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SUBACUTE 28 DAYS REPEATED TOXICITY ASSESSMENT OF THYMOQUINONE (VOLATILE OIL OF BLACK SEED) IN WISTAR RATS

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ABSTRACT

Thymoquinone is a major active principle of *Nigella sativa* (volatile oil of black seed or kalonji Seed) and constitutes about more than 30 % its volatile oil. The kalonji seed are believed to be commonly used as natural remedy for all disease except death. The study was subjected to subacute toxicological testing to dose dependant toxicity to use as traditional medicine and establishing dose criteria to human being. Thymoquinone orally administered to male and female wistar rat at dose level of 2.5, 5.0, 10.0 mg/kg body weight for 28 days repeatedly, did not produce any sign of toxicity, mortality, pathological and blood parameters changes. It is suggested that thymoquinone at higher dose level is well tolerated by male and female rats. The Higher dose of thymoquinone 10.0 mg/kg body weight determined to be no observed adverse effect level (NOAEL).

KEYWORDS : Thymoquinone, Toxicological Studies, No Observed Adverse Effect Level (NOAEL)

Thymoquinone (*Nigella sativa* seed) is a member of the ranunculaceae family growing in countries bordering the mediterranean sea, India, Pakistan, and Iran. For many centuries, *Nigella sativa* seeds (also called black seeds or black cumin) have been used as a food additive as well as for medicinal purposes in many countries. This plant is one of the most extensively studied, both phytochemically and pharmacologically (Riaz et al., 1996; Siddiqui and Sharma, 1996). Most properties of whole seeds or their extracts are mainly attributed to quinone constituents, of which, thymoquinone is more abundant compound (Ali and Blunden, 2003; Filippo D'Antuono et al., 2002). In addition to its pharmacological action, *Nigella sativa* has also been investigated for other properties that include immune stimulant, antiinflammatory, anticancer, antimicrobial, antioxidant and antiparasitic action (Ghazanfar, 1994; Nickavar, 2003 and El-Dakhakhny,1963) .

There are only few studies reporting subacute toxicity of thymoquinone. Black seeds of kalonji have been widely accepted as medicine and food additive, but data on its long term toxicity is still lacking. To evaluate this, it is deemed necessary to conduct the long term toxicity study of its active constituent in laboratory animals.

MATERIALS AND METHODS

Male and female albino wistar rat (*Rattus norvegicus*) of 6-7 week age and acclimatized for a period of 7 days prior to dosing. All animals were housed and maintained in controlled environment at temperature 22 ± 3ºc, relative humidity 60 ± 10 % and a light/dark cycle of 12 hr each. Animal were provided with purified drinking water and feed (Pelleted Diet, Pranav Agro Industries) ad *libitum* throughout the study period. The test item (thymoquinone) was purchased from sigma aldrich India and peanut oil was procured from the local market (Hayes, 1994).

Acute Oral Toxicity

Nine female rats were divided into 3 groups with three rats each (between 8 to 9 weeks). Following overnight fasting, the animals were administered with thymoquinone formulated in peanut oil at the dose levels of 2000, 300 and 300 mg/kg body weight respectively as per the OECD guideline 423, adopted on OECD 17th 2001. The maximum dose volume was maintained at 10 ml/kg body weight. All animals were observed twice daily for signs of toxicity, morbidity and mortality over a period of 14 days (OECD N° 423., 2001).

Subacute 28-day Oral Toxicity in Rats

Male and female rats (110 ± 20 g body weight) were randomized on weight basis into six groups (Group I to VI) with 5 male and 5 female rats in each group. Group I served as control and Group II served as vehicle control group animals were daily administered peanut oil. The groups III, IV, V and VI were administered 2.5, 5.0, 10 and 10 (satellite group) mg/kg body weight of thymoquinone

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respectively for a period of 28 days. Rats were observed twice daily for signs of toxicity, morbidity and mortality throughout the study period. Body weight, food and water intake by individual rat were recorded weekly for each group. After 28 days of repeated dosing, all the five group (I to V) and after 14 days post dosing, satellite group VI were humanely sacrificed by using CO_2 exposure. Blood samples were collected from abdominal aorta in EDTA solution and non-oxalate tubes for the estimation of haematological and biochemical parameter respectively from all the groups on the day of study termination. All the vital organs were collected, weighed and fixed in buffered formalin for histopathology (OECD N° 407., 1995).

Organ Body Weight Ratio

The vital organ such as liver, kidney, brain, heart, lung, spleen, adrenal of rats and the male reproductive organs (testis, epididymis, prostrate and seminal vesicle) and female reproductive organs (ovary, uterus, cervix and vagina) were quickly removed and weigh individually. The organ to body weight ratio was recorded.

Biochemical Study

Biochemical analysis was performed on animal serum for the determination of parameters that include Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, serum bilirubin, serum albumin. All analyses were carried out using Sysmex Automated Clinical Chemistry Analyzer.

Hematological Study

Blood samples collected in EDTA were analyzed for total erythrocytes (TEC) and total leucocytes (TLC) counts using the method of Winfrobe and Landsberg whereas, total haemoglobin (Hb) and differential leucocytes counts (DLC) were estimated.

Statistical Analysis

Statistical significance were presented between control and experimental values as mean ± SEM (n=5). Statistical comparison of body weight changes was made using one way ANOVA (Seigel,1996).

RESULTS

Acute Toxicity Study

Based on the mortality observed in set I treated with 2000 mg/kgbodyweight of thymoquinone whereas, no mortality and toxicity sign was observed in Set II and set III treated with 300 mg/kgbodyweight. The LD50 (cut of value) was found 500 mg/kgbodyweight according to OECD guidelines 423.

Subacute Oral Toxicity

Repeated oral administration of different dose for 28 days did not produce any toxicity sign and mortality. Body weight, food and water were also comparable to their respective controls.

Organ Body Weight Ratio

The absolute body weights of thymoquinone treated male and female rats no significant changes were observed while, comparable to controls rats. The relative organ weights (organ to body weight ratio) of animals exposed to different dose of thymoquinone did not indicate

Table 1: Relative Organ Body Weight of Male Rats Orally Administration Thymoquinone For 28 Days

	Dose (mg/kg body weight)					
Organ	**Control**	**Vehicle Control**	**Low Dose**	**Mid Dose**	**Higher Dose**	**Satellite group**
Liver	3.13 ± 0.24	2.90 ± 0.21	3.07 ± 0.24	3.09 ± 0.27	3.13 ± 0.24	3.16 ± 0.24
Kidney	0.75 ± 0.20	0.77 ± 0.34	0.76 ± 0.03	0.77 ± 0.07	0.77 ± 0.34	0.76 ± 0.27
Lungs	0.73 ± 0.01	0.71 ± 0.21	0.73 ± 0.03	0.72 ± 0.05	0.71 ± 0.21	0.70 ± 0.28
Brain	0.78 ± 0.03	0.89 ± 0.19	0.78 ± 0.03	0.81 ± 0.05	0.89 ± 0.19	1.48 ± 0.40
Testis	1.12 ± 0.68	1.18 ± 0.63	1.13 ± 0.09	1.11 ± 0.13	1.18 ± 0.63	1.17 ± 0.05
Epididymis	0.31± 0.54	0.38± 0.44	0.41± 0.64	0.39± 0.52	0.38± 0.22	0.37± 0.64
Seminal Vesicle	0.48± 0.56	0.48± 0.41	0.52± 0.61	0.54± 0.12	0.52± 0.21	0.49± 0.54
Spleen	0.24 ± 0.01	0.25 ± 0.01	0.24 ± 0.03	0.26 ± 0.06	0.25 ± 0.01	0.52 ± 0.26
Heart	0.29 ± 0.00	0.33 ± 0.02	0.32 ± 0.03	0.31 ± 0.03	0.33 ± 0.02	1.45 ± 1.40
Adrenal	0.02 ± 0.00	0.03 ± 0.07	0.03 ± 0.01	0.03 ± .004	0.03 ± 0.07	0.17 ± 0.31

Table 1 (continue): Relative Organ Body Weight of Female Rats Orally Administration Thymoquinone For 28 Days

Dose (mg/kg body weight)						
Organ	**Control**	**Vehicle Control**	**2.5 (Low)**	**5.0 (Mid)**	**10.0 (High)**	**Satellite Group**
Liver	2.99 ± 0.18	2.88 ± 0.16	3.04 ± 0.27	3.00 ± 0.22	2.99 ± 0.15	2.88 ± 0.16
Kidney	0.74 ± 0.06	0.72 ± 0.08	0.75 ± 0.05	0.74 ± 0.06	0.74 ± 0.06	0.72 ± 0.08
Lungs	0.70 ± 0.80	0.69 ± 0.07	0.71 ± 0.04	0.72 ± 0.07	0.71 ± 0.05	0.69 ± 0.07
Brain	0.81 ± 0.00	0.79 ± 0.03	0.78 ± 0.02	0.77 ± 0.03	0.80 ± 0.04	0.80 ± 0.01
Ovary	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.01	0.07 ± 0.01	0.72 ± 0.00	0.07 ± 0.00
Uterus	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.02
Spleen	0.24 ± 0.01	0.22 ± 0.02	0.24 ± 0.03	0.25 ± 0.05	0.24 ± 0.01	0.23± 0.00
Heart	0.28 ± 0.02	0.28 ± 0.02	0.31 ± 0.02	0.30 ± 0.03	0.32 ± 0.02	0.30 ± 0.02
Adrenal	0.29 ± 0.26	0.02 ± 0.00	0.03 ± .004	0.03 ± 0.01	0.08 ± 0.00	0.03 ± 0.01

any significant changes and value are shown in (Table 1).

Biochemical Study

The results of serum biochemical parameters of male rats are shown. There was no change in clinic-chemical parameters of male and female rats exposed to different dose of thymoquinone for 28 days and the values were comparable to controls rats (Table 2).

Haematology

The results of haematological parameters in male and female rats exposed to different doses thymoquinone are shown. There was no significance changes in Hb RBC, WBC and differential leukocyte count (DLT) (Table 3).

Table 2 : Serum Biochemical Parameter In Rats Treated Orally With Thymoquinone For 28 Days

Parameter	**Dose mg/kg body weight**					
	(Control)	**(Vehicle Control)**	**2.5 (Low)**	**5.0 (Mid)**	**10.0 (High)**	**Satellite Group**
AST	19.67±14.45	16.62±14.50	15.94±19.28	21.04±19.67	16.94±30.27	17.84±29.27
ALT	67.70±10.20	67.64±10.25	57.62±10.50	57.42±10.52	62.67±11.16	62.77±12.16
ALP	60.67±14.45	60.62±14.50	60.94±19.28	61.04±19.67	53.94±30.27	53.44±28.27
S-Bilirubin (mg %)	1.20±0.21	1.08±0.11	1.05±0.16	1.38±0.12	1.41±0.19	1.31±0.21
S- Cholesterol (mg %)	46.80±5.01	47.00±5.43	56.00±11.04	54.80±10.13	51.50±9.48	52.54±9.58
S-Albumin (g%)	4.18±0.19	4.18±0.19	4.48±0.31	4.48±0.28	4.29±0.18	4.31± 0.19
S-Protein(g/dl)	7.48±0.21	7.42±0.26	7.66±0.19	7.56±0.11	7.50±0.22	7.66±0.19

Table 3 : Haematological Parameters In Rats Treated Orally With Thymoquinone For 28 Days

Parameter	**Dose mg/kg body weight**					
	0 (Control)	**0 (Vehicle Control)**	**2.5(Low)**	**5.0 (Mid)**	**10.0 (High)**	**Satellite Group**
Hb (mg/dl)	14.69±0.28	14.23±0.38	15.11±0.48	15.29±0.38	14.89±0.26	15.99±0.29
RBC ($x10^6/\mu L$)	8.13±0.18	7.92±0.22	7.18±0.08	7.52±0.28	6.93±0.21	6.83±0.24
WBC (mm^3)	9.07±1.48	9.15±1.28	9.48 ± 2.12	12.41 ±2.48	14.78±1.71	14.66±1.61
Neutrophil (%)	41.47±3.18	39.77± 2.78	37.72± 2.12	36.52± 2.02	36.02± 1.98	36.82± 2.01
Leucocytes (%)	28.01±1.22	29.12±1.52	24.45±1.62	22.82±1.72	18.82±2.78	19.01±2.68
Monocyte (%)	0.35±0.12	0.55±0.55	0.39±0.18	0.52±0.28	0.72±0.24	0.74±0.23
Eosionophil (%)	1.12±0.13	0.94±0.22	0.71±0.28	0.98±0.07	1.0±0.25	1.0±0.25

Table 4 : Histopathological Observation In Tissue Of Rats Treated Treated Orally With Thymoquinone For 28 Days

Tissue	Number of Lesion											
	Dose mg/kg body weight											
	0 (Control)		0 (Vehicle Control)		2.5 (Low)		5.0 (Mid)		10.0(High)		Satellite Group	
	M	F	M	F	M	F	M	F	M	F	M	F
Liver	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Lungs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Ovary	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Testis	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Intestine	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

Histopathology

Autopsy of treated animals after 28 days of exposure revealed no significance change in their vital organs. Microscopic examination of liver, kidney, brain, testes, and ovary of rats treated with the different doses of thymoquinone for 28 days did not shown any significant tissue damage and were comparable with those of controls rats. While, the gross pathological examination observed slightly uterus distention in two control and one treated female rat which, is spontaneous and physiological/cycle nature and did not effect on outcome of study (Table 4).

DISCUSSION

Traditional medicine has maintained greater popularity all over developing world prompted by the increase awareness and interest in medicinal plant and the use is rapidly increase generation by generation (Ogbonnia., et al., 2008). More often, this has led to indiscriminate use with out appropriate dose resulting in abuse. The incidence of adverse effects of these herbal remedies and sometimes life-threatening conditions has been reported among various ethnic groups (Elvin-Lewis, 2001; Kazmi et. al 2003). This has made it imperative to ascertain the toxicity profile of medicinal plants even though they have been used for ages to enable for scientific documentation on their safety/risk potentials The need to evaluate the toxicity profile of thymoquinone (volatile oil of kalonji seed) was prompted by its widespread use in the management of various disease except death as well as use in food additive in the world. The observed behavioral signs of lethargy and abdominal breathing and mortality in the rats that received 2000 mg /kg body weight suggests the involvement of central nervous system in its toxic effect. A single dose toxicity test can contribute initial biological information for classification of chemicals and labeling. The acute toxicity along with subacute toxicity are considered important for the assessment of risk posed by new chemicals substance, natural and synthetic agents in the human environment. This is initial report on subacute dosing of thymoquinone to rats. The present study did not entirely meet the recommended design. There is a lack of long term data of thymoquinone by the oral route in laboratory animals for the evaluation of safety margin, it is necessary to known that the no observed adverse effect level (NOAEL) at the higher dose level without toxic effect. A 28-days oral toxicity study was carried out in rats to determine the potential of thymoquinone to produce toxicity in man.

CONCLUSION

The need to evaluate the toxicity profile of thymoquinone active constitute of black seed of kalonji and lack of long term toxicity data that the present study suggest that thymoquinone is non-toxic since, no marked changes as hematological, biochemical, and histopathological parameters were observed at this dose level of 10 mg/kg body weight thus, at normal therapeutic doses is considered to be safe for long term used as a remedy for all disease except death since, traditional practionaer recommended generation by generation.

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REFERENCES

Ali B. H. and Blunden G., 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res., **17** (4): 299-305.

El-Dakhakhany M., 1963. Studies on the Egyptian *Nigella sativa* L: Somepharmacological properties of its seed's active principle incomparison to its dihydro-compound and its polymer. ArzneimForsch Drug Res., **15**:1227-1229.

Elvin-Lewis M., 2001. Should we be concerned about herbal remedies. J. Ethnopharmacol, **75**: 141-164.

Filippo D'Antuono L., Moretti A. and Lovato AFS, 2002. Seed yield, yield components, oil content and essential oil. content and composition of *Nigella sativa* L. and *Nigella damascena* L. Indust Crops Prod., **15**: 59-69.

Ghazanfar SA., 1994. Handbook of Arabian Medicinal Plants . Boca Raton, FL: CRC Press :180-181.

Hayes A.W, 1994.Principle and Method for Research in Toxicology, 3rd Edition. Raven Press, New York.

Kazmi, F., A. R. Shakoori, M. A. Hafeez and S. S. Ali, 2003. Short-term effects of chlorpyrifos on hematology and biochemical components of blood of the Sprague-Dawley rats. Pak. J. Zool., **35:** 245-257**.**

Nickavar B., Mojab F., Javidnia K. and Amoli M.A., 2003. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Z Naturforsch, **58** (9-10): 629-631.

OECD, 1995: OECD N° 407, "Repeated Dose 28-day Oral Toxicity Study in Rodents". The Organisation for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals, Adopted by the council on 27th July, 1995.

OECD, 2001. OECD N° 423, "Acute toxic class method". The Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, Adopted by the Council on 17th December, 2001.

Ogbonnia S., Adekunle A., Bosa M. K. and Enwuru V. N., 2008. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. African J. Biotech. ,**7** (6): 701 -705.

Riaz M., Syed M. and Chaudhary F.M., 1996: Chemistry of themedicinal plants of the genus *Nigella*. Hamdard Medicus, **39**: 40-45.

Siddiqui A.A. and Sharma P.K.R., 1996. Clinical importance of *Nigella sativa* L. A review. Hamdard Medicus, **39**: 38-42.