04	77.38±0.02a^{ns}	77.47±0.02b^{ns}	77.32±0.01c^{ns}	77.56±0.02d^{ns}	77.66±0.01e^{ns}
MCH(pg)	26.38±0.04	26.58±0.04a^{ns}	26.77±0.05b^{ns}	26.36±0.02c^{ns}	26.61±0.05d^{ns}	26.79±0.04e^{ns}
MCHC(g/dl)	32.63±0.02	32.62±0.02a^{ns}	33.61±0.01b^{ns}	33.16±0.03c^{ns}	32.95±0.01d^{ns}	33.46±0.01e^{ns}
PCV (%)	44.82±0.01	48.73±0.03a^{ns}	46.36±0.03b^{ns}	48.85±0.06c^{ns}	48.48±0.03d^{ns}	47.41±0.03e^{ns}
Platelet(10^{9})	874.15±0.03	884.83±0.07a^{ns}	878.6±0.08b^{ns}	874.33±0.81c^{ns}	878.33±0.05d^{ns}	885.53±0.75e^{ns}
Neutrophil (%)	49.35±0.18	49.23±0.04a^{ns}	49.38±0.06b^{ns}	49.34±0.02c^{ns}	50.40±0.01d^{ns}	49.53±0.02e^{ns}
Lymphocytes (%)	80.59±0.07	80.65±0.02a^{ns}	80.59±0.13b^{ns}	80.59±0.05c^{ns}	80.72±0.01d^{ns}	80.66±0.01e^{ns}

Values are expressed as mean ±S.D of six animals

Statistical comparisons

a- Group II is compared with group I b-Group III is compared with group I

b- Group IV is compared with group 1 d-Group V is compared with group I

e - Group VI compared with group I ns – non significant

Table 5: Haematological values of the rats treated with *Daikon* (vegetable) extract for 90 days.

Haematological parameters	Dose (mg/kg b.w.)/ Groups					
	Control mg/kg Group I	150mg/kg Group II	250mg/kg Group III	350mg/kg Group IV	450mg/kg Group V	550mg/kg Group VI
RBC($*10^{12}$/L)	7.45±0.01	7.63±0.01a^{ns}	7.45±0.02b^{ns}	7.15±0.02c^{ns}	7.34±0.02d^{ns}	7.57±0.06e^{ns}
Hb(g/dl)	14.15±0.04	14.83±0.02a^{ns}	14.27±0.07b^{ns}	14.52±0.4c^{ns}	14.12±0.02d^{ns}	14.31±0.19e^{ns}
WBC(10^9/L)	5.78±0.11	5.56±0.01a^{ns}	5.83±0.20b^{ns}	5.75±0.11c^{ns}	5.50±0.06d^{ns}	5.55±0.01e^{ns}
MCV(µM^6)	16.66±0.01	76.56±0.02a^{ns}	76.32±0.02b^{ns}	76.47±0.02c^{ns}	76.38±0.02d^{ns}	76.43±0.04e^{ns}
MCH(pg)	27.82±0.03	27.61±0.05a^{ns}	27.35±0.03b^{ns}	27.77±0.05c^{ns}	27.58±0.04d^{ns}	27.24±0.13e^{ns}
MCHC(g/dl)	33.46±0.01	33.94±0.02a^{ns}	34.16±0.03b^{ns}	34.61±0.01c^{ns}	33.62±0.02d^{ns}	34.21±0.01e^{ns}
PCV(%)	45.41±0.03	48.47±0.04a^{ns}	47.80±0.05b^{ns}	49.35±0.04c^{ns}	48.73±0.03d^{ns}	48.82±0.01e^{ns}
Platelet(10^9)	891.15±0.02	893.8±0.02a^{ns}	892.1±0.01b^{ns}	892.4±0.02c^{ns}	893.5±0.09d^{ns}	893.49±0.05e^{ns}
Neutrophil (%)	51.53±0.02	52.38±0.19a^{ns}	52.34±0.02b^{ns}	51.39±0.06c^{ns}	51.23±0.04d^{ns}	51.35±0.18e^{ns}
Lymphocytes (%)	80.66±0.01	80.75±0.02a^{ns}	80.75±0.02b^{ns}	80.64±0.08c^{ns}	80.65±0.02d^{ns}	80.79±0.22e^{ns}

Values are expressed as mean ±S.D of six animals

Statistical comparisons

a- Group II is compared with group I b-group III is compared with group I

b- Group IV is compared with group 1 d-Group V is compared with group I

e - Group VI compared with group I ns – non significant

The Haematological parameters were analyzed for different dosage in 30 & 90th days. The result shows administration of methanolic extract of *Daikon* at all the selected concentrations did not produce any significant change in all the parameters tested, such as RBC,Hb,MCV,MCHC,WBC, platelets, neutrophils and lymphocytes.

Table 6: Liver marker enzymes and biochemical parameters of rats treated with *Daikon* (vegetable) methanolic extract for *90 days.*

Biochemical parameters	Control mg/kg Group I	150mg/kg Group II	250mg/kg Group III	350mg/kg Group IV	450mg/kg Group V	550mg/kg Group VI
AST	41.15±0.03	41.54±0.01a^{ns}	41.48±0.01b^{ns}	41.26±0.03c^{ns}	40.95±0.02d^{ns}	40.96±0.01e^{ns}
ALT	38.73±0.02	38.94±0.3a^{ns}	39.29±0.03b^{ns}	39.17±0.01c^{ns}	38.76±0.01d^{ns}	39.10±0.02e^{ns}
ACP	59.19±0.01	59.65±0.02a^{ns}	59.65±0.01b^{ns}	59.67±0.01c^{ns}	59.85±0.05d^{ns}	69.08±0.02e^{ns}
Urea	14.55±0.02	14.65±0.02a^{ns}	14.67±0.02b^{ns}	14.66±0.02c^{ns}	14.74±0.02d^{ns}	14.76±0.01e^{ns}
Uric acid	4.45±0.25	4.17±0.02a^{ns}	4.06±0.01b^{ns}	3.96±0.03c^{ns}	4.05±0.03d^{ns}	4.17±0.01e^{ns}
Protein	6.71±0.01	6.88±0.02a^{ns}	6.95±0.01b^{ns}	6.36±0.01c^{ns}	6.48±0.03d^{ns}	6.55±0.03e^{ns}

Values are expressed as mean ±S.D of six animals

Statistical comparisons

c- Group II is compared with group I b-group III is compared with group I

d- Group IV is compared with group 1 d-Group V is compared with group I

e -Group VI compared with group I ns – non significant

The biochemical parameters were analyzed for different dosage in 90th day also. The result shows administration of methanolic extract at all the selected concentrations did not produce any significant change in all the parameters such as ALP, AST, ALP were tested.

CONCLUSION

Our results had demonstrated that the methanolic extract of *Daikon* (vegetable) contains significant active metabolites. Methanolic extract of *Daikon* possesses non-toxic effect in rat model. No deaths or signs of toxicity were observed in the rats that received the extract up to an oral acute limit dose of 2000 mg/kg. Body weight did not cause any adverse effects and it can be considered as nontoxic and safe. The biochemical and haematological parameters (RBC, Hb, WBC, MCV, MCH, Platelets, neutrophils and lymphocytes) did not showed any significant alterations in the treated groups when compared to control.

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