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BIOCHEMICAL ANTHELMINTIC ACTION OF CARICA *PAPAYA* AQUOUS SEED EXTRACT IN TRICHOSTRONGYLUS COLUBRIFORMIS

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ABSTRACT

The biochemical mechanism of anthelmintic action of aqueous extract of *C. papaya* seeds has been investigated on gastrointestinal parasite, Trichostrongylus colubriformis. Aqueous extract inhibited the glucose uptake and depleted the glycogen content in the presence of glucose indicating that *C. papaya* aqueous extract affects the energy generating mechanism of the parasite. It also significantly increased the lactate dehydrogenase enzyme suggesting inhibition of ATP production or accumulation of lactic acid. Aqueous extract of *C. papaya* also significantly inhibit AChE activity, In conclusion, the possible mechanism of anthelmintic action of *C. papaya* aqueous extract may be related to inhibition of energy metabolism as well as alteration in the AChE activity of the parasite.

KEYWORDS: *C. papaya*, Acetyl cholinesterase, lactate dehydrogenase, Trichostrongylus colubriformis

INTRODUCTION

Carica papaya (Family Caricaceae) originated in Central America. The application of *papaya* latex that is probably of most interest to livestock producers is as an anthelmintic (dewormer). In traditional veterinary medicine, *papaya* seeds also are used as dewormers. In Indonesia and the Philippines, air-dried seeds are ground and mixed with water - 3 g of seeds/kg body weight. The efficacy of *papaya* latex against *Ascaris suum* tested in 16 pigs. [1] The anthelmintic study of *papaya* latex conducted by against *Ascaridia galli* in chickens. [2] The fruit juice is regarded as a medicine in all countries where the tree is found and the milky

juice of the unripe fruit is thought to possess powerful anthelmintic properties, particularly against roundworms. The boiled green leaves of *papaya* are used against malaria and as an anthelmintic. The seeds as a vermifuge and tea of the fallen leaves against hypertension.

MATERIALS AND METHODS

Collection and processing of Carica *papaya*

Seeds of *Carica papaya* were obtained from the Department of Aromatic and Medicinal plants, College of Agriculture, J.N.K.V.V Jabalpur, and India. The hot and cold aqueous extract of the seeds were used for the study. Hot aqueous extract was prepared [3]. Seed powder of *C. papaya* (100 gm) was kept in a flask to which 1000 ml of distilled water was added. The flask was kept on heating mantle for boiling and heated till the contents were reduced to half. The content was cooled and filtered through an ordinary filter paper and then complete evaporation of water was done by using rotary vaccum evaporator at 40^0 C (100 rpm) to prepare hot extract. Cold aqueous extract was prepared. [3] With some modification. Seed powder of *C. papaya* (100 gm) was kept in a flask to which 1000 ml of distilled water was added. The flask was kept for overnight and homogenized for 10 minutes. The content was filtered through an ordinary filter paper and then water was evaporated using rotary vaccum evaporator at 40^0 C (100 rpm) to prepare cold extract.

Collection of parasites

The gastrointestinal parasite; *Oesophagostomum, Bunostomum, Trichuris spp*. and *Trichostrongylus colubriformis* were collected from the intestines of freshly slaughtered goats obtained from the Government slaughter house Jabalpur. All adult parasites were immediately transferred into separate beaker having Hank's balance salt solution (HBSS) maintained at 38.5°C temperature.

Gross motility and mortality of parasites

The parasite viz; *Oesophagostomum, Bunostomum, Trichuris spp*. and *Trichostrongylus colubriformis* were incubated at different concentrations of aqueous extract of *C. papaya* (500, 1000, 1500 and 2000 µg/ml). The mass motility of *Oesophagostomum, Bunostomum, Trichuris spp*. and *T. colubriformis* after 4 h of incubation in different concentrations of aqueous extract of *C. papaya* was visually graded as 0, ±, +, ++, +++ and ++++, representing nil, feeble, poor, moderate, good and vigorous motility.

BIOCHEMICAL STUDIES

The biochemical parameters in terms of glucose uptake, glycogen content, and enzymes namely LDH and AChE were determined in *T. colubroformis* parasite incubated with hot and cold aqueous extract of *C. papaya*. Difference concentrations of aqueous extract of *C. papaya* (500, 1000, 1500 and 2000 µg/ml) were used to study their effect on various biochemical parameters. The worms were incubated in Petri dish containing various concentrations of drug in HBSS for 4 h at 38.5^{O}C in BOD incubator. *T. colubriformis* worms of equal number (200-300 mgs) were transferred to each incubation medium, After 4 h of incubation, mass motility and mortality of *T. colubriformis* was examined and graded. The incubated worms were used for biochemical studies.

Glucose uptake of *T. colubriformis*

T. colubriformis worms were incubated in four different concentrations of aqueous extract of *C. papaya* (500, 1000, 1500 and 2000 µg/ml) in drug-free HBSS medium (control) for 4 hrs. Glucose uptake was estimated [4] using glucose left in the Incubate which was estimated by the Erba biochemical autoanalyser kit. The value thus obtained was subtracted from the quantity of glucose present at the time of incubation. The subtracted value was expressed as mg of glucose taken up by 100 mg of wet weight of *T. colubriformis* (mg %) in 4 hrs.

Glycogen content of *T. colubriformis*

T. colubriformis parasites were incubated as described above. After incubation, the worms were washed three times with normal saline and hydrolyzed [5] and the hydrolyzed glucose was estimated by using Erba diagnostic kit. The glycogen content of worms was expressed as mg glycogen per 100 mg of wet weight of *T. colubriformis.*

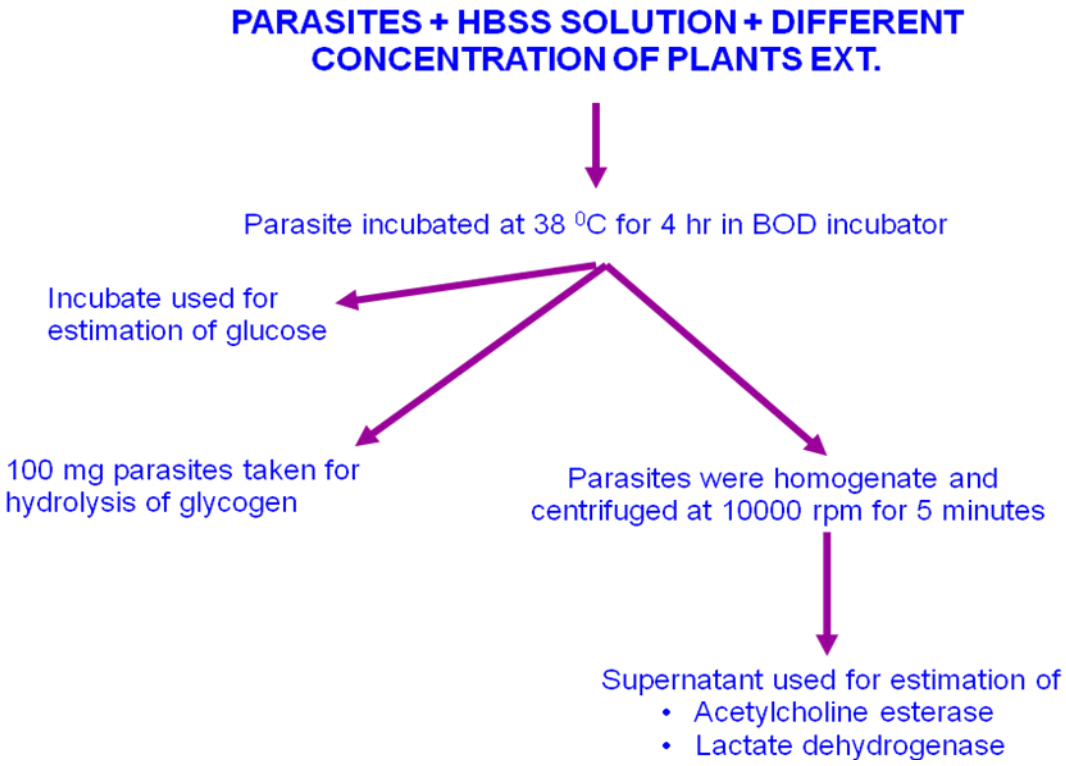
Lactate dehydrogenase activity of *T. colubriformis*

Trichostrongylus colubriformis worms were incubated in glucose containing medium for 4 hrs at 38.5^{0}C and then 200mg of worms were washed with normal saline, homogenated and centrifuged at 5000 rpm for 5 minutes. The lactate dehydrogenase content was estimated by using Crest Biosystems Kits and expressed as IU/L of homogenate.

AChE activity of *T. colubriformis*

Trichostrongylus colubriformis parasites were incubated in different concentrations of extract at 38.5^{0}C for 4 hrs at. After incubation 200 mg of worms were washed with normal saline, homogenated and centrifuged at 5000 rpm for 5 minutes. The activity of AChE was estimated

in whole worm homogenate. [6] The AChE activity was expressed as IU/L of homogenate.



Statistical analysis

The data obtained from experiment was subjected to analysis variance [7].

RESULTS

Mass motility and mortality of parasites

The observations indicated that cold and hot aqueous extract of *C. papaya* showed 100 percent efficacy against *Trichuris sp.* in terms of gross motility (Table 1 and Table 3) and mortality (Table 2 and Table 4). However, *in vitro* anthelmintic efficacy *C. papaya* extract were ranging between 70-90 percent against *Trichostrongylus, Bunostomum, Oesophagostomum* and *Trichuris spp* of parasites (Figure 1).

Table-1: Observations on gross motility of parasites treated with different concentrations of *C. papaya* (cold aq. ext.).

Parasite	500 µg/ml		1000 µg/ml		1500 µg/ml		2000 µg/ml	
	0hr	4hr	0hr	4hr	0hr	4hr	0hr	4hr
Trichostrongylus spp.	++++	++	++++	++	++++	++	++++	0
Bunostomum spp.	++++	+++	++++	++	++++	+	++++	±
Oesophagostomum spp.	++++	+++	++++	++	++++	+	++++	±
Trichuris spp.	++++	++	++++	+	++++	±	++++	0

Nil= 0, Feeble= ±, Poor= +, Moderate= ++, Good= +++ and Vigorous motility= ++++

Table-2: Observations on gross mortality of parasites treated with different concentrations of *C. papaya* (cold aq. ext.).

Parasite	500µg/ml		1000µg/ml		1500µg/ml		2000µg/ml	
	0hr	4hr	0hr	4hr	0hr	4hr	0hr	4hr
Trichostrongylus spp.	10	8	10	7	10	4	10	0
Bunostomum	10	10	10	9	10	7	10	2
Oesophagostomum	10	9	10	7	10	5	10	1
Trichuris	10	6	10	3	10	0	10	0

Table-3:- Observations on gross motility of parasites treated with different concentrations of *C. papaya* (hot aq. ext.).

Parasite	500 µg/ml		1000 µg/ml		1500 µg/ml		2000 µg/ml	
	0hr	4hr	0hr	4hr	0hr	4hr	0hr	4hr
Trichostrongylus	++++	++++	++++	+++	++++	+	++++	+
Bunostomum	++++	++++	++++	++++	++++	++	++++	+
Oesophagostomum	++++	++++	++++	++++	++++	++	++++	+
Trichuris	++++	+++	++++	+++	++++	+	++++	0

Nil= 0, Feeble= ±, Poor= +, Moderate= ++, Good= +++ and Vigorous motility= ++++

Table-4:- Observations on gross mortality of parasites treated with different concentrations of *C. papaya* (hot aq. ext.).

Parasite	500µg/m		1000µg/ml		1500µg/ml		2000µg/ml	
	0hr	4hr	0hr	4hr	0hr	4hr	0hr	4hr
Trichostrongylus	10	9	10	8	10	4	10	2
Bunostomum	10	10	10	10	10	6	10	4
Oesophagostomum	10	10	10	9	10	5	10	3
Trichuris	10	8	10	6	10	2	10	0

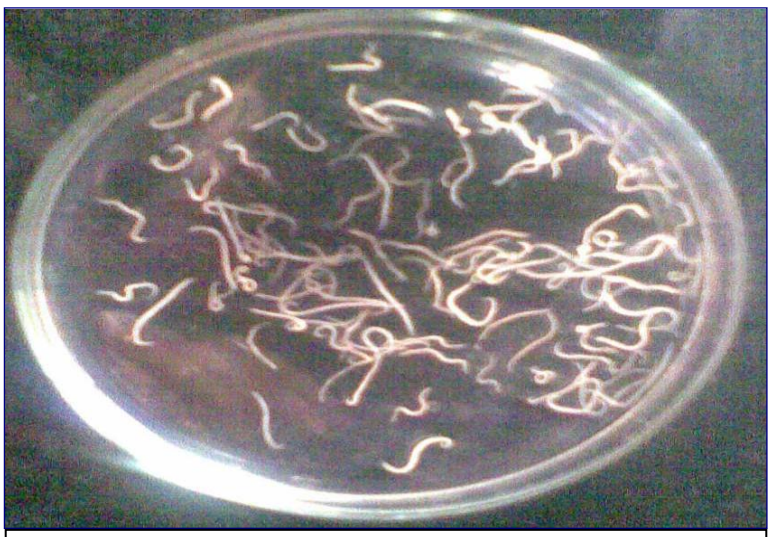


Figure 1: Incubation of parasite in HBSS to observe motility and mortality with extract of C. *papaya* seeds

Effect of *C. papaya* extracts on glucose uptake of *T. colubriformis*

Incubation of *T. colubriformis* with cold aqueous extract *C. papaya* (500 and 1000, 1500 and 2000 µg/ml) for 4 hrs in HBSS resulted in significant ($P < 0.05$) inhibition of glucose uptake (Table 5). On the contrary, hot aqueous extract of *C. papaya* (500 µg/ml) exhibited non-significant effect on glucose uptake but inhibited glucose uptake on subsequent concentration of 1000, 1500 and 2000 µg/ml (Table 6).

Effect of *C. papaya* extract on tissue glycogen content of *T. colubriformis*

Cold aqueous extract *C. papaya* at the concentration of 500 µg/ml significantly ($P < 0.05$) depleted the tissue glycogen content of *T. colubriformis.* The effect on glycogen content of the parasite was significantly enhanced with increase in concentration of plant extract (Table 5). Hot aqueous extract *C. papaya* at lower concentration (500 µg/ml) was non-significant in depleting tissue glycogen; however, at subsequent concentrations it depleted tissue glycogen (Table 6).

Effect of *C. papaya* extracts on lactate dehydrogenase activity of *T. colubriformis*

Aqueous extract of *C. papaya* significantly increased the lactate dehydrogenase activity of *T. colubriformis*. Cold aqueous extract of *C. papaya* significantly increased LDH at lower concentration and further subsequently increased LDH activity with increase in concentration of plant extract (Table 5). However, hot aqueous extract was found non-significant at 500 µg/ml concentration of plant extract (Table 6).

Effect of *C. papaya* extracts on AChE activity of *T. colubriformis*

The cold and hot aqueous extracts of *C. papaya* significantly ($p < 0.05$) inhibited AChE activity at concentration of 1000, 1500 and 2000 µg/ml of plant extract which was found to be dose dependent (Table 5 and Table 6).

Table-5:- Effect of different concentrations of cold aqueous extract of *C. papaya* on various biochemical parameters of *T. colubroformis.*

Parameters	Control	500µg/ml	1000 µg/ml	1500 µg/ml	2000 µg/ml
Glucose Uptake	6.11^{A}	5.79^{B}	4.90^{C}	3.88^{D}	2.02^{D}
Tissue Glycogen	3.07^{A}	2.80^{B}	1.65^{C}	1.01^{D}	0.88^{E}
Lactate dehydrogenase(IU/L)	36.76^{D}	40.42^{D}	85.67^{C}	139.87^{B}	161.87^{A}
Acetylcholine esterase (IU/L)	4150.83^{A}	4092.50^{A}	3288.67^{B}	1894.33^{C}	1259.83^{D}

Same superscript showing Non-significant

Table-6: Effect of different concentrations of hot aqueous extract of *C. papaya* on various biochemical parameters of *T. colubroformis.*

Parameters	Control	500µg/ml	1000 µg/ml	1500 µg/ml	2000 µg/ml
Glucose Uptake	6.11^{A}	6.02^{AB}	5.88^{B}	5.23^{C}	4.46^{D}
Tissue Glycogen	3.07^{A}	3.06^{A}	2.80^{B}	2.17^{C}	1.92^{D}
Lactate dehydrogenase(IU/L)	36.76^{M}	37.57^{M}	66.90^{L}	96.93^{H}	105.97^{G}
Acetylcholine esterase (IU/L)	4150.83^{A}	4106.00^{AB}	4009.83^{B}	3845.64^{C}	3251.00^{D}

Same superscript showing Non-significant

DISCUSSION

The results suggested that aqueous extract of *C. papaya* induces energy crisis consequent to the blockade of glucose up take; however, parasites still mobilize their glycogen source for the production of ATP as suggested. [8] In view of this possibility, the effect of aqueous extract of *C. papaya* was studied on tissue glycogen level of *T. colubriformis which* produced a significant ($P < 0.05$) reduction in tissue glycogen content of *T. colubriformis.* The biochemical mechanism of action of benzylisothiocynate, an active component obtained from *C. papaya,* has been suggested by inhibition of glucose uptake and depletion of glycogen content. [9] Aqueous extract of *C. papaya* significantly caused inhibition of glucose uptake and tissue glycogen content of parasite *Ascaridia galli.* [10]

The rise in tissue lactate dehydrogenase catalysing the conversion of pyruvate to lactate is fetal to parasite. [11] The increased tissue lactate dehydrogenase level implies that either the malate pathway is inhibited or the excretion of the lactic acid from the parasite is blocked. [12] Keeping in view the reports of mentioned co-workers, anthelmintic action of aqueous extract of *C. papaya* might be due to result from the inhibition of ATP production because of the effect on the malate-succinate pathway or due to the deleterious effect of accumulated lactic acid in *T. colubriformis.* The role of ACh as an excitatory neurotransmitter in the activity of intestinal parasites has been is well established [13, 14]. Hence assessment of AChE activity is one of the major determinants of the functional status of the neurotransmission. Certain organophosphates and carbamates have been reported to act through inhibition of AChE. [15] The aqueous extract of *C. papaya* also inhibited AChE activity in *T. colubriformis,* which leads to accumulation of acetylcholine at neuromuscular junction to result in spastic paralysis of parasite. [10]

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