

Acute toxicity and antihyperlipidemic activity of rhizome of *Tectaria coadunata* (*Kukkutnakhi*): A folklore herb

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Abstract

Background: *Tectaria coadunata* (Wall. Ex Hook and Grev.) C. Chr (*Kukkutnakhi*) is a pteridophyte fern which is found in Western Ghats, Kerala Ghats, and Mahendragiri forest region. It is used by many *Vaidyas* in hyperlipidemic conditions and obesity. **Aim:** This study aimed to evaluate the acute toxicity and antihyperlipidemic activity of *T. coadunata* in experimental animals. **Materials and Methods:** Oral acute toxicity study was carried out in female Wistar rats as per OECD 425 guideline. Antihyperlipidemic activity of powder of *T. coadunata* (540 mg/kg) was carried out in high-fat diet-induced hyperlipidemia in Wistar albino rats. **Results:** *T. coadunata* rhizome powder at the dose of 2000 mg/kg did not produce any mortality and toxic effects during acute toxicity study in female rats. Test drug produced highly significant ($P < 0.001$) reversal in the triglycerides and very-low-density lipoprotein (VLDL)-cholesterol along with nonsignificant decrease in the cholesterol level in rats fed with hyperlipidemic diet. Further, *T. coadunata* has shown nonsignificant decrease in serum urea, serum glutamic pyruvic transaminase, alkaline phosphatase, and bilirubin direct while statistically significant decrease in bilirubin total in comparison to cholesterol control group. Histopathological study has shown reversal of adverse changes induced by hyperlipidemic diet in heart, liver, and kidney. **Conclusion:** It is concluded that drug is safe up to the dose level of 2000 mg/kg in rats. Rhizome of *T. coadunata* has shown antihyperlipidemic activity in rats, which suggest its potential role in hyperlipidemia and associated conditions.

Keywords: Acute toxicity, hyperlipidemia, *Kukkutnakhi*, *Tectaria coadunata*

Introduction

Tribal as well as folklore claims and sources of herbal medicines were utilized by *Acharyas* of *Ayurveda* while documenting *Ayurvedic* materia medica. *Acharya Sushruta* suggests to collect the information about the drugs from cowherds owner, caretakers of grazing animals, sages, hunters and other forest dwellers.^[1] Several folklore claims are being subjected to scientific evaluation^[2] and the present drug *Tectaria coadunata* (Wall. Ex Hook and Grev.) C. Chr. Dryopteridaceae, commonly known as *Kukkutnakhi*, is being used by certain folks of Maharashtra for the management of *Arbuda* (tumor), *Granthi* (cyst), and other inflammatory diseases.^[3] This drug is least explored with respect to its therapeutic utility. Rhizome of the *T. coadunata* is widely used in folklore practice, but till date, there is no any evidence of experimental research carried out on antihyperlipidemic activity as well as safety on acute administration. This prompted us to evaluate antihyperlipidemic activity of *T. coadunata* in experimental animals. Further, it was evaluated for its acute toxicity study for validation of safety profile in animals.

Materials and Methods

Drug and chemicals

Rhizomes of *T. coadunata* [Figure 1a and b] were collected from its natural habitat, Ambaghat, Kolhapur (Maharashtra), during December 2012 and identified with the help of taxonomist and text pteridophyte flora of Western Ghats, South India. A herbarium sheet of the plant and sample specimen was deposited in Pharmacognosy laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar, Specimen No. PHM 6067/2/01/2013 for future references.^[4] Rhizomes were washed, shade dried, powdered, sieved through BSS no. 100 mesh and preserved in an air-tight glass jar. Hyperlipidemic diet

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Figure 1: Photomicrographs of *Tectaria coadunata* (Wall. Ex Hook and Grev.) (a) and its rhizome part (b)

includes cholesterol in coconut oil and hydrogenated vegetable oil.^[5] All chemicals used in the study were of analytical grade.

Animals

Healthy female rats, nulliparous and nonpregnant, weighing 190 ± 20 g for acute toxicity and Wistar strain albino rats of either sex weighing 200 ± 30 g for antihyperlipidemic activity were obtained from animal house attached to the Institute. Animals were exposed to 12 h light and dark cycles with ideal laboratory condition in terms of ambient temperature ($22^\circ\text{C} \pm 3^\circ\text{C}$) and relative humidity (50%–70%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and drinking water was given *ad libitum*. The experimental protocols were approved by Institutional Animal Ethics Committee (M. D./IAEC/14/2013/21) in accordance with the guideline formulated by Committee for the Purpose of Control and Supervision of Experiments on Animals, India.

Dose fixation

The human therapeutic dose mentioned in Ayurvedic Pharmacopoeia of India for the powder drug is 3–6 g.^[6] Considering this, the dose for animal experimentation was calculated by extrapolating the human dose to animal dose based on the body surface area ratio by referring to the standard table of Paget and Barnes.^[7] Accordingly, the rat dose is 540 mg/kg body weight for antihyperlipidemic activity. The test drug (TD) was administered orally by oral catheter.

Acute oral toxicity study

Acute oral toxicity study for *T. coadunata* rhizome powder was carried out as per OECD 425 guideline.^[8] A total of five overnight-fasted female rats were sequentially dosed once with TD at 2000 mg/kg, orally and observed for 14 days. Mortality, gross behavior and other parameters were closely observed for the first 4 h and up to 8 h on the 1st day and thereafter every 24 h up to 14 days.

Antihyperlipidemic activity

Protocol designed in earlier works has been followed in the present study with some modifications as per experimental need.^[9] Wistar strain albino rats of either sex were randomly divided into four groups each comprised of six rats. Group I was kept as normal control (NC) group, received distilled water as vehicle at a dose of 10 ml/kg, orally. Group II was kept as cholesterol control (CC) group, received vehicle. Group III was administered with TD, *T. coadunata* rhizome powder (540 mg/kg, po), in the form of stock solution. Group IV was administered with standard drug (SD), atorvastatin (7.2 mg/kg, po).^[10]

TDs and vehicle were administered to respective groups at morning hours which continued for 21 days. The hyperlipidemic diet was administered to all groups to induce hyperlipidemia except NC group. Cholesterol extra pure powder was made into 20% suspension in coconut oil (Parachute coconut oil, Batch No. PP018, Goa). The suspension was administered (0.5 ml/100 g) at morning hours to the rats daily for 21 days after 1 h of drug administration. Hydrogenated vegetable oil (Vanaspati ghee –“Raag” brand, Adani Wilmar Ltd., Gujarat) was administered at a dose of 0.5 ml/100 g in evening hours. At the end of experimental periods, blood was withdrawn from the retro-orbital puncture under light ether anesthesia using capillary tube for estimation of serum biochemical and hematological parameters. The body weight of each rat was noted on the last day and rats were sacrificed. The abdomen was opened through midline incision to record the autopsy changes followed by dissecting out the important organs.

Serum biochemical parameters were carried out using fully automated biochemical random access analyzer (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The parameters were total cholesterol,^[11] triglyceride,^[12] high-density lipoprotein (HDL) cholesterol,^[13] very-low-density lipoprotein (VLDL) cholesterol,^[14] apolipoprotein B,^[15,16] blood urea,^[17] serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT),^[18] alkaline phosphatase,^[19] total bilirubin^[20] and direct bilirubin.^[21]

Further, important internal organs were carefully dissected, namely liver, kidney and heart. After noting any sign of gross lesion and ponderal changes, all were transferred to 10% phosphate-buffered formalin solution for fixation and later on subjected to dehydrating, wax embedding, sectioning and staining with hematoxylin and eosin for histological evaluation by light microscopy.

Statistical analysis

The data was expressed as mean \pm standard error of mean for six rats per experimental group. The data generated during the study were analyzed by employing one-way ANOVA followed by Dunnett's multiple *t*-test for unpaired data to determine significance of difference between groups at $P < 0.05$.

Results

Acute toxicity study

T. coadunata rhizome powder at the dose of 2000 mg/kg did not produce any mortality and other toxic effects during the entire duration of study (14 days) in female rats. Gross behavior of all the animals was found to be normal during the study period.

Antihyperlipidemic activity

T. coadunata rhizome powder was evaluated for its effect on experimentally produced hyperlipidemia in rats. A progressive increase in body weight was seen in all animals during experimental period [Table 1]. CC group showed moderate nonsignificant increase in relative weight of liver while nonsignificant decrease in relative weight of kidney in comparison to NC group. Treatment with TD led to marginal decrease in relative weight of liver, heart and kidney in comparison to CC group [Table 2].

Statistically significant increase in serum cholesterol, triglyceride and VLDL cholesterol level was seen in CC group in comparison to NC group. TD group has shown nonsignificant decrease in serum cholesterol while statistically significant decrease in both serum triglyceride and VLDL levels

Table 1: Effect of test drugs on the body weight in experimentally induced hyperlipidemia in albino rats

Groups	Body weight (g)		
	Initial	Final	Percentage change
NC	177.33 \pm 5.90	188.00 \pm 4.67	6.17 \pm 1.12
CC	212.00 \pm 5.42	222.00 \pm 12.22	4.75 \pm 5.23
<i>Tectaria coadunata</i>	193.2 \pm 15.82	201.2 \pm 9.56	5.37 \pm 4.42
Atorvastatin	179.67 \pm 1.745	189.33 \pm 3.17	5.46 \pm 2.32

Data: Mean \pm SEM. NC: Normal control, CC: Cholesterol control, SEM: Standard error of mean

Table 2: Effect of test drugs on relative weight of liver, heart, and kidneys in experimentally induced hyperlipidemia in albino rats

Groups	Relative weight (g/100 g body weight)		
	Liver	Heart	Kidney
NC	3.38 \pm 0.18	0.316 \pm 0.017	0.763 \pm 0.020
CC	3.58 \pm 0.08	0.317 \pm 0.001	0.713 \pm 0.004
<i>Tectaria coadunata</i>	3.28 \pm 0.12	0.309 \pm 0.006	0.686 \pm 0.021
Atorvastatin	3.49 \pm 0.21	0.328 \pm 0.005	0.694 \pm 0.030

Data: Mean \pm SEM. NC: Normal control, CC: Cholesterol control, SEM: Standard error of mean

in comparison to CC group. Treatment with TD apparently increased serum HDL level in comparison to cholesterol. Apolipoprotein-B was seen to be marginally decreased in TD group in comparison to CC group [Table 3].

Statistically significant increase in serum urea, SGOT, alkaline phosphatase and bilirubin levels was seen in CC group in comparison to NC group. *T. coadunata* rhizome powder-treated group has shown nonsignificant decrease in serum urea, SGPT, alkaline phosphatase and bilirubin direct while statistically significant decrease in bilirubin total in comparison to CC group [Table 4].

In addition to the above, histopathological studies have shown fatty degenerative changes, sinusoidal inflammation and cell infiltrations in liver [Figure 2b], moderate fatty degenerative changes in majority of sections of heart of animals in CC group [Figure 3b], fatty degeneration, edematous changes, and intense cell infiltration in kidney [Figure 4b and c] in comparison to NC rats [Figures 2a, 3a and 4a, respectively]. In contrast, hyperlipidemic diet-produced adverse changes which were markedly attenuated by treatment with *T. coadunata* rhizome powder [Figures 2c, 3c, and 4c, respectively] and by treatment with SD, atorvastatin [Figures 2d, 3d and 4d, respectively].

Discussion

Acute toxicity study was employed as per OECD 425 guidelines using female rats. Literature survey of conventional LD₅₀ tests shows that usually there is little difference in sensitivity between the sexes, but in those cases where differences were observed, females were generally slightly more sensitive.^[22] Hence, in the present study, acute toxicity was evaluated in female rats. The study proved that the drug is nontoxic orally up to the dose level of 2000 mg/kg in rats. This dose is many fold higher than the therapeutic equivalent dose of TDs in

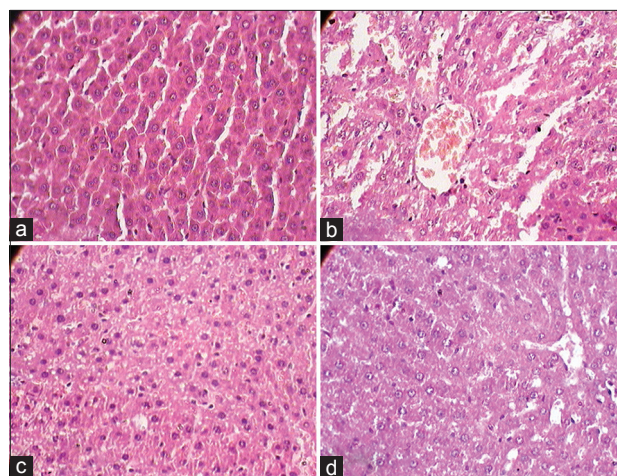


Figure 2: Photomicrographs of representative section of liver. Fatty degenerative changes, sinusoidal inflammation, and cell infiltrations in cholesterol control group (b) in comparison with normal control group (a). *Tectaria coadunata* rhizome powder-treated (c) and atorvastatin-treated (d) rat shows mild fatty changes in comparison with cholesterol group (1 \times 400)

Table 3: Effect of test drugs on lipid profile in experimentally induced hyperlipidemia in albino rats

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	VLDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)	Apolipo protein-B (mg/dl)
NC	46.00±3.22	96.5±14.05	19.30±2.81	39.17±1.85	-
CC	77.33±6.36*	176.0±9.59*	35.2±1.92*	41.67±4.00	17.00±2.08
<i>Tectaria coadunata</i>	62.60±3.39	95.40±5.16 [#]	20.77±1.89 [#]	42.50±5.44	15.75±0.63
Atorvastatin	56.50±2.63 [#]	104.67±11.82 [#]	20.93±2.36 [#]	43.83±6.16	14.17±1.33

Data: Mean±SEM. * $P<0.01$ compared to normal control group, [#] $P<0.01$ compared to cholesterol control group (ANOVA followed by Dunnett's multiple *t*-test). VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, NC: Normal control, CC: Cholesterol control, SEM: Standard error of mean

Table 4: Effect of test drugs on serum parameters in experimentally induced hyperlipidemia in albino rats

Groups	Urea (mg/dl)	SGPT (IU/L)	SGOT (IU/L)	Alkaline phosphatase (IU/L)	Bilirubin total (mg/dl)	Bilirubin direct (mg/dl)
NC	43.50±1.48	53.00±2.86	145.17±6.48	269.00±46.83	0.65±0.1	0.17±0.02
CC	61.5±1.65**	52.33±3.48	219.83±15.77**	535.00±87.81**	1.07±0.08*	0.28±0.05*
TD	59.20±3.43	27.17±12.29	228.83±19.37	372.83±63.00	0.67±0.13 [#]	0.18±0.03
SD	41.00±3.20 ^{##}	57.60±5.66	154.83±8.77 ^{##}	426.00±46.81	0.62±0.05 ^{##}	0.15±0.02 [#]

Data: Mean±SEM. * $P<0.05$, ** $P<0.01$ compared to normal control group. [#] $P<0.05$, ^{##} $P<0.01$ compared to cholesterol control group (ANOVA followed by Dunnett's multiple *t*-test). SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, NC: Normal control, CC: Cholesterol control, SEM: Standard error of mean, TD: Test drug, SD: Standard drug

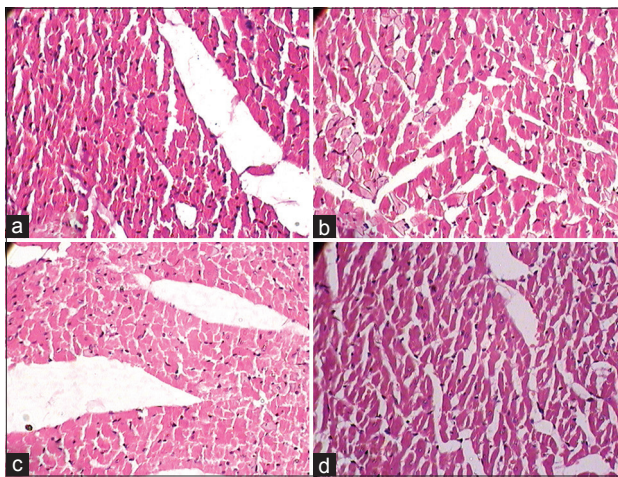


Figure 3: Photomicrographs of representative section of heart. Moderate fatty degenerative changes in cholesterol control group (b) in comparison with normal control group (a). *Tectaria coadunata* rhizome powder-treated (c) and atorvastatin-treated (d) rat shows almost normal cytoarchitecture in comparison with cholesterol group (1 × 400)

rats, implicating that the TD is relatively safe for clinical use at therapeutic dose level.

A progressive increase in body weight was observed in all animals during experimental period in almost similar fashion, which suggests that the rats were devoid of drastic adverse changes during experimental period. Decrease in the organ weight is indicative of degenerative changes or loss of tissue of that particular organ, while increase in the weight may be due to hyperfunctioning of that organ or edematous changes.^[23] In the present study, administration of hyperlipidemic diet showed moderate increase in relative weight of liver whereas significant decrease in relative weight of kidney. The observed changes corroborate with the

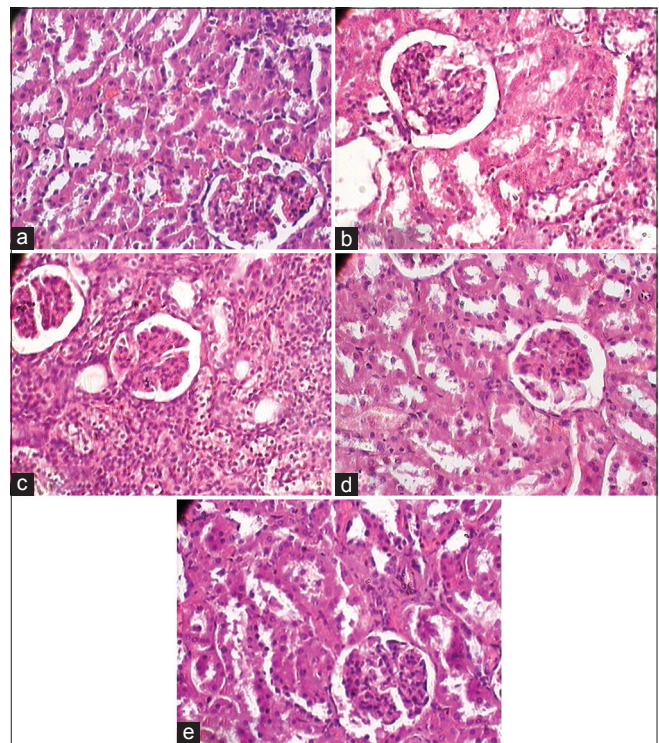


Figure 4: Photomicrographs of representative section of kidney. Fatty degeneration, edematous changes, and intense cell infiltration in kidney in cholesterol control group (b and c) in comparison to normal control group (a). *Tectaria coadunata* rhizome powder-treated (c) and atorvastatin-treated (d) rat shows almost normal cytoarchitecture in comparison with cholesterol group (1 × 400)

adverse effects observed in histopathological studies of the above organs. Treatment with TD leads to marginal decrease in relative weight of liver, heart, and kidney in comparison to CC group.

Elevated levels of different types of lipids have been implicated in the production of atherosclerosis. In this condition, the blood vessel wall thickens due to accumulation of lipid and this leads to loss of elasticity of blood vessel wall and becomes the cause of many cardiovascular system complications such as myocardial infarction, stroke, and peripheral vascular disease which account for significant mortality in developed and developing countries.^[24] In the present study, administration of hyperlipidemic diet led to significant elevation of serum cholesterol, serum triglycerides, serum low-density lipoprotein (LDL), and serum VLDL levels in comparison to NC rats on normal diet. This establishes the efficacy of the experimental protocol to induce hyperlipidemic condition. TD produced significant decrease in the triglycerides and in VLDL levels along with nonsignificant decrease in the cholesterol level. Surprisingly, a nonsignificant increase in HDL cholesterol level was observed in TD group. This activity can have good therapeutic application since elevation of HDL cholesterol level with concomitant decrease in LDL cholesterol level will be quite useful in patients with hypercholesterolemic conditions.

Elevation of serum transaminase is indicative of liver injury due to leakage of this enzyme from the tissue into the serum. Increased activity is observed in inflammatory, degenerative, and neoplastic lesions of the liver.^[25]

Hyperlipidemic status was associated with liver and kidney injury as marked elevation of hepatic enzymes; transaminase and serum alkaline phosphatase as well as serum urea and bilirubin level in rats. *T. coadunata* rhizome powder-treated group has shown nonsignificant decrease in serum urea, SGPT, alkaline phosphatase and bilirubin direct while statistically significant decrease in bilirubin total in comparison to CC group which suggests the liver and kidney protective role of TD in rats.

Based on previous works, mechanisms have been proposed to explain antihyperlipidemic activity of *Kuklutnakhi*. It might interfere with the formation of lipoproteins by inhibiting biosynthesis of cholesterol in the liver.^[26] Secondly, by interfering with the formation of endogenous triglycerides in the tissues and by inhibiting the enzyme diacyl glycerol transferase, it may increase fecal excretion of bile acids and cholesterol, substantially decreasing the rate of absorption of fat and cholesterol in the intestine^[27] and finally, by inhibiting the activity of the lipoprotein lipase at different sites and by inhibiting the activity of the rate-limiting enzyme in cholesterol biosynthesis-3-hydroxy 3-methyl 3-methylglutaryl CoA.

Conclusion

From the present study, it can be concluded that drug *T. coadunata* is relatively safe for clinical use at therapeutic dose level. This study demonstrated the antihyperlipidemic activity of rhizome of *T. coadunata* (*Kuklutnakhi*) in high-fat diet-induced hyperlipidemia in rats which suggests its potential

role in hyperlipidemia and associated conditions, hence proved the folklore claims scientifically in rats.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

टेक्टेरिया कोएडूनेटा के कंद (कुक्कुटनखी)की तीव्र विषाक्तता परीक्षण तथा हायपरलिपिडेमिया प्रतिरोधी क्रिया – एक लोक साहित्यिक वनस्पति

हार्दिक मोरी, के. निष्ठेश्वर, बी. आर. पटेल, मुकेश नारिया

टेक्टेरिया कोएडूनेटा .चर .सी (.एण्ड ग्रेव.हुक.एक्स.वाल) कुक्कुटनखी यह टेरिडोफाइट फर्न है जो की पश्चिमी घाट, केरला घाट तथा महेन्द्रगिरी जंगल में मिलता है इसे बहुत से वैद्यों द्वारा हायपरलिपिडेमिया तथा स्थौल्य नियंत्रण के लिए प्रयुक्त किया गया है। प्रस्तुत अध्ययन में टेक्टेरिया कोएडूनेटा के कंद का प्रयोगिक जानवरों में तीव्र विषाक्तता परीक्षण तथा हायपरलिपिडेमिया प्रतिरोधी क्रिया का अवलोकन किया गया जिसमें मादा विस्टर चूहों में तीव्र विषाक्तता का अध्ययन ओ.ई.सी.डी. ४२५ के मार्गदर्शन के तहत किया गया। टेक्टेरिया कोएडूनेटा के कंद चूर्ण का ५४० मि मात्रा में .ग्रा.कि./ग्रा. हायपरलिपिडेमिया प्रतिरोधी क्रिया के अध्ययन हेतु ज्यादा मात्रा में वसा युक्त भोजन देकर तैयार किए गए हायपरलिपिडेमिया ग्रस्त विस्टर अल्बिनो चूहों में देखा गया तथा वजन में बदलाव, जैवरासायनिक मानक तथा ऊतीविकृत परीक्षण किए गए। परिणाम स्वरूप टेक्टेरिया कोएडूनेटा के कंद चूर्ण की २००० मि.ग्रा./कि.ग्रा. ने मादा प्रदत्त चूहों में विषाक्तता में कोई प्रतिक्रिया नहीं दिखाई तथा कोई अवांछनीय लक्षण स्पष्ट नहीं हुये। चिकित्सा औषधि चूहों में अधिक मात्रा में तथा वी.एल.डी.एल. के स्तर में कमी आई तथा कोलेस्ट्रॉल के स्तर में अल्प कमी आई। टेक्टेरिया कोएडूनेटा ने रक्तगत यूरिया, एस जी पी टी, अल्केलाइन फॉस्फेट में आवश्यक कमी तथा कुल विलीरूबिन में सांख्यिकीय दृष्टि से कमी कोलेस्ट्रॉल नियंत्रण समूह की तुलना में स्पष्ट दिखाई। ऊती विकृति परीक्षण, ज्यादा लिपिड दिये गए भोजन से पैदा हुये हृदय, यकृत तथा वृक्क के अवांछित बदलाव पूर्ववत हो गए। इस प्रकार यह निष्कर्ष निकाला गया कि टेक्टेरिया कोएडूनेटा के कंद चूर्ण की २००० मि.ग्रा./कि.ग्रा. तक की मात्रा चूहों में सुरक्षित है टेक्टेरिया कोएडूनेटा के कंद चूर्ण ने हायपरलिपिडेमिया प्रतिरोधी क्रिया को चूहों में दिखाया तथा यह हायपरलिपिडेमिया तथा उससे संबन्धित अवस्थाओं में कारगर सिद्ध होता है।