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AMELIORATING EFFECT OF *CARICA PAPAYA* L. FRUIT EXTRACT ON ENTAMOEBA HISTOLYTICA IN MICE

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Abstract

Entamoeba histolytica is a major public health problem in tropical and subtropical countries. Treatment failure with specific chemotherapy suggested the development of new plant origin therapy. Different parts *of Carica papaya* Linn. have anti-parasitic agent. The study evaluated the efficacy of *C. papaya* fruit methanolic and aqueous extracts against *Entamoeba histolytica* as compared with the used Albendazole® in experimentally infected mice. Male CD-1 mice were divided into two groups of 50 each; immunocompetent (IC) and dexamethasone immunosuppressed (IS). Each group was subdivided into 5 subgroups, normal, infected and infected treated with crude & pure *C. papaya* extracts (500mg/kg) or albendazole (200mg/kg). Each mouse was infected orally with 1000 *E. histolytica* cysts. Six weeks after infection, treatment with pure and crude extracts (3doses/ week) for 3 weeks which started from the 3rd week after oral administration of dexamethasone, significantly reduced the trophozoites (78.20% & 76.29%, respectively) in IC mice compared to albendazole (88.72% & 81.81%). Serum IgG, IgM, IgA antibody levels and IFN-γ significantly ($P< 0.001$) increased in treated mice compared to normal ones. Histopathological results showed improvement in the hepatic and intestinal architecture of infected mice treated with papaya extracts and albendazole with various degrees. Results of IS groups were the same as those of IC ones but, with lower levels.

Keywords: *Entamoeba histolytica, Carica papaya*, Mice, Immune responses, Polyclonal antibody, ELISA

Introduction

E. histolytica, an anaerobic protozoan parasite of the genus *Entamoeba*, infects humans and other primates mostly by the ingestion of cysts in fecal contaminated food or drink (Sen *et al,* 2007). *Entamoeba histolytica* causes intestinal amoebiasis and extra-intestinal manifestations, although 90% infections were asymptomatic, nearly 50 million people become symptomatic, with about 100,000 deaths annually (Chou and Austin, 2022). Children and elderly populations are the most susceptible to opportunistic infections due to low cellular immunity (Dey *et al,* 2012). Dogs and cats are infected for a short time, but they don't play a marked role in zoonotic transmission (WHO, 1997). The ingested organism excysts into active trophozoite in large intestines reproduce by simple division and encyst as they progress in large intestine, to pass with feces and can live in the environment for weeks to months (Pritt and Clark, 2008), and under fingernails for up to 45 minutes (Khalil *et al,* 2017)

Amoebiasis symptoms, including the intestinal ulcerations, bloody diarrhea, weight loss, fever, gastrointestinal obstruction, peritonitis and may develop anemia (Kucik *et al,* 2004). *E. histolytica* causes liver abscesses in patients (95%) within 5 months that may rupture into the pleural space, peritoneum, or pericardium (Tharmaratnam *et al,* 2020). Invasive amoebiasis may be the commonest among HIV/AIDS patients, calling for routine HIV testing to diagnose co-infection (Hsu *et al,* 2008).

The human immunological response is activated once the trophozoites enter the intestinal epithelium. The parasite's immune evasion strategies rely on immunomodulation

by reducing interferon-gamma (IFN-α) production, removing immune cells and soluble immune mediators, and changing metabolic pathways to avoid reactive oxygen and nitrogen species (Nakada-Tsukui and Nozaki 2016). They invade mucosa and submucosal regions by phagocytosis of dead cells and degradation of extracellular matrix (ECM) via prostaglandin E2 (PGE2) releases, which bind to prostaglandin E receptor-4 in enterocytes (Dey and Chadee, 2008). *E. histolytica* also have mechanisms to eradicate complement proteins and antibodies through capping formation by the elimination of membrane surface proteins that antibodies recognize (Baxt *et al,* 2008). It activates signaling pathways, causing a rise in intracellular calcium, production of reactive oxygen species (ROS), apoptosis, and the loss of mem-brane integrity (Carrero *et al,* 2020).

Adequate therapy for the amoebic colitis is a must to reduce illness, prevent complicated disease and extraintestinal spread, and decrease transmission (Gonzales *et al,* 2019). Albendazole has been the drug of choice long ago (Chávez *et al,* 1992). Also, it is used abroad (Lemee *et al,* 2000), and in Egypt for helminthic and protozoa infections (Fahmy and Diab, 2021). Albendazole and mebendazole are generally safe with few side effects; but, when they were used for prolonged time (>14-28 days) or even only 1 time, liver toxicity and others may occur (Ba-nsal *et al,* 2006). Beside, adverse side effects of chemical drugs including metallic taste, headache, nausea, glossitis, urticaria, and pruritus, as well as carcinogenic and teratogenic properties (Upcroft and Upcroft, 2001). So, the development of an alternative medicinal plant or herbs for *E. histolytica* safe with mild side effects, effective, and afford- able was a must (Shrivastav *et al,* 2020).

Carica papaya (Family: Caricaceae) is a medicinal plant widely cultivated in tropical and subtropical countries, including Egypt as edible fruits (Ali *et al,* 2011). Its parts and extracts have many biological and pharmacological properties due to the carotenoids, minerals, phenolic acids, flavonoids, and vitamins (Sagadevan *et al,* 2019). *C. papaya* seeds and/or fruits extracts proved to be effective in treating infectious diseases; as *E. histolytica* (Jaime, 2007; Quiñones-Gutiérrez *et al,* 2013), and *Trypanosoma cruzi* (Jiménez-Coello *et al,* 2014).

This study aimed to evaluate the alcoholic and aqueous extracts of papaya fruit in treating *Entamoeba histolytica* in Swiss Albino mice (immuno-suppressed/IC) versus albendazole administration. This was based on parasitological ones (trophozoite burden in intestines and cyst in stools), serological ones (antibody IgA, IgM, & IgG levels; and cytokine response IFN-ɤ) and histopathological aspects in hepatic and intestinal tissues.

Materials and Methods

Crude extract preparation: Fruits of *C. papaya* plant were purchased freshly from a local market in Cairo. Then they were washed and cut into small pieces, dried at a temperature not exceeding 60ºC using hot air ovens (Universal Hot Air Oven), and finely powdered by evaporation. Dry powder was kept at 27°C for extraction process. 700gm of ground fruit were dissolved in 7 liters of distilled water to get the crude *C. papaya* extract solution (Oduola *et al,* 2007).

Purified extract preparation: Seven hundred grams of the finely powdered *C. papaya* fruits were extracted with 3 liters of 85% methanolic (MeOH) at room temperature. 85% MeOH extract was filtrated via a Whatman No.1 filter paper (Sigma-Aldrich, Camlab House, Cambridge CB24 5WE, the United Kingdom), and concentrated to dryness under reduced pressure using a rotatory evaporator (BUCHI, Switzerland) at 45°C. Extraction was repeated for three successive times (Lee *et al,* 2009), and then the extract was defatted with petroleum ether, and thus the MeOH purified papaya extract was prepared.

Animals: One hundred clean laboratory bred Swiss Albino male mice (CD-1 strain), 3 to 5 weeks old and an average weight of 24

±2g were purchased from the Schistosome Biological Supply Program, Theodor Bilharz Research Institute, Giza. Mice were kept under experimental controlled conditions in an institution responsible for animal ethics (Bayne and Turner 2013). Handling and dealing with mice were conducted according to the Ethical guidelines of Ain-Shams University, which agreed with the Helsinki declarations (2000).

Immune suppression: Mouse immune suppression was performed by using dexamethasone orally at a dose of 0.025mg/g/day for 14 successive days prior to infection (Rehg *et al,* 1988). Dexamethasone tablets were purchased from Memphis Pharm. & Chemical Ind., Cairo, and grinded into a white powder and aquatic suspension was freshly prepared.

Doses: Three doses of papaya extracts and albendazole per week were given for 3 weeks, started from the 3rd week after oral administration of dexamethasone. Albendazole was purchased from Egyptian Int. Pharmaceutical Industries CO, Cairo, grinded into a white powder, and aquatic suspension was freshly prepared and given at a dose of 200mg/kg (Khan *et al,* 2019). The crude and purified *C. papaya* fruit extracts were given at dose of 500mg/kg (Abdel-Lateef *et al,* 2018). Mice were orally administered using a stainless-steel oral cannula.

Parasites: Feces of highly infected *E. histolytica* patients were collected and purified to isolate cysts (Bassily *et al,* 1987). Fresh stools were broken up in tap water, and 3ml of fecal suspension was layered on 2.5ml of 1 M sucrose (specific gravity 1.11) in a 75- by 12mm plastic tube and centrifuged at 400 xg for 15min at 20°C. Cysts concentrated at the water-sucrose interface were carefully removed with a Pasteur pipette, washed in 4 ml of normal saline, and precipitated by centrifugation at 600xg for 10min. Supernatant was removed and cysts were suspended in 4ml of phosphate buffer saline (PBS-pH 7.2) for mice infection.

Experimental design: One hundred Swiss Albino mice were divided into two main groups of 50mice/each: dexamethasone immunosuppressed (IS) & immunocompetent groups (IC); each was subdivided into five groups of ten mice each: G1: uninfected untreated (control negative), G2: infected untreated (control positive), G3: infected and treated with Albendazole® (200mg/kg), G4: infected treated with crude *C. papaya* fruit extract (500mg/kg), and G5: infected and treated with purified *C. papaya* fruit extract (500mg /kg). Infection was done by orally introducing 1000 *E. histolytica* cysts/mouse (Abdou *et al,* 2013). After approving *E. histolytica* infection, all mice were sacrificed by cervical dislocation under ether anesthesia for parasitological, immunological, and histopathological examined.

Serum preparations: Heart blood was obtained from each mouse, left at room temperature and centrifuged at 2000rpm for 20 min. Sera were separated and stored at -20˚C until needed for immunological study. Liver and small intestine were dissected out kept in 10% formalin and processed for histopathological examination.

Parasitological examinations: Intestinal fluid was obtained from all mice groups. Smears were examined in normal saline and stained in Giemsa stain (El Shazly *et al,* 2006). Cysts were detected and counted (Kuk *et al,* 2012). Each stool sample (0.24g) was dissolved in 3ml of saline, stained in Logol iodine and microscopically examined.

Drugs' efficacy was calculated as follows: Efficacy % = (Total cysts of positive control-Total cysts after treatment/ Total cysts of positive control) x100 (Orenstein *et al,* 1985).

Preparation of *E. histolytica* antigen: 9ml Diamond's TYI-S-33 culture medium (Cerva, 1989) in a screw-capped tube was inoculated with 10,000 amoebae/ml. of the medium at the tube bottom, at 37°C, and after 72 hr growth were used for crude antigen preparation. Tubes of grown *E. histolytica* were chilled by dipping in ice-cold water for 10 min to dislodge amoebae that adhered to gla-

ss surface. Cysts were obtained by centrifugation at 550g for 15min., washed three times in 50ml of 0·25 M sucrose, resuspended in PBS pH 7.2, centrifuged at 550g for 20min, dispensed into 5ml screw-capped tubes and then frozen and stored at -4°C (Das *et al,* 1979).

ELISA: ELISA wells micro-plates were coated with 50µl/well *E. histolytica* antigen (20µg/ml) in coating buffer. Plates were sealed and incubated for 2hr at room temperature. After three times washing with washing buffer (PBS/Tween contained 0.15 M-PBS pH 7.2 & 0.05% Tween 20), 200µl/well blocking buffer (1% bovine serum albumin (BSA in PBS-D) was added and incubated for 2hr at room temperature. Plates were twice washed with washing buffer and incubiuted for 1hr with 100µl/well serum 1/250 in diluted buffer (PBS/T with traces of 1% BSA) at room temperature. 100µl/well of peroxidase-conjugated anti-human IgG, IgM & IgA conjugate in dilutions of 1/500, 1/100, & 1/250 respectively, was added to each well after 5x washing. After 1hr incubation at room temperature, wells were washed (5x) and developed by adding a specific substrate (Sun Red Biotechnology Co, Shanghai, China). 100µl of substrate solution, one tablet of O-phenylene diamine dihydrochloride (OPD) (Sigma) dissolved in 25ml of 0.05M phosphate citrate buffer, pH 5 with peroxidase H_2O_2 (Sigma) was added to each well. Plates were incubated in dark at room temperature for 30min. 50µl/well of 8 N H_2SO_4 was added to stop reaction, and absorbance was measured at 492nm by ELISA reader (Bio-Rad microplate reader, Richomond, Co.) according to Engvall and Perlmann (1971).

Sandwich ELISA: Detected sera IFN-γ, capture anti-IFN-γ antibody (Sun Red Biotechnology Co, China) (100µl/well), diluted in 50mM carbonate buffer pH 9.6, was coated onto wells of ELISA plates overnight at 4°C. Plates were washed 4x with PBS/T, blocked with 200µl blocking buffer/ well, and immediately washed 4 times with PBS/T before adding 100µl/well of serum (1/200 dilution) in PBT/T with 0.5% BSA. After 1hr, plates were washed 5x with PBS/T before adding 100µl/well biotinylated anti-rabbit diluted in PBS/T plus BSA. After 1hr, plates were washed 5x with PBS/ T before adding 100ml/ well of streptavidin horseradish peroxidase. After 1h at 24°C, 100µl/well of tetramethylbenzidine (TMB) substrate was added after washing 4x with PBS/T. After 30 min, reaction was stopped by adding 100µl of 2M $H_2 SO_4$ per well (Sawyer *et al,* 2007). Absorbance was measured at 492nm by an ELISA reader.

Histopathological examination: Liver and intestine assessed amoebiasis changes and compared treatment healing after *C. papaya* fruit extract and albendazole (Oduola *et al,* 2010).

Statistical analysis: Data was presented as the mean ± standard deviation (Mean ± SD). The mean values of each group were calculated from mean values of individual mice. Mean groups were compared by analysis of variance. Comparison between two groups was done using the student's *t*-test. Results were significant if P-values < 0.05. Analyses were done by SPSS computer program (GraphPad InStat, San Diego, CA, USA).

Ethics approval: This study followed the recommendations of the legal ethical guidelines of the Animal Ethics Committee of the TBRI (Approval No. 6019/2016).

Results

Six weeks post infection with *E. histolytica* untreated and treated mice groups were evaluated individually for parasitological parameters. Mean of total number of trophozoites burden in intestinal fluid of untreated IC infected mice is lower than that of untreated dexamethasone IS infected mice. Oral administration of albendazole to IC and IS infected mice significantly reduced trophozoites burden to 88.72% & 86.40%, respectively, compared to infected control. Treatment of IC infected mice with pure and crude extract of *C. papaya* fruits significantly decreased mean of total the trophozoites

number in infected mice by 78.20% and 76.29%, respectively, compared to infected control. IS mice displayed a reduction of 68.68% & 60.72% up on treatment with the pure and crude form of *C. papaya*, respectively, compared to control ones.

Cysts count per gram of stool (cpg) was done at six weeks post infection. Feces of each mice group were collected, and microscopic examination, as stained and unstained to identify all *E. histolytica* forms. Cysts shedding in stool of non-treated IS infected group was significantly higher than in untreated IC infected group in stained and unstained samples. Compared to infected untreated IC mice, the stained speceimens of the groups treated with pure and crude papya gave a significant reduction (70.83% & 60.00%, respectively) in cpg, but lower than that of albendazole group (83.33%). In the unstained specimens, treatment with pure and crude papya extracts significantly reduced cpg by 69.23% & 61.53%, respectively that was less than that caused by albendazole (82.3%). IS infected mice treated with pure and crude papya extracts gave significant reduction of 64.4% & 56.8%, respectively in the stained cpg and 67.27% & 60.00%, respectively, in unstained cpg compared to infected untreated ones. Although reduction rates were significant but, was lower than that by albendazole (around 82%). Trophozoites and cysts number in pure *C. papaya* fruit extract treated mice was lower than that in crude extract treated ones, but that was not significant.

E. histolytica infection elicited significant ($P < 0.001$) increased in IgG, IgM & IgA serum levels against *E. histolytica* antigen in IC & IS mice compared with untreated uninfected ones. IC mice treatment with albendazole or papaya extracts significantly ($P< 0.05$) caused production of serum antibodies (IgG, IgM & IgA) against immunogenic compared to normal negative control. But, IC mice treated with either albendazole or papaya extracts (crude or pure) significantly ($P< 0.001$) reduced serum IgG, IgM & IgA antibodies levels against *E. histolytica* antigen as compared to positive control ones. However, only IgG and IgM levels induced in all treated IS mice serum compared to negative control.

In the IgA level, there was no significant difference between treated IS mice as compared to levels in untreated uninfected ones. By comparing with untreated infected IS mice, treatment with papaya or albendazole reduced serum IgG, IgM & IgA levels. IS mice humoral immune responses against antigen were lower than in IC ones. There was a significant increase ($P < 0.05$) in serum IFN-γ level in infected untreated mice compared with uninfected untreated ones. Six weeks post-infection and three weeks post last treatment, *C. papaya* extracts or albendazole elevated circulating IFN-γ levels as compared with negative control in both IC & IS mice. Conversely, serum IFN-γ levels significantly decreased in mice treated with *C. papaya* extract or albendazole compared to those detected in positive controls. But, cytokines levels in IC mice were higher than those in IS ones.

In infected untreated IC mice, *E. histolytica* caused deteriorated changes in liver's histology; many granulomatous loci, mainly contained lymphocytes and some macrophages, chromatin aggregations under the hepatic nuclear membranes, prominent inflammatory infiltrates around many portal zones relatively with dilatations in sinusoidal spaces. Infected untreated IS mice showed more pathological changes than infected untreated IC ones, as larger and numerous micro-abscesses, more trophozoite-mediated necrotic areas especially in centro-lobular zones. Treated infected mice showed improvement in hepatic architecture mainly when treated by papaya purified extract or albendazole. Treating infected IS mice with *C. papaya* crude extract gave a less recovery degree compared to the infected IC ones, as the former showed hydropic degeneration in some hepatocytes with pyknotic nuclei and few local inflammatory infiltrates.

Infected untreated IC mice villi were blunt,

short, hypertrophy, with epithelial ulcerations. Chronic inflammatory infiltration near lamina propria sometimes developed to lymphoid aggregation in submucosal region. In infected untreated IS mice, intestinal tissues showed more pathological changes than in infected untreated IC ones, such as severe villi ulcerations, more trophozoites in lumen, crypts necrotic degeneration, lymphoid aggregations with fibrosis around some trophozoites, local necrosis in muscular layer induced by invasive trophozoites and degener ation with necrotic fluids in villous epithelia.

Infected IC & IS mice showed histological improvements (normal villous epithelia; no ulceration, or trophozoites, normal crypts) when treated with purified/crude extracts or albendazole. Treatment of IC & IS mice by crude extracts were histological improved, but with less pathological changes, as few chronic inflammatory infiltrations in villi, degradation in crypts basal regions and few ulcerations in villous epithelia.

Details were given in tables (1, 2, & 3) and figures (1, 2, 3, 4, & 5).

Table 1: Effect of albendazole, aqueous & defatted methanolic extract of *Carica papaya* on trophozoite of infected mice's intestinal fluid

	Groups	Mean no. of trophozoite	SD±	% Reduction
Immuno-competent	Control (+ve)	379.14	72.96	-
	Pure *Papaya*	82.63	26.75	78.20
	Crude *Papaya*	89.86	09.01	76.29
	Albendazole	42.75	15.84	88.72
Immuno-suppressed	Control (+ve)/D	447.00	78.42	-
	Pure *Papaya* /D	140.00	50.96	68.68
	Crude *Papaya* /D	175.57	72.82	60.72
	Albendazole/D	60.75	38.84	86.40

SD, standard deviation; *D,* dexamethasone

Table 2: Effect of albendazole, aqueous and defatted methanolic extract of *Carica papaya* on cysts in stool of infected mice

	Groups		Mean No. of cysts/gm	SD±	% Reduction
Immunocompetent	Control (+ve)	Stain	30,000	3914.72	-
		No stain	32,500	4727.50	-
	Pure *Papaya*	Stain	8,750	2922.22	70.83
		No stain	10,000	4012.00	69.23
	Crude *Papaya*	Stain	12,000	2591.61	60
		No stain	12,500	3053.54	61.53
	Albendazole	Stain	5,000	2439.00	83.33
		No stain	5,750	2172.73	82.3
Immunosuppressed	Control (+ve)/D	Stain	62,500	3917.02	-
		No stain	68,750	5454.36	-
	Pure *Papaya*/D	Stain	22,250	2722.03	64.4
		No stain	22,500	2718.10	67.27
	Crude *Papaya*/D	Stain	27,000	3567.10	56.8
		No stain	27,500	3854.22	60
	Albendazole/D	Stain	11,250	4531.34	82
		No stain	12,500	2027.43	81.81

Table 3: Effect of albendazole, aqueous, defatted methanolic extract of *C. papaya* on mice serum isotype responses to *E. histolytica* antigen

group	IgG	IgM	IgA
Immunocompetent mice groups			
Control -ve (Mean±2SD)	0.240±0.021 (0.282)	0.123±0.003 (0.129)	0.048±0.023 (0.094)
Control +ve	0.987 ± 0.020*	0.479 ± 0.023*	0.275 ± 0.017*
mmPure Papaya	0.310± 0.007*	0.185 ± 0.003*	0.152 ± 0.010*
Crude Papaya	0.389 ± 0.011*	0.183 ± 0.005*	0.209 ± 0.005*
Albendazole	0.572 ± 0.026*	0.262 ± 0.035*	0.155 ± 0.008*
Immunosuppressed mice groups			
Control -ve/D (Mean±2SD)	0.134±0.01 (0.154)	0.065±0.013 (0.091)	0.275±0.017 (0.309)
Control (+ve)/D	0.800 ± 0.017*	0.301 ± 0.010*	0.277 ± 0.003
Pure Papaya/D	0.288 ± 0.005*	0.175 ± 0.001*	0.115 ± 0.010
Crude Papaya/D	0.284 ± 0.018*	0.129 ± 0.008*	0.222 ± 0.008
Albendazole/D	0.422 ± 0.019*	0.200 ± 0.002*	0.200 ± 0.002

Ten mice/ group ± standard deviation (SD) around mean. Cut off= Mean + 2SD. *D,* Dexamethasone, * Significant ($P < 0.05$)

Discussion

Albendazole is a known drug treating amoebiasis in animal (Al-Mukhtar and Barwari, 2008) and humans (Khan *et al,* 2019). Formulations based on plant products were used in disease prevention and treatment since ancient Egyptian times (Rahmani *et al,* 2016).

Nagaty *et al.* (1959) in Egypt successfully treated dog *Ascaris* in vivo with the latex *Ficus carica* and *Papaya* from Giza Zoo garden. Abou-Shady *et al.* (2014) in Egypt reported that *C. papaya* has significant anti-cestodal properties that enable its seed extract was an effective alternative to PZQ for *Hymenolepis nana.* Oloyede *et al.* (2015) in Nigeria found that the pretreatment of male rats with aqueous extract of *Carica papaya* seed exhibited anti-ulcerogenic and antioxidant effects due to the enhanced antioxidant enzymes. Peachey *et al.* (2016) in UK reported increasing interest in the evaluation of traditional 'ethnoveterinary' medicines as alternatives to chemical anthelmintic. The cysteine proteinases (CPs), a group of enzymes derived from fruits such as papaya (*C. papaya*), pineapple (*Ananas comosus*) and figs (*Ficus* spp.), gave good efficacy against adult stages of a range of parasitic nematodes, *in vitro* and *in vivo*. Pandey *et al*. (2017) in Australia suggested that the selective anti-proliferative and anti-metastatic attributed of the papaya leaf juice extract against prostatic diseases.

Singh *et al.* (2020) in India reported that papaya leaf extract possesses the strong medicinal properties as antibacterial, antiviral, anti-tumor, hypoglycaemic and anti-inflammatory activities. They added that tleaf juice increased the platelet counts in the dengue fever patients. Od-Ek *et al.* (2020) in Thailand reported that papaya has the potential to reduce obesity risk associated with adiposity, anti-inflammation and anti-oxidation.

Mansour *et al.* (2022) in Saudi Arabia reported that dietary supplementation with *C. papaya* extract at a level of 250mg/kg body weight succeeded to alleviate the negative effects of Chlorpyrifos (CPF) on the physiological, immunological, and antioxidant status of female catfish. Also, CP extracts alleviated the endocrine disruption and hepatic DNA damage and counteracted sub-chronic CPF toxicity in female African catfish, and so, *C. papaya* extract is used as feed additive in aquatic diet.

Different parts of *C. papaya,* such as leaves, barks, roots, latex, fruit, flowers, and seeds were used in folk medicine to treat a variety of diseases (Jaiswal *et al,* 2010). Phenolic compounds in *C. papaya* extract explained its pharmacological properties as anti-parasitic action (Canini *et al,* 2007). Abdel-Lateef *et al.* (2018) reported 13 polyphenolic compounds and flavonoids of *C. papaya* fruit defatted MeOH extract were identified by HPLC-ESI-MS. Khor *et al.* (2021) identified 11 lipophilic constituents in *C. papaya* leaf supercaritical carbon dioxide ($scCo_2$) extract with 5% ethanol (CPSCE), which have antioxidative effect. Abdel-Halim *et al.* (2021) found that *C. papaya* leaves have the best bioactive polyphenols extract. Kadiri (2017) identified six phenolic compounds in *C. papaya* seeds extract; including: p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, quercetin-3-galactoside, and kaemp-ferol-3-glucoside. Ferulic acid is the major phenolic compound in samples, followed by caffeic acid and p-coumaric acid, as they constitute the large proportion of the total phenolic content. They deduced that aqueous methanol was a better solvent for extraction of antioxidant compounds in all samples. Both Siddiq *et al.* (2005) and Al-Reza *et al.* (2009) reported that polar extracts possess strongest activity than non-polar ones. McGaw *et al.* (2007) found that ethanol extracts gave best activity against parasite than water extracts, but they didn't mention its *E. histolytica* action.

Besides, *C. papaya* has certain chemical components of high anthelminthic attributes in poultry with satisfactory efficacy at dose of 1200mg/bird (Fajimi and Taiwo, 2005; Adu *et al,* 2009). The fruits and specially the mature seeds of *C. papaya* have the excellent anthelminthic & anti-amoebic effects,

but with less pronounced than metronidazole (Sarker *et al,* 2010; Ahmed *et al,* 2011). The papaya contains many biologically active compounds in its different parts. Carpaine and benzylisothiocyanate are found mainly in seeds that may be active ingredient of papaya extract in its anti-amoebic effect (Mohammed *et al,* 2014). However, Runnie *et al.* (2004) reported that a very high phenolic content of the MeOH extract of *C. papaya* leaf was observed. Besides, leaves, seed, latex and fruit of *C. papaya* contain anthelmintic alkaloid and carpaine effective in expelling alimentary canal worms (Dotto and Abihudi, 2021).

In the present study, reduction of parasite loads with aqueous extracts of *C. papaya* was attributed to papain, which was capable to digest bacteria and parasitic cells (Fajimi and Taiwo, 2005). However, Okeniyi *et al.* (2007) reported that more large-scale intervention studies comparing *C. papaya* with standard anti-parasitic drugs was a must. Also, Cock *et al.* (2018) reported that many plant species used to treat amebic diarrhea must be tested against the *E. histolytica* itself.

In the present study, aqueous and MeOH *C. papaya* fruits extracts went with albendazole in treating amoebiasis in IC & IS mice. This agreed with Miller *et al.* (2007) who reported testing the effect of suppressing the ability of immune system to curb parasite, which spread in genetically IS animals and in dexamethasone ones. The present results showed that administration of albendazole (200mg/kg) to mice significantly ($P < 0.05$) reduced the trophozoites number in intestinal fluid and cysts in stool of infected mice by 80.40 to 88.72% in IS and IC groups respectively. While mice treated with pure or crude papaya extracts (500mg/ kg) had a reduction of 65 to 78%, respectively, but mice subjected to dexamethasone had a lower reduction of 56 to 69%, respectively. This agreed with Sarker *et al.* (2010), reported that mature *C. papaya* seeds exhibited an anti-amoebic action but less prominent than metronidazole. Aqueous extracts of *C. papaya* seeds caused a significant reduction in parasite burden of the mice attributed to the papain, which is capable of digesting bacteria and parasitic cells, hence its use as anthelmintic and antibiotic (Fajimi and Taiwo 2005). Abdel-Lateef *et al*. (2018) reported that oral administration of defatted MeOH extract of *C. papaya* fruits (500mg/kg) decreased *Schistosoma mansoni* number in CD-1 mice by 36.4% and ova in intestine and liver tissues by 39.7% & 49.8%, respectively, but lower than praziquantel (94-96%). Also, the protective capacity of oral administration of crude extracts, MeOH, EtOAc or BuOH of *C. papaya* (100mg/kg) to mice infected with *S. mansoni* was 60.3%, 38.7%, 68.2%, & 52.7%, respectively. But, 500mg/kg PZQ treatment on two consecutive days at 6 weeks post-infection induced a 92.8% reduction in total worm burden (Aly *et al,* 2020).

Generally, protozoa infection is associated with IgG and IgM production, and in helminths and arthropod dermatitis there is IgE. IgA produces response to intestinal protozoa, such as *E. histolytica* and *Giardia lamblia* (Evering, 2006). The present study found that IgG, IgM, and/or IgA serum antibody levels were higher in the untreated and treated compared with the negative control. But, their levels were significantly ($P< 0.001$) reduced in mice treated with *C. papaya* extracts or albendazole compared to levels in positive control mice. The elevated IgG responses in positive control were associated with a high parasite burden, led to a high circulating parasite antigens level, but many of them didn't relate to protection (Njoroge *et al,* 2010). Thus, high IgG level didn't confer protective immunity in positive control as shown by highest number of recovery parasites (Aly *et al,* 2020). This also agreed with Amin *et al.* (2019) who found significant decrease in serum IgM levels of *C. papaya* pulp or seed MeOH extracts treated mice compared to positive control ones (bacterial infected). But, they found high IgG serum levels in *C. papaya* extracts treated group compared to positive control mice.

Infected mice group increased serum IgM levels was attributed to the acute resistance and disease severity (Amin *et al,* 2019). Furthermore, high IgM levels may be a desired therapeutic strategy for selective IgM deficiency, since they have antibacterial and immunomodulatory effects on the autoimmune symptoms of the disease (Gupta and Gupta, 2017). But, drop in IgM levels after treatment with papaya extracts indicated class switching of IgM. Because B-lymphocyte produced IgM antibodies on their own, but need interactive help from T- lymphocytes to control transition from IgM to IgG, IgA, or IgE (Amin *et al,* 2019).

Secretory IgA is one of the abundant Igs produced by plasma cells prevents pathogens adhere and remove to the mucosal barrier (Lamm, 1998). Shalash *et al.* (2016) reported that fecal IgA in cryptosporidiosis infected mice untreated was significantly higher compared to normal ones, post paromomycin or/and nitro-anilines treatment IgA level decreased, but IgA level was significantly associated with infection and cyst shedding.

E. histolytica colonizes in the large intestinal lumen, triggering an immunological response to overcome and remove the infection by the release of cytokine mediators and immunoglobulins. These cytokines have an important role in parasite control (Asgharpour *et al,* 2005). The host immune response to parasites was shown to be a T-cell dependent process (Taylor-Robinson and Phillips 1992). Classically, the host initially responds with a Th1 type response, which shown to be directed against early stages of the parasite and important for inducting the cell mediated protective immunity to the parasites (WHO, 2002). Thus, in the present study, the infected control and treated mice showed significantly higher IFN-γ responses to *E. histolytica* antigen than the negative control ones. However, both *C. papaya* and albendazole treated IC& IS groups showed significantly decreased IFN-γ responses compared to infected untreated group. But, the effect of the immune response in IS mice was lower than that in IC. This was interpreted by that dexamethasone is a type of corticosteroid that inhibited cytokines' secretion of Th1 cells more than that of Th2 cells (Franchimont *et al,* 1998). This also agreed with Abou-el-Nour *et al.* (2017) who found a significant increase in IFN-γ levels in *E. histolytica* infected group compared to negative control. But, treatment with medicinal plants (garlic and ginger) significantly decreased IFN-γ levels compared to infected untreated ones. Also, this agreed with McDonald (2000), who reported the immune system of infected mice overcame and removed the infection by secreting Th1 cytokines as IFN-γ, which played a very effective role in the innate and adaptive immune responses to *C. parvum* infection but, without well-known mechanism (Aliberti *et al,* 1996). Besides, the IFN-γ secreted from activated the natural killer T cells (NKT) caused in considerable protection against *E. histolytica* in CD1d mice, whereas CD1d mice developed severe abscesses, when *S. mansoni* 0-3hr & SWAP antigens was used independently to stimulate the lymph node and spleen cells (Wong-Baeza *et al,* 2010). Mose *et al.* (2013) reported that at the endpoint, cells from the infected control and all treated had IFN-γ and IL-5 proliferative responses larger than negative control. They added that the IFN-γ responded to both antigens was reduced in *C. papaya* aqueous and MeOH extracts, but without significant difference.

Besides, the present results agreed with both Powell *et al.* (2010) and Mohammed *et al.* (2014), who reported that *C. papaya* aqueous extract re-constructed the destroyed liver and intestinal tissues of mice. Moreover, the papaya extracts generally improved the epithelial cell profile either in intestinal villi or epithelia of central veins of the hepatic tissues. This agreed with Ezike *et al.* (2009) who reported that aqueous and methanolic extracts of papaya fruit possesses anti-ulcero-genic effect. Also, Hakim *et al.* (2019) who reported that aqueous extract of the *C. papaya* fruit had a significant effect on epit-

helization and fibrillation.

In the present study, albendazole, like *C. papaya* purified extract, induced the re-construction of damaged villi. This agreed with Mohammed *et al.* (2014) who reported that *C. papaya* aqueous exhibited anthelminthic and anti-amoebic activities, without significant side effects. Also, Mahdy *et al.* (2017) reported that histopathological changes were improved after administration of powdered papaya seeds extract in treating *Aspiculuris tetraptera*, they referred such improvement to the alkaloid components in papaya seeds (benzyl isothiocyanate) which was responsib le for anthelmintic activity of papaya plant.

Abdullah *et al.* (2011) reported that papaya fruits increase regulatory T cells (mediated in inflammations decrease) and reduced IFN-γ, CD^4, T cells. Amazu *et al.* (2010) reported anti-inflammatory activity of the methanolic extract of the seeds of *C. papaya* in experimental animals, they interpreted these data by inhibitory action of extract against some mediators such as histamine, prostaglandins, nitric oxide, cytokines, platelets activating factor. HPLC-ESI-MS analysis of methanolic extract of *C. papaya* fruit caused a first-ordered compound p-hydroxybenzoic acid-tri-O-hexoside which is isomeric with 2-hydroxybenzoic or salicylic acid, a precursor to aspirin (Abdel-Lateef *et al,* 2018). The latter is a medication used to reduce pain, fever, or inflammation, so, anti-inflammatory effect of MeOH papaya fruit extract was attributed for the presence of p-hydroxybenzoic-acid-tri-O-hexoside in large amount in the extract. HPLC-ESIMS analysis referred to a second leveled-high phenolic compound, Protocatechuic-di-O-hexosylglucuronide, which that was considered an antioxidant against free radicals (López-Martínez *et al,* 2015). Fractionation of MeOH papaya fruits extract yielded a major valuable phenolic compound (Abdel-Hady *et al,* 2014), the protocatechuic acid derivatives with antioxidant and anti-inflammatory properties (Rivera-Pastrana *et al,* 2010).

Consequently, the present histological improvement in the hepatic & intestinal tissues of infected mice treated with purified methanolic extract was attributed to these derivatives.

Conclusion

Generally speaking, plants were extensively studied since ancient times and numerous important chemical constituents with tremensdous therapeutic potential were identified.

C. papaya extract proved to be effective in treating and preventing the risky *Entamoeba histolytica*. *Carica papaya*-based medicines use as anti-*Entamoeba histolytica* agent was recommended.

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Explanation of figures

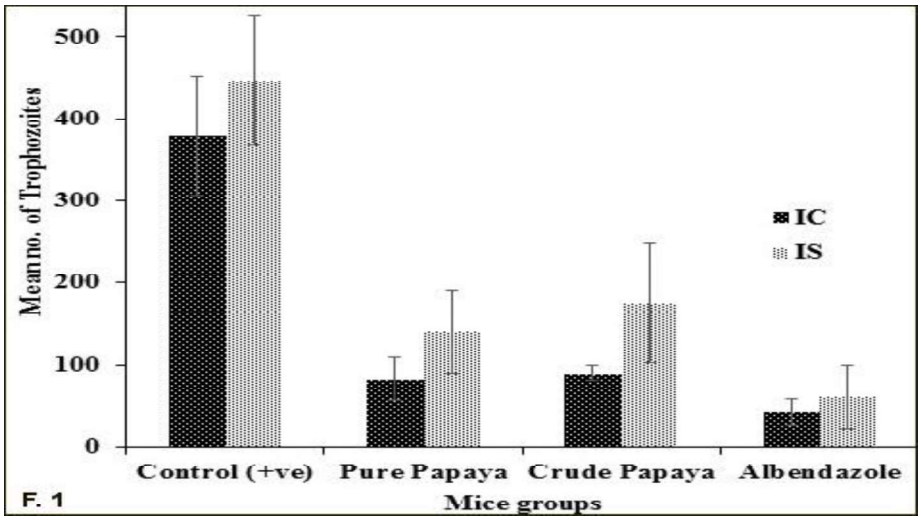
Fig. 1: Efficacy of albendazole and aqueous and defatted methanolic extract of *Carica papaya* on trophozoite count of CD-1 mice, evaluated 6 weeks post infection with 1000 *Entamoeba histolytica* cysts. Each column represents mean the number of intestinal trophozoites of 10 individual mice. Vertical bars represent the standard deviation around the mean. IC, Immunocompetent mice; IS, Immunosuppressed mice

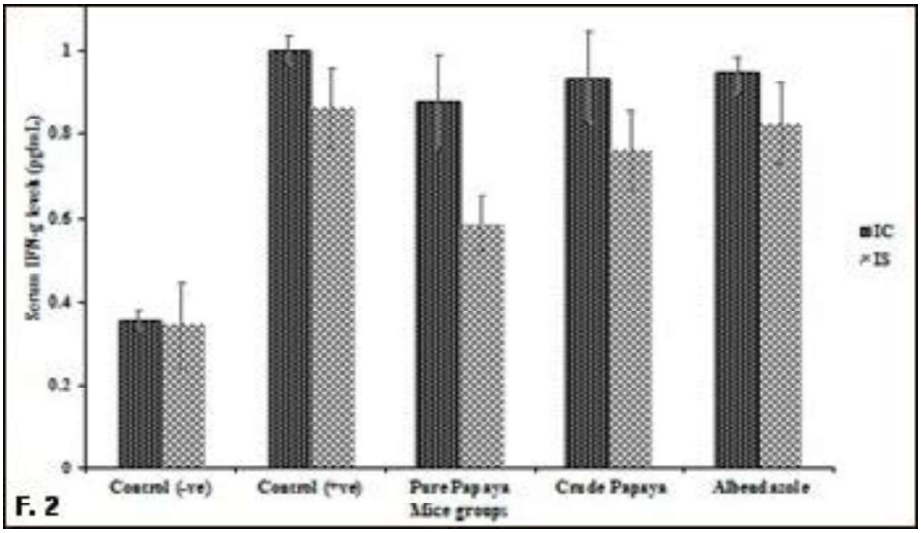
Fig. 2: Efficacy of albendazole, aqueous and defatted methanolic extract of *Carica papaya* on *Entamoeba histolytica* infected mice sera IFN-ɣ levels. Each column represents mean of cytokine concentration (pg/mL) of mouse (10 per group) sera diluted 1:200, assayed on an individual basis in duplicates, 3 weeks after treatment. Vertical bars denote standard deviation about mean. IC, Immunocompetent mice; IS, Immunosuppressed mice

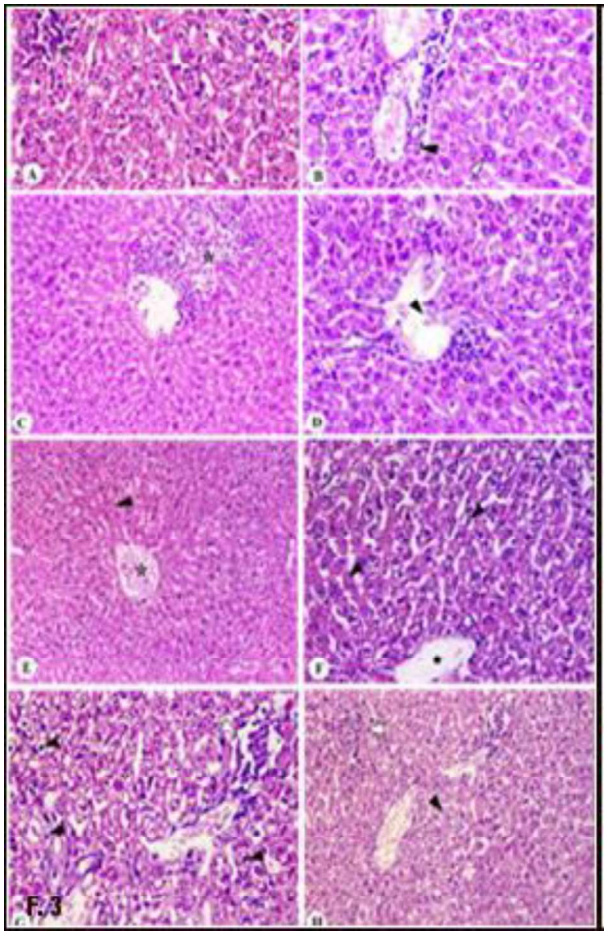
Fig. 3: Sections of liver (H & E). A, B: Positive control of IC showed granulomatous loci (asterisk), chromatin aggregations under hepatic nuclear membranes (white arrowhead), and inflammatory infiltrates around portal zone (black arrowhead) (X400 & X400, respectively). C, D: Positive control of IS showed a micro abscess (asterisk), trophozoite-mediated necrotic areas in a cenro-lobular zone (black arrowhead) (X200 and X400, respectively). E, F: Pure extract-treated and albendazole-treated IC showing highly improved hepatic architecture [healthy central vein (asterisk) & normal hepatic strands (black arrowheads)] (X200 & X400, respectively), G, H: Crude extract-treated IS showed hydropic degeneration in some hepatocytes with pyknotic nuclei (black arrowheads), local inflammatory infiltrates (white arrow heads) (X200 & X400, respectively)

Fig. 4: Sections of intestine (H & E). A: Positive control of IC showed ulcerations in epithelia of villi (white arrowhead), hypertrophy (black arrow), shortening of villous (asterisk), cryptic degeneration (black arrowhead) (X400), B: Lymphoid aggregation in sub mucosal region (white arrowheads) (X100). C: Ulcerations in villi (Black arrowheads) (X100), D: Positive control of IS showed *E. histolytica* trophozoites in the intestinal lumen (Black arrowheads) (X400), E: Necrotic degeneration in crypts (white arrowheads) (X400), F: Lymphoid aggregations with fibrosis around a trophozoite (asterisk) (X400), G: Local necrosis inside the muscular layer induced by invasive trophozoites (white arrowheads) (X400), H: Degeneration with necrotic fluids in villous epithelia (black arrowhead) (X400)

Fig. 5: Sections of intestine (H & E). A: Albendazole-treated IS group showed normal villous epithelia (black arrowheads), neither ulceration nor trophozoites detected, normal crypts (white arrowheads) (X100), B: Purified extract-treated IS group showed normal villous epithelia (black arrowheads), normal crypts (white arrowheads) (X100), C: Purified extract-treated IC group showed healthy villous epithelia (black arrowheads) (X100), D: Crude-treated IC group showed few chronic inflammatory infiltrations in villi (white arrowheads), degradation in basal regions of some crypts (black arrowhead) (X200), E: Crude-treated IS group showed few ulcerations in villous epithelia (white arrowheads) (X100)



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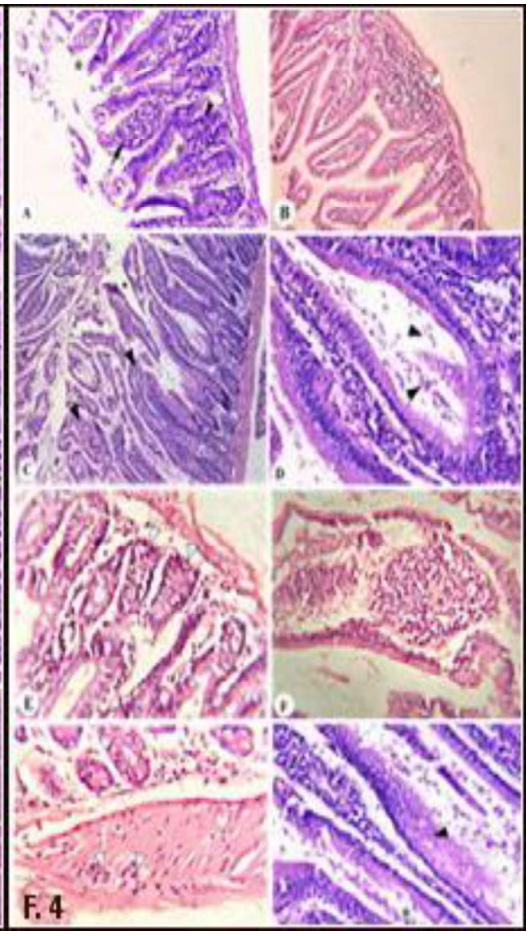
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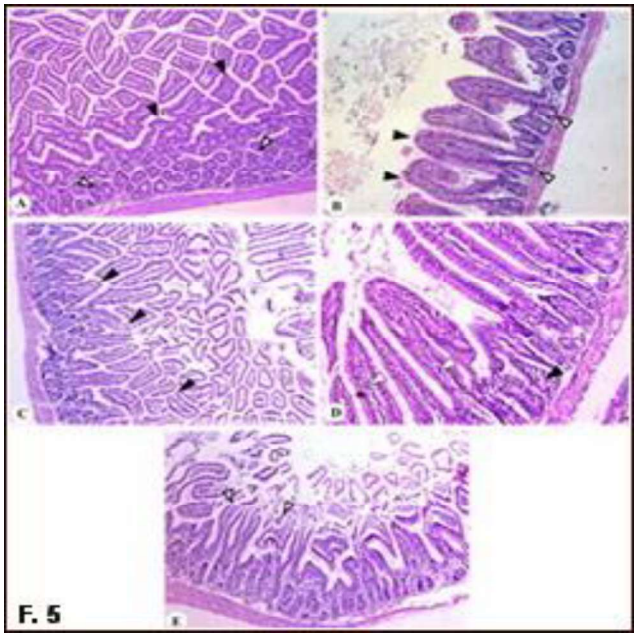
F. 5