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TOXIC EFFECTS OF CASSIA ALATA ON BIOCHEMICAL AND ENZYMATIC PARAMETERS OF TRIBOLIUM CASTANEUM (HERBST)

Upadhyay R.K.*, Gupta R.K., Prajapati K.K. and Ahmad S.

Department of Zoology, D D U Gorakhpur University, Gorakhpur 273009, U.P. India.

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***Corresponding Author**
Upadhyay R.K.
Department of Zoology, D D U Gorakhpur University, Gorakhpur 273009, U.P. India.

ABSTRACT

In the present investigation different Cassia alata solvent extracts were evaluated for its toxic effects on *T. castaneum* in the laboratory. Its effect was observed on changes in the level of different bio-molecules and certain metabolic enzymes of *T. castaneum* at different time periods i. e 4hr, 8hr, 12hr and 16hr following sub-lethal (40% and 80% 0f 24-h LD_{50}) exposures different solvent extracts of *C. alata* was studied. Six extracts were applied for obtaining LD_{50} values. The LD_{50} values of acetone, chloroform, petroleum ether, methanol, hexane and water was found to be very low i.e. 0.840µg/gm, 0.510µg/gm, 0.590µg/gm, 1.57µg/gm 0.310 µg/gm and 2.50µg/gm respectively. Activity of different bio-molecules i. e. glycogen, protein, DNA, RNA and amino acids in treated beetles (*T. castaneum*) was significantly ($p<0.05$) decreased after 16 hr treatment in comparison to control beetles. Hexane extract have shown higher depletion i.e. 51.39%, 40.82%, 28.25%, 32.18% and 28.33% in glycogen, protein, DNA, RNA and amino acids respectively. Same Above extract have potentially inhibit the activity of acid phosphatase (54.85%), alkaline phosphatase (60.71%), glutamate pyruvate transaminase (62.69%) and glutamate oxaloacetate transaminase (72.37%), lactic dehydrogenase (85.46%) and acetylcholinesterase (63.51%) and obstruct the physiological homeostasis. Low LD_{50} values obtained in different fractions and its action on enzymes and other biomolecules indicates that *Cassia alata* possess potential candidates that can be used for control of *T. castaneum* and other stored grain insects.

KEYWORDS: *Tribolium castanem*, *Cassia alata*, Enzymatic changes, bio pesticides.

INTRODUCTION

Tribolium castaneum is the first to colonize a new stock and according to an estimate the overall damage caused by the *Tribolium* annually in stored grains approximately account for 10-40 % of loss Worldwide.[1] It is also known to cause up to an economic damage rate of 34 and 40% respectively of stored millet and wheat flour.[2] Among all the stored grain insects *Tribolium* is a dangerous stored grain pest that damages food grains, occurs in storehouses and godowns and has a worldwide distribution.[3] It eats the entire content of the grain and leave the hollow shell of grain behind.[4] For protection of stored grains and other agricultural products from insect infestation, various synthetic pesticides especially in the form of fumigant were used against insect pests. But it has been reported that exposed insects have acquired resistance against most of the insecticides.[5] The massive use of these synthetic pesticides is not ecologically safe and causes various biochemical and behavioral changes in animals and humans.[6] Thus it is necessary to develop certain new alternatives that might have no adverse effects on environment and non-target animals.[7] Plants are composed of certain active chemicals which are directly beneficial for its the growth and development of the plant. Rather, they are part of the plant's defense against plant-feeding insects and other herbivores.[8] Plant materials that have been reported to be efficacious against insect pests include.[9] Furthermore, different insects react in varying ways to different plant products. Few major stored grain pests are *Sitophilus oryzae* Linn. (Rice weevil), *Trogaderma grariarium* (Khapara beetle), *Rhizopertha dominca* (Fabr), *Tribolium castaneum* (Herbst) (Rust red flour beetle), *Sitotraga cerealella*.[10] Grain and flour moth, *Bruchus chinensis* (Pulse beetle).

In this connection it has already been established that many plant products have insecticidal properties at against insect pests of field crops[11] as well as stored grains.[12] The present study deals with effects of *Cassia alata* extracts on different bio-molecules and metabolic enzymes of a strain of *T. castaneum* (Herbs).

MATERIALS AND METHODS

Insect culture

Adult insects of *Tribolium castaneum* (Herbst) were collected from the food grain store houses available in local market in Gorakhpur. The beetles were reared on healthy, clean and un-infested wheat seeds in glass jars and capped with muslin cloth for ventilation. Culture was maintained in laboratory under controlled temperature (28 ± 2^0C), relative humidity

(75±5% RH) and a photoperiod of 12: 12 (L:D) h in B.O.D. Insects were reared in glass jars on gram seeds and each time early age beetles were used for the experiments.

Collection of plant material

Fruits of *Cassia alata* of were collected from different places of western part of India especially from state of Rajasthan. Specimens were identified by applying standard taxonomic key specially by observing inflorescence and family formula with the help of a taxonomic expert. Fresh plant material was used to prepare extracts. Plant material was dried, chopped, grounded and milled to make powder in domestic grinder.

Preparation of extracts

Fruits of *T. castaneum* were collected and chopped in to small pieces, dried and pulverized to make fine powder in an electric grinder. The powdered stem (200 gm) was then extracted with various solvent according to their polarity. Extracts were allowed to evaporate in a speed vac to get residue. It was dried and weighed and re-dissolved in known volume of different solvents. Dissolved residues were stored in cold at 4^0C temperature for experimental purpose.

Toxicity bio-assays

Adults of *Tribolium castaneum* were exposed with various increasing concentrations of each plant extracts separately. For this purpose, separate filter paper strips (1 cm^2) were coated with different concentrations of plant extracts were placed in the glass culture tubes and open ends were plugged with cotton balls. The coated filter paper strips were air-dried before application. Only solvent treated filter papers were strips used to set control. Ten adult insects were released culture in glass culture tubes (10 cm Height X 4 cm diameter). For each extract, five different concentrations were used and for each concentration six replicates were set. Mortality in *Tribolium castaneum* was recorded after 24 hr in presence and absence of various plants extracts separately. LD_{50} values were determined by Probit method.[13] LD_{50} values were calculated in µg/gm body weight of the insect.

Determination of glycogen

Glycogen contents were measured according to method of Dubois et al.[14] For this purpose 500 mg of *T. castaneum* were homogenized in 2ml of 5% Tri-chloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50ml of 5% TCA and 6

ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100gm of body weight of *T. castaneum*. Three treatments were performed at three trials. Data obtained was statistically analyzed by using ANOVA method.

Determination of total free amino acid

Level of free amino acids was determined following Spies.[15] A total 500 mg of *T. castaneum* were homogeninzed in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at 15,000 X g for 20 minutes and supernatant was separated. For estimation of total free amino acids 0.1 ml of supernatant was taken and to it 0.1 ml of distilled water and 2.0 ml Ninhydrin reagent were mixed. The reaction mixture was kept in boiling water for 15 minutes. A total of 2 ml of 5.0% ethyl alcohol was added to the above boiled mixture. A violet color was developed in the reaction mixture which was measured at 575 nm. For calculating the total free amino acid content standard curve was prepared by using known amount of glycine and was expressed in gm/100gm body weight of *T. castaneum*. Three replicates were used and data is statistically analyzed by ANOVA method.

Determination of nucleic acids

Level of nucleic acids in the whole body extracts of *T. castaneum* was estimated according to method of Scheidner.[16] For this purpose a total 500 mg of *T. castaneum* were fed with 40% and 80% of LD_{50} of different solvent extracts of *C. alata* separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at 15,000Xg for 25 minutes.

DNA estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H_2SO_4) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue color was developed in the solution which is measured at 595 nm (O.D.).

RNA estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8ml of distilled water. Now 2ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green color was developed, which was measured at 660nm. In both cases three replicates were set and data obtained was statistically analyzed by ANOVA method.

Determination of total protein

Total proteins of *T. castaneum* were estimated according to Lowry et al.[17] For this purpose 500 mg of *T. castaneum* were treated with 40% and 80% of LD_{50} of different solvent extracts of *C. alata* These treated *T. castaneum* were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000Xg for 15 minutes. Each experiment was performed three times. Standard curve was prepared by using 10 µg, 20 µg, 40 µg, 80 µg and 100µg of Bovine serum albumen. Data obtained was statistically analyzed by ANOVA method.

***In vivo* Determination of enzymatic parameters**

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided sub-lethal doses (40% and 80% of LD_{50}) of different solvent extract of *C. alata* was provided. Insects were sacrificed at the 4 h interval up to 16 h for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged at 4^0C for 25 minutes at 15,000 X g. Supernatant was isolated in a glass tube and used as enzyme source.

Determination of acid and alkaline phosphatase

Level of alkaline phophatase was determined according to the method of Bergmeyer.[18] For this purpose 500 mg of *T. castaneum* were homogenized in 1 ml of PBS buffer at 4^0C and centrifuged at 15,000 X g for 15 min. A 0.2 ml of supernatant was taken in a test tube and 1.0 ml of acid buffer substrate solution was added. Contents were mixed thoroughly and incubated for 30 minutes at 37^0C. Now 4.0 ml of 0.10N NaOH solution was added to the incubation mixture. Similarly, for determination of ALP, 0.10 ml of supernatant was taken in a test tube and 1.0 ml of alkaline buffer substrate was mixed with it. The mixture was mixed thoroughly and incubated for 30 minutes at 37^0C. Now 5.0 ml of 0.02 N NaOH was added to the incubation mixture. The reaction was stopped by adding excess of NaOH. The p-nitrophenol formed as result of hydrolysis of p-nitrophenyl phosphate gave a yellow colour with NaOH. Optical density was measured at 420 nm. Standard curve was drawn with the help of different concentrations of p-nitrophenol. Enzyme activity was expressed as µ moles of p-nitrophenol formed /30min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogenase was measured according to the method of Annon.[19] For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5)

in ice bath and centrifuged at 10000 X g for 30 minutes in cold centrifuge at 4^0C. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Now the contents were incubated at 37^0C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixed and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Enzyme activity has been expressed as μ moles of pyruvate reduced/45min/mg protein.

Determination of glutamate pyruvate transaminase and glutamic-oxaloacetic transaminase

GPT and GOT activity was measured according to the method of Reitman and Frankel.[20] A total of 500 mg *T. castaneum* were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 X g for 15 min at 4^0C. For determining the activity of GPT, 0.10 ml of enzyme source was taken and 0.50 ml of GPT substrate. Similarly, for determination of GOT, 0.10 ml of enzyme source was taken and 0.50 ml of GOT substrate was added to it. Now 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and contents were left stand for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 20 minutes. The optical density was read at 505 nm after setting the blank. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was expressed in units of glutamate pyruvate transaminase or glutamate oxaloacetate transaminase activity/ hr/mg protein.

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al.[21] For this purpose 500mg treated *T. castaneum* were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at 1000 X g for 30 minutes in cold centrifuge at 4^0C. To the supernatant mixed 0.10 ml (5×10^{-4} M) of freshly prepared acetylcholinethioiodide solution, 0.05 ml of DTNB reagent (chromogenic agent) and 1.45 ml of PBS (pH 6.9) were added. The changes in optical density were monitored at 412 nm regularly for three minutes at 25^0C. Enzyme activity has been expressed as μ moles 'SH' hydrolysed per minute per mg protein.

Statistical analysis

The LD_{50} for each extract was determined by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied.[22]

RESULTS

Toxicity determination

The solvent extracts of *C. alata* have shown potent toxicity against the insect *T. castaneum* and displayed very low LD_{50} i.e. 1.5 µg/gm, 1.2µg/gm, 1.2 µg/gm, 1.57 µg/gm, 0.3 µg/gm and 2.0 µg/gm of body weight of *T. castaneum* for acetone, chloroform, petroleum ether, methanol, hexane and water extracts respectively (Table 1).

Determination of bio-molecules

Treatment of *T. castaneum* with 40% of 24-h LD_{50} of *C. alata* acetone, chloroform, petroleum ether, methanol hexane and water extracts have significantly depleted the glycogen content up to 65.32%, 38.01%, 58.55%, 62.68%, 59.85%, and 63.33%after 16 hr of 24-h LD_{50} of the extracts (Table2-7). For the same a higher depletion was observed when insect were treated with 80% o 24-h LD^{50} i. e., 31.09%, 32.24%, 48.26 and 48.41%, 51.39% and 47.41% (Table 2-7). Same extracts have also cut down the protein synthesis and up to 52.82%, 45.39%, 56.13%, 46.86%, 41.69% and 63.53% after 16 hr of 40% of 24-h LD_{50} treatment in comparison to control. More depletions were caused by 80% of 24-h LD_{50} treatment that were found to be 50.72%, 45.17%, 52.76%, 45.28% 40.32% and 57.50% (Table 2-7). Similarly, 40% of 24-h LD_{50} of the solvent extracts significantly ($p<0.05$) inhibited the DNA content up to 56.69%,45.13%, 46.90%, 49.90%, 40.17% and 50.81%, while 80% of LD^{50} have shown more percent depletions i. e., 46.49%, 35.40%, 44.94%, 44.94%, 28.25% and 43.66% in comparison to control (Table 2-7). In a similar consequence RNA content was inhabited by 40% of 24-h LD_{50} up to 43.35%, 43.35%, 41.04%, 42.77%, 33.14% and 36.94%, while these were 28.32%, 34.49%, 33.91%, 33.14%, 32.18% and 33.14% in insects treated with 80% of 24-h LD_{50} (Table 2-7) The insects treated with 40% of LD_{50} have displayed reduced amino acid contents i. e. 61.50%, 36.68%, 63.92%, 41.52%, 34.98%, 57.14% and a dose of 80% of 24-h LD_{50} have shown depletion of the same parameters up to 46.49%, 33.53%, 57.99%, 38.62%, 28.33% and 44.13% in comparison to control (Table 2-7).

Determination of enzymes

The solvent and aqueous extracts of *C. alata* have exerted significant ($p<0.05$) inhibitory activity against certain metabolic enzymes of *T. castaneum*. Among the extracts hexane extract has caused higher inhibitory activity against the enzymes and significantly it's 40% of 24-h LD_{50} have reduced the body content of ACP (55.49%), ALP (69.34%), GPT (64.46%), GOT (76.32%), LDH (85.56%) and AChE (64.76), while 80% of 24-h LD_{50} have cut down ACP (54.85%), ALP (60.71%), GPT (62.69%), GOT (72.37%), LDH (85.46%) and AChE (63.51%) (Table 8-13) Contrarily, aqueous extract have shown lower activity against the same enzymes and have shown lesser inhibition after treatment with 40% of 24-h LD_{50} i. e., 81.54%, 87.37%, 94.36%, 92.74%, 96.69% and 91.58% (Table 8-13). Further, 80% of LD_{50} of aqueous extract have also not found to be effective against the enzymes and shown inhibition i.e. 80.86%, 85.57%, 91.97%, 88.25%, 94.88% and 90.74% (Table 8-13) However, acetone chloroform petroleum ether and methanol have also shown inhibitory activity against these metabolic enzymes having moderate activity data presented in tables 8-13.

DISCUSSION

For effective and fast control of stored grain insect synthetic pesticides are being used, which show very high lethality, but subsequently, it is contaminating the environment and causing food poisoning. However in the present investigation natural extracts isolated from *C. alata* have been used to observe its lethality in *T. castaneum.* When *T. castaneum* were treated with 40% and 80% of 24-h LD_{50} of acetone, chloroform, petroleum ether, methanol hexane and aqueous extracts of *C. alata*, each of them significantly ($p<0.05$) reduced the level of glycogen, protein, DNA, RNA and amino acids. Hexane extract have shown higher activity and showed depletions. 51.39%, 40.82%, 28.25%, 32.18% and 28.33% in glycogen, protein, DNA, RNA and amino acids (Table 2-8). Similar results were reported in *Pimpla turionella* wasp when its larvae, pupae and adult females were treated with cypermethrin. Cypermethrin reducesd the level of glycogen, protein, nucleic and acids.[23] Few organophosphorus insecticides such as chloropyrifos, thiamethoxam, fipronil and malathion caused disintegration in protein and carbohydrate metabolism in silk worm *Bombyx mori*.[24] Depletion of glycogen indicates more and more utilization of food reserves to cope up the insecticide induced stress. This decrease in glycogen level may be due to high release of glucagon, corticosteroids and catecholamines which stimulate glucose production to combat energy demand. Normally in the body free glycogen floats in the haemolymph/blood that

after breakdown help to maintain glucose level in blood. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment.[25] Similarly protein and nucleic acid synthesis may also block at cellular level and catabolism get increased which results into low availability of proteins and nucleic acid.[26] However, certain metabolic enzymes such as ACP, ALP GPT, GOT, LDH and AChE have been found to be reduced after the treatment of *C. alata* extracts. Similar to bio-molecules the hexane extracts were found to have highest activity. It showed inhibition of 54.85%, 60.71%, 62.69%, 72.37% 85.46% and 63.51% in ACP, ALP GPT, GOT, LDH and AChE respectively (Table 8-13) This inhibition indicates obstruction in their chemical pathways that led to the formation of abnormal state in the insects and make insects unable to survive.[27]

After the treatment of *C. alata* extracts insects have displayed a high degree of stress and to fight against this stress insect requires induction in hydrolytic activities within the body tissues which did not occur due to cut down of the acid and alkaline phosphatase level and the toxic stress persists even up to 16 hrs.[28] An increase in glycogenesis causes a significant decrease in free amino acid level and transamination of amino acids was get reduced, hence the level of glutamate pyruvate transaminase and glutamate oxaloacetate transaminase get decreased. As a result of obstruction in transaminase activity, protein synthesis was retarded. Therefore, a sharp decrease or increase in the level of above enzymes effect oxygen consumption in insects. In the present study change in the level of various enzymes in whole body extract of *Tribolium castaneum* may be due to physiological alterations which are induced by compounds isolated from different solvent extracts of *C. alata*. However, elevation or reduction in enzyme level is associated with physiological imbalance in insects.[29] Similarly, Chanda[30] have also reported the changes in the levels of certain enzyme during the course of insecticidal effect of malathion and carbaryl. However, *C. alata* it can be concluded that solvent extracts of *C. alata* work as potent molecular toxicant showing very high lethality on body tissues of *Tribolium castaneum*.

CONCLUSION

Table 1: LD_{50} of different extracts of *Cassia alata* against *Tribolium castaneum* (Herbst)

Solvent extract	LD_{50} (µg/gm)	UCL	LCL	Slope function
Acetone	0.840	1.334	0528	1.7
Chloroform	0.510	0.747	0.347	1.55
Petroleum ether	0.590	0.903	0.385	1.63
Methanol	1.57	0.843	0.320	1.74
Hexane	0.310	0.480	0.200	1.65
Water	2.50	3.665	1.705	1.55

Table 2: Effect of 40% and 80% of LD_{50} of *Cassia alata* acetone fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Tribolium castaneum*(Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
Glycogen	2.01±0.04 (100)	1.697±0.043 (84.42)	1.457±0.035 (72.48)	1.623±0.023 (80.74)	1.310±0.026 (65.17)	1.390±0.038 (69.15)	1.110±0.026 (55.22)	1.313±0.059 (65.32)	1.027±0.035 (51.09)
Protein	9.187±0.070 (100)	7.277±0.043 (79.21)	6.136±0.067 (57.40)	6.687±0.099 (72.79)	5.273±0.099 (57.40)	5.403±0.109 (58.81)	4.803±0.066 (52.28)	4.853±0.075 (52.82)	4.660±0.056 (50.72)
DNA	0.545±0.001 (100)	0.432±0.0032 (79.25)	0.408±0.003 (74.85)	0.378±0.0026 (69.34)	0.341±0.0029 (62.56)	0.331±0.0021 (60.72)	0.295±0.0029 (54.12)	0.309±0.0021 (56.69)	0.248±0.002 (45.49)
RNA	0.519±0.007 (100)	0.373±0.0037 (71.87)	0.275±0.0017 (52.99)	0.308±0.0037 (59.34)	0.238±0.0035 (45.86)	0.248±0.0043 (47.78)	0.195±0.0018 (37.57)	0.225±0.0024 (43.35)	0.147±0.0007 (28.32)
Amino acid	0.826±0.005 (100)	0.795±0.0015 (96.24)	0.708±0.0023 (85.71)	0.711±0.0018 (86.07)	0.668±0.0018 (80.87)	0.594±0.0023 (71.91)	0.441±0.0018 (53.39)	0.508±0.0043 (61.50)	0.384±0.0046 (46.49)

Values are mean ±SE of three replicates.

Table 3: Effect of 40% and 80% of LD_{50} of *Cassia alata* chloroform fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Tribolium castaneum*(Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
Glycogen	2.01±0.04 (100)	1.3±0.047 (64.67)	0.974±0.0032 (48.46)	1.09±0.0017 (54.22)	0.867±0.0035 (43.13)	0.928±0.0043 (46.17)	0.751±0.011 (37.36)	0.764±0.0058 (38.01)	0.648±0.0017 (32.24)
Protein	9.187±0.070 (100)	7.197±0.093 (78.34)	6.617±0.067 (72.03)	6.697±0.067 (72.90)	5.541±0.011 (60.31)	5.683±0.035 (61.86)	5.334±0.105 (58.06)	4.170±0.015 (45.39)	4.150±0.072 (45.17)
DNA	0.545±0.001 (100)	0.435±0.00018 (79.80)	0.334±0.0038 (61.27)	0.369±0.0018 (67.69)	0.275±0.0029 (50.45)	0.310±0.0008 (56.87)	0.237±0.0006 (43.48)	0.246±0.0014 (45.13)	0.193±0.0018 (35.40)
RNA	0.519±0.007 (100)	0.409±0.0029 (78.81)	0.381±0.0048 (61.46)	0.346±0.0045 (66.67)	0.319±0.0037 (61.46)	0.318±0.0032 (61.27)	0.253±0.0029 (48.75)	0.225±0.0018 (43.35)	0.179±0.0047 (34.49)
Amino acid	0.826±0.005 (100)	0.724±0.003 (87.65)	0.667±0.0024 (80.75)	0.525±0.0042 (63.56)	0.439±0.0047 (53.14)	0.471±0.0044 (57.02)	0.340±0.0084 (41.16)	0.303±0.004 (36.68)	0.277±0.0035 (33.53)

Values are mean ±SE of three replicates

Table 4: Effect of 40% and 80% of LD_{50} of *Cassia alata* petroleum ether fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Tribolium cataneum*(Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
Glycogen	2.01±0.04 (100)	1.53±0.006 (76.12)	1.490±0.023 (74.13)	1.403±0.026 (69.80)	1.263±0.04 (62.83)	1.297±0.038 (64.52)	1.16±0.023 (57.71)	1.177±0.02 (58.55)	0.970±0.036 (48.26)
Protein	9.187±0.070 (100)	8.830±0.074 (96.11)	8.557±0.082 (93.14)	7.663±0.0027 (83.41)	7.323±0.062 (79.71)	6.307±0.096 (68.65)	6.2±0.095 (67.49)	5.157±0.066 (56.13)	4.847±0.07 (52.76)
DNA	0.545±0.001 (100)	0.482±0.003 (88.42)	0.385±0.0014 (70.63)	0.421±0.0027 (77.23)	0.364±0.0023 (66.77)	0.347±0.0024 (63.66)	0.296±0.0035 (54.30)	0.253±0.0018 (46.41)	0.245±0.0058 (44.94)

RNA	0.519±0.007 (100)	0.364±0.0046 (70.13)	0.318±0.0046 (61.27)	0.329±0.0029 (63.39)	0.235±0.0018 (45.28)	0.254±0.0033 (48.94)	0.204±0.0061 (39.31)	0.213±0.0046 (41.04)	0.176±0.0043 (33.91)
Amino acid	0.826±0.005 (100)	0.779±0.0018 (94.30)	0.589±0.0018 (71.30)	0.681±0.0035 (82.44)	0.52±0.0017 (62.95)	0.592±0.0032 (71.67)	0.5±0.0023 (60.53)	0.528±0.005 (63.92)	0.479±0.0026 (57.99)

Values are mean ±SE of three replicates.

Table 5: Effect of 40% of LD_{50} of *Cassia alata* methanol fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Tribolium castanium*(Herbst).

Parameters	**Time (in h)**								
	0 (Control)	**4**		**8**		**12**		**16**	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.730±0.032 (86.07)	1.456±0.032 (72.78)	1.570±0.035 (78.11)	1.317±0.026 (65.52)	1.367±0.032 (68.01)	1.213±0.035 (60.35)	1.260±0.04 (62.68)	0.975±0.038 (48.41)
Protein	9.187±0.070 (100)	8.43±0/065 (91.76)	7.687±0.078 (83.67)	7.403±0.063 (80.58)	6.143±0.039 (66.87)	5.883±0.046 (64.04)	5.680±0.046 (61.83)	4.303±0.041 (46.86)	4.160±0.053 (45.28)
DNA	0.545±0.001 (100)	0.41±0.023 (75.21)	0.361±0.0018 (66.69)	0.347±0.0014 (63.66)	0.315±0.0018 (57.79)	0.311±0/004 (57.05)	0.289±0.004 (53.02)	0.272±0.002 (49.90)	0.245±0.001 (44.94)
RNA	0.519±0.007 (100)	0.369±0.0024 (71.10)	0.315±0.004 (60.69)	0.323±0.0018 (62.23)	0.242±0.002 (46.63)	0.247±0.0024 (47.59)	0.239±0.0024 (46.05)	0.222±0.0055 (42.77)	0.172±0.002 (33.14)
Amino acid	0.826±0.005 (100)	0.798±0.0033 (96.60)	0.730±0.0014 (88.37)	0.534±0.0035 (64.65)	0.496±0.0026 (60.04)	0.433±0.0035 (52.42)	0.384±0.0037 (46.49)	0.343±0.0026 (41.52)	0.319±0.0035 (38.62)

Values are mean ±SE of three replicates.

Table 6: Effect of 40% and 80% of LD_{50} of *Cassia alata* hexane fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Tribolium castaneum*(Herbst).

Parameters	Time (in h)							
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
Glycogen	2.01±0.04 (100)	1.763±0.018 (87.71)	1.470±0.025 (73.13)	1.527±0.023 (75.97)	1.323±0.037 (65.82)	1.370±0.021 (68.16)	1.237±0.026 (61.54)	1.203±0.026 (59.85)	1.033±0.056 (51.39)
Protein	9.187±0.070 (100)	6.493±0.040 (70.68)	6.40±0.035 (69.66)	6.067±0.035 (66.04)	5.283±0.052 (57.50)	5.610±0.043 (61.06)	4.447±0.023 (43.40)	3.830±0.057 (41.69)	3.750±0.046 (40.82)
DNA	0.545±0.001 (100)	0.516±0.0023 (94.66)	0.345±0.0018 (63.29)	0.436±0.0018 (79.98)	0.281±0.0033 (51.55)	0.331±0.0024 (60.72)	0.238±0.0088 (43.66)	0.219±0.010 (40.17)	0.154±0.0055 (28.25)
RNA	0.519±0.007 (100)	0.315±0.004 (67.98)	0.249±0.0029 (47.98)	0.242±0.002 (46.63)	0.200±0.0023 (38.54)	0.239±0.0024 (46.05)	0.184±0.0047 (35.45)	0.172±0.002 (33.14)	0.167±0.004 (32.18)
Amino acid	0.826±0.005 (100)	0.811±0.0018 (98.18)	0.746±0.0036 (90.31)	0.573±0.0037 (69.37)	0.337±0.0052 (40.80)	0.426±0.0018 (51.57)	0.311±0.004 (37.65)	0.289±0.0053 (34.98)	0.234±0.0038 (28.33)

Values are mean ±SE of three replicates.

Table 7: Effect of 40% and 80% of LD_{50} of *Cassia alata* aqueous fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Tribolium castaneum*(Herbst).

Parameters	Time (in h)							
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
Glycogen	2.01±0.04 (100)	1.863±0.040 (92.68)	1.440±0.023 (71.64)	1.707±0.038 (84.92)	1.267±0.020 (63.03)	1.533±0.026 (76.27)	1.127±0.050 (56.07)	1.273±0.0067 (63.33)	0.953±0.033 (47.41)
Protein	9.187±0.070 (100)	8.417±0.038 (91.62)	8.343±0.038 (90.81)	8.397±0.037 (83.58)	7.167±0.045 (78.01)	7.253±0.050 (78.95)	6.860±0.035 (74.67)	5.837±0.046 (63.53)	5.283±0.044 (57.50)
DNA	0.545±0.001 (100)	0.426±0.003 (78.15)	0.414±0.0039 (75.95)	0.387±0.0012 (70.99)	0.367±0.0024 (43.16)	0.342±0.0037 (62.74)	0.313±0.0046 (57.42)	0.277±0.0017 (50.81)	0.238±0.002 (43.66)

RNA	0.519±0.007 (100)	0.334±0.002 (64.35)	0.240±0.0039 (46.24)	0.275±0.0017 (52.99)	0.224±0.0069 (43.16)	0.213±0.0024 (41.04)	0.192±0.003 (36.99)	0.192±0.0023 (36.94)	0.172±0.0014 (33.14)
Amino acid	0.826±0.005 (100)	0.814±0.0037 (98.54)	0.756±0.0023 (91.52)	0.683±0.0015 (82.68)	0.515±0.0058 (62.34)	0.585±0.0029 (70.82)	0.456±0.002 (55.20)	0.472±0.0046 (57.14)	0.366±0.0033 (44.31)

Values are mean ±SE of three replicates.

Table 8: Effect of 40% and 80% of LD_{50} of *Cassia alata* acetone fraction on ACP, ALP, GPT, GOT LDH and AChE of *Tribolium castaneum*(Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
ACP	2.346±0.003 (100)	2.018±0.0024 (86.01)	1.921±0.057 (81.88)	1.918±0.013 (81.75)	1.917±0.0233 (81.71)	1.915±0.0083 (81.62)	1.913±0.012 (81.54)	1.882±0.013 (80.22)	1.77±0.0003 (75.44)
ALP	1.853±0.047 (100)	1.517±0.0057 (81.86)	1.502±.0.0081 (80.05)	1.481±0.011 (79.11)	1.466±0.041 (79.11)	1.424±0.003 (76.84)	1.419±0.0013 (76.57)	1.406±0.032 (75.87)	1.396±0.0023 (75.33)
GPT	4.289±0.0046 (100)	4.031±0.0018 (93.98)	4.027±0.0012 (93.89)	4.005±0.012 (93.37)	3.912±0.003 (91.21)	3.909±0.002 (91.14)	3.886±0.0013 (90.60)	3.844±0.01 (89.62)	3.812±0.05 (88.87)
GOT	3.117±0.0012 (100)	2.849±0.004 (91.40)	2.839±0.0024 (91.08)	2.821±0.031 (90.50)	2.816±0.01 (90.34)	2.807±0.012 (90.05)	2.801±0.022 (89.86)	2.786±0.0019 (89.38)	2.756±0.0014 (88.41)
LDH	8.316±0.0022 (100)	8.281±0.0087 (99.57)	8.259±0.015 (99.31)	8.251±0.024 (99.21)	8.241±0.05 (99.09)	8.131±0.0017 (97.77)	8.001±0.011 (96.21)	7.952±0.021 (95.62)	7.851±0.03 (94.40)
AChE	0.962±0.0009 (100)	0.909±0.031 (94.49)	0.891±0.035 (92.61)	0.879±0.0014 (91.37)	0.852±0.007 (88.56)	0.846±0.0013 (87.94)	0.831±0.003 (86.38)	0.822±0.013 (85.44)	0.809±0.04 (84.09)

Values are mean ±SE of three replicates.

Table 9: Effect of 40% and 80% of LD_{50} of *Cassia alata* chloroform fraction on ACP, ALP, GPT, GOT LDH and AChE of *Tribolium castaneum*(Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
ACP	2.346±0.003 (100)	2.008±0.0019 (85.59)	1.891±0.018 (80.60)	1.788±0.003 (76.21)	1.751±0.0013 (74.63)	1.735±0.001 (73.95)	1.723±0.021 (73.44)	1.715±0.0022 (73.10)	1.706±0.02 (72.71)
ALP	1.853±0.047 (100)	1.502±0.0012 (81.05)	1.479±.008 (79.81)	1.471±0.0031 (79.38)	1.455±0.006 (78.52)	1.413±0.05 (76.25)	1.411±0.0023 (76.14)	1.406±0.009 (75.87)	1.391±0.0081 (75.06)
GPT	4.289±0.0046 (100)	3.661±0.0018 (85.35)	3.437±0.002 (80.13)	3.405±0.012 (79.38)	3.212±0.023 (74.88)	3.189±0.013 (74.35)	3.127±0.0043 (72.90)	3.111±0.007 (72.53)	3.091±0.006 (72.06)
GOT	3.117±0.0012 (100)	2.809±0.0019 (90.01)	2.793±0.002 (89.60)	2.721±0.013 (87.29)	2.696±0.011 (86.49)	2.687±0.0021 (86.20)	2.485±0.022 (79..72)	2.391±0.005 (76.70)	2.311±0.021 (74.14)
LDH	8.316±0.0022 (100)	8.101±0.0021 (97.41)	7.959±0.015 (95.70)	7.851±0.016 (94.40)	7.641±0.011 (91.88)	7.432±0.037 (89.36)	7.111±0.031 (85.50)	7.106±0.057 (85.44)	7.056±0.031 (84.84)
AChE	0.962±0.0009 (100)	0.885±0.031 (91.99)	0.871±0.025 (90.54)	0.857±0.0023 (89.08)	0.841±0.019 (87.42)	0.836±0.0013 (86.90)	0.811±0.008 (84.30)	0.801±0.01 (83.26)	0.786±0.001 (81.70)

Values are mean ±SE of three replicates.

Table 10: Effect of 40% and 80% of LD_{50} of *Cassia alata* Petroleum ether fraction on ACP, ALP, GPT, GOT LDH and AChE of *Tribolium castaneum*(Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
ACP	2.346±0.003 (100)	1.865±0.016 (79.49)	1.856±0.0019 (79.11)	1.674±0.018 (71.35)	1.629±0.0001 (69.43)	1.612±0.0013 (68.71)	1.595±0.0012 (67.98)	1.583±0.012 (67.47)	1.546±0.031 (65.89)
ALP	1.853±0.047 (100)	1.502±0.036 (81.05)	1.459±0.0022 (78.73)	1.439±.0081 (77.65)	1.421±0.0011 (76.68)	1.401±0.036 (75.60)	1.391±0.015 (75.06)	1.365±0.03 (73.66)	1.312±0.0021 (70.80)

GPT	4.289±0.0046 (100)	3.711±0.01 (86.34)	3.632±0.0083 (84.68)	3.412±0.012 (79.55)	3.398±0.0021 (79.22)	3.201±0.0021 (74.63)	3.179±0.002 (74.11)	3.109±0.033 (72.48)	3.10±0.011 (72.27)
GOT	3.117±0.0012 (100)	2.792±0.03 (89.57)	2.768±0.0019 (88.80)	2.756±0.002 (88.41)	2.702±0.033 (86.68)	2.659±0.001 (85.30)	2.652±0.003 (85.08)	2.414±0.002 (77.44)	2.396±0.036 (76.86)
LDH	8.316±0.0022 (100)	8.048±0.012 (96.77)	8.035±0.0083 (96.62)	7.788±0.045 (93.65)	7.771±0.016 (93.44)	7.523±0.011 (90.46)	7.369±0.017 (88.61)	7.242±0.011 (87.08)	7.186±0.0081 (86.41)
AChE	0.962±0.0009 (100)	0.781±0.0011 (81.18)	0.772±0.031 (80.24)	0.765±0.045 (79.52)	0.731±0.014 (75.98)	0.712±0.019 (74.01)	0.703±0.03 (73.07)	0.687±0.011 (71.41)	0.676±0.04 (70.27)

Values are mean ±SE of three replicates.

Table 11: Effect of 40% and 80% of LD_{50} of *Cassia alata* methanol fraction on ACP, ALP, GPT, GOT LDH and AChE of *Tribolium castaneum* (Herbst).

Parameters	**Time (in h)**								
	0 (Control)	**4**		**8**		**12**		**16**	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.004±0.04 (85.42)	1.898±0.009 (80.90)	1.787±0.018 (76.17)	1.766±0.0013 (75.27)	1.731±0.0023 (73.78)	1.715±0.002 (73.10)	1.703±0.011 (72.59)	1.689±0.012 (71.99)
ALP	1.853±0.047 (100)	1.546±0.016 (83.43)	1.492±0.005 (80.51)	1.468±.0009 (79.22)	1.454±0.03 (78.46)	1.447±0.016 (78.08)	1.403±0.05 (75.71)	1.391±0.0023 (75.06)	1.301±0.0057 (70.21)
GPT	4.289±0.0046 (100)	3.717±0.001 (86.66)	3.641±0.0081 (84.89)	3.417±0.0032 (79.66)	3.401±0.021 (79.23)	3.209±0.003 (74.81)	3.181±0.0032 (74.16)	3.116±0.0013 (72.65)	3.107±0.0081 (72.44)
GOT	3.117±0.0012 (100)	2.802±0.015 (89.89)	2.779±0.009 (89.15)	2.764±0.0012 (88.67)	2.711±0.0013 (86.97)	2.676±0.01 (85.85)	2.666±0.034 (85.53)	2.425±0.021 (77.79)	2.411±0.016 (77.35)
LDH	8.316±0.0022 (100)	8.059±0.032 (96.90)	8.041±0.0031 (96.69)	7.869±0.05 (94.62)	7.848±0.016 (94.37)	7.591±0.002 (91.28)	7.397±0.0009 (88.94)	7.261±0.0021 (87.31)	7.117±0.03 (85.58)
AChE	0.962±0.0009 (100)	0.872±0.0011 (90.64)	0.862±0.005 (89.60)	0.853±0.01 (88.66)	0.846±0.004 (87.94)	0.839±0.009 (87.21)	0.822±0.013 (85.44)	0.801±0.001 (83.26)	0.779±0.021 (80.97)

Values are mean ±SE of three replicates.

Table 12: Effect of 40% and 80% of LD_{50} of *Cassia alata* hexane fraction on ACP, ALP, GPT, GOT LDH and AChE of *Tribolium castaneum* (Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
ACP	2.346±0.003 (100)	1.564±0.009 (66.66)	1.446±0.008 (61.63)	1.421±0.0013 (60.57)	1.382±0.0031 (58.90)	1.355±0.001 (57.75)	1.311±0.05 (55.88)	1.302±0.0081 (55.49)	1.287±0.012 (54.85)
ALP	1.853±0.047 (100)	1.431±0.0012 (77.22)	1.425±.0001 (76.90)	1.412±0.021 (76.20)	1.391±0.006 (75.06)	1.372±0.03 (74.04)	1.351±0.0023 (72.90)	1.285±0.009 (69.34)	1.125±0.0083 (60.71)
GPT	4.289±0.0046 (100)	3.522±0.008 (82.11)	3.401±0.0012 (79.29)	3.359±0.015 (78.31)	3.195±0.003 (74.49)	3.172±0.002 (73.95)	2.979±0.0013 (69.45)	2.756±0.021 (64.46)	2.689±0.02 (62.69)
GOT	3.117±0.0012 (100)	2.658±0.0019 (85.27)	2.639±0.0022 (84.66)	2.621±0.013 (84.08)	2.619±0.04 (84.02)	2.602±0.001 (83.47)	2.401±0.032 (77.02)	2.379±0.0013 (76.32)	2.256±0.011 (72.37)
LDH	8.316±0.0022 (100)	7.735±0.008 (93.01)	7.728±0.006 (92.92)	7.702±0.016 (92.61)	7.513±0.011 (90.34)	7.319±0.0009 (88.01)	7.214±0.0011 (86.74)	7.116±0.006 (85.56)	7.107±0.0018 (85.46)
AChE	0.962±0.0009 (100)	0.766±0.01 (79.62)	0.754±0.015 (78.37)	0.711±0.004 (73.90)	0.703±0.009 (73.07)	0.685±0.0013 (71.20)	0.672±0.021 (69.85)	0.623±0.0016 (64.76)	0.611±0.008 (63.51)

Values are mean ±SE of three replicates.

Table 13: Effect of 40% and 80% of LD_{50} of *Cassia alata* aqueous fraction on ACP, ALP, GPT, GOT LDH and AChE of *Tribolium castaneum* (Herbst).

Parameters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	80%	40%	80%	40%	80%	40%	80%
ACP	2.346±0.003 (100)	2.134±0.0061 (90.96)	2.038±0.002 (86.87)	1.941±0.003 (82.77)	1.938±0.013 (82.60)	1.931±0.023 (82.31)	1.925±0.038 (82.02)	1.913±0.019 (81.54)	1.897±0.0023 (80.86)
ALP	1.853±0.047 (100)	1.786±0.006 (96.38)	1.717±0.005 (92.66)	1.686±.0001 (90.98)	1.671±0.0009 (90.17)	1.666±0.04 (89.90)	1.624±0.031 (87.64)	1.619±0.0002 (87.37)	1.582±0.0083 (85.37)

GPT	4.289±0.0046 (100)	4.139±0.005 (96.50)	4.131±0.0031 (96.31)	4.127±0.0021 (96.22)	4.115±0.011 (95.94)	4.112±0.0013 (95.87)	4.109±0.002 (95.80)	4.056±0.013 (94.36)	3.945±0.014 (91.97)
GOT	3.117±0.0012 (100)	3.006±0.002 (96.43)	2.949±0.003 (94.61)	2.915±0.005 (93.51)	2.911±0.031 (93.39)	2.906±0.021 (93.23)	2.897±0.0034 (92.94)	2.891±0.012 (92.74)	2.751±0.01 (88.25)
LDH	8.316±0.0022 (100)	8.297±0.012 (99.77)	8.285±0.0083 (99.62)	8.269±0.001 (99.43)	8.256±0.006 (99.27)	8.248±0.03 (99.18)	8.149±0.017 (97.99)	8.041±0.031 (96.69)	7.891±0.005 (94.88)
AChE	0.962±0.0009 (100)	0.926±0.001 (96.25)	0.919±0.031 (65.53)	0.911±0.024 (94.69)	0.909±0.0023 (94.49)	0.901±0.04 (93.65)	0.896±0.0023 (93.13)	0.881±0.006 (91.58)	0.873±0.004 (90.74)

Values are mean ±SE of three replicates.

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