

Comparative study of antidiarrheal effect of proposed new formulae from *D.innoxia* hydroalcoholic leave extract against *E.coli* O:157 induced diarrhea in goats

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Abstract

New proposed formulation of 5% and 7.5% of hydroalcoholic leave extract of *D.innoxia* were tested as new drug in order to treat induced diarrheal infection by pathogenic *E.coli*O:157 strain isolate in 16 healthy local goats divided in to equal four groups according to following treatment groups T1 and T2 represented (25 and 37.5 mg/kg *D.innoxia*), ciprofloxacin (5mg/kg) and DMSO vehicle group 0.5 ml /kg as positive control .Dosing continue orally daily for 14 days after induction of diarrheal infection .The efficacy of different treatment were evaluated according to improvement in clinical symptoms in which the formulation showed dose dependent effect with superiority of higher dose in T2 over ciprofloxacin in reduction body temperature and respiration rate , improvement in body weight gain and dehydration signs with reduction of fecal bacterial count (*E.coli*) to nearly normal with completely ceasing diarrhea at the end of treatment . This was due to the presence of multiphytochemical that have not only antibacterial effect but also astringent ,anti-inflammatory ,analgesic ,antispasmodic, narcotic ,antipyretic and antiallergic that help to over come the stress that accompanied diarrheal infection.

Key word :Datura, diarrhea,dehydration ,formula,E.coliO:157

الخلاصة:

تركيبة جديدة مقترحة بتركيز 5% , 7.5% من الخلاصة الكحولية المائية لاوراق نبات الداتورا انوكسيا جربت لعلاج اصابة اسهال مخمجة بعثرة مرضية من جرثومة *E.coli*O:157 حيث تم اصابة ستة عشر ماعز محلي قسمت الى اربع مجاميع متساوية كالتالي (T1,T2 اعطيت جرعة 25, 37.5 ملغم اغمم من خلاصة النبات ومجموعة ciprofloxacin بجرعة 5ملغم اغمم ومجموعة DMSO تمثل السيطرة الموجبة 0.5 مل اغمم حيث استمر البرنامج العلاجي يوميا لمدة اربع عشر يوما بعد الاصابة الجرثومية وظهور اعراض الاسهال, اما كفاءة العلاج للاسهال تم تقديرها حسب التحسن في الاعراض المرضية التي شهدت الكفاءة للتركيبة وحسب الجرعة المستعملة حيث سجلت الجرعة العالية للمستخلص النباتي (T2) افضلية في تخفيض درجات الحرارة الجسم ومعدل التنفس وتحسن في زيادة الوزن وأختفاء اعراض الجفاف وانخفاض في اعداد بكتيريا الايكولاي الى تقريبا الاعداد الطبيعية مع توقف كامل للاسهال في نهاية العلاج. الافضلية سجلت بسبب احتواء المستخلص على مواد فعالة ليست ذات تاثير مضاد لنمو البكتيريا فقط بل تحتوي على مواد ذات فعالية قابضة, مضادة للالتهابات, مضادة للحرارة والالام ومضادة للتقلصات والحساسية والتي يمكن ان تساعد في ازالة الاجهاد والعلامات السريرية المصاحبة للاسهال الجرثومي .

Introduction

Datura innoxia (Family: Solanaceae, locally known as Elsakran) is used for many medicinal purposes. *Datura* precise and natural distribution is uncertain, owing to its extensive cultivation and naturalization throughout the temperate and tropical regions. It contains atropine alkaloids such as scopolamine, hyoscyamine, hyoscine norscopolamine, meteloidine (1) and (2). Traditional medicine uses flowers, leaves and seed of *Datura innoxia* (D.innoxia) medically treat for skin eruptions and infection, colds, nervous disorders, antipyretic, anti-inflammatory, analgesic, spasmolytic, astringent and antidiarrheal effect.

Material and methods

D.innoxia preparation and extraction

D.innoxia leaves were collected from collage agriculture farms. The plant classification was done in Ministry of Agriculture/ State Board for Seeds Testing and Certification (S.B.S.T.C) in Abu Graib /Baghdad. The leaves dried in shade, blended to powder and extracted by using hydroalcoholic method in 30% ethanol according to (3) the yield of extract was calculated according to the dry plant powder.

Preparation of *D.innoxia* formulae

D.innoxia leaves extract formula was prepared in two concentrations 5% and 7.5% by dissolving the extract in 50% (dimethyl sulphoxide) DMSO and after supported ingredients. Different trials were done to prepare the best formulation that its ingredients kept secret for know how purpose?. The formulae were prepared under sterilized condition under UV light and using 0.5 millipore filter paper to filtrate extract that filled in sterilized well sealed bottle of 25 ml to be given for treatment of diarrheal goats.

Detection of phytochemical components

Different phytochemical components were determined according to methods of detection mentioned by (3) for alkaloid, flavonoid, steroid, terpenoid, resin, phenol, saponin, tannins as well as glycosides.

Isolation and identification of pathogenic *E.coli* isolate

Pathogenic bacteria isolates were collected from severely infected diarrheal goats with hemorrhagic diarrhea. Analytical profiles Index (API-20) as well as different biochemical methods were used for identification of *E.coli* growth while the special growth colour in chrome and sorbitol macConkey agars were used for identification of *E.coli* O:157 strain.

In -Vivo study of antidiarrheal effect of *D. innoxia* formulations

Sixteen healthy local goats weighed (22-25)kg examined clinically for normal signs as well as for fecal bacterial growth that showed normal flora but without *E. Coli* O:157 strain growth. The goats divided equally into four treatment groups that kept in separated places in well closed table with special fodder and water given ad libitum. Treatment begins after 48 hrs. of inducing infection after appearance signs of diarrhea that treated according to the following groups:

Group (T1): infected and treated orally daily with 25 mg /kg b.w (5%) formulation for two weeks.

Group (T2): infected and treated orally daily with 37.5 mg /kg b.w (7.5%) formulation for two weeks

Group (cipro): infected and treated orally daily with 5 mg /kg b.w of ciprofloxacin at concentration 50 mg/ml. (safcoceieb/ Syria) Company

Group(DMSO): infected and treated with formulated vehical (DMSO) with

out plant extract at dose 0.5 ml /kg b.w

The following parameters have been studied (before , after infection and after treatment)

A- Physiological and clinical symptoms observation (body weight change ,body tempareture , respiratory rate)

B-fecal bacterial count for E.coli bacteria .

Inducing Infection (Diarrhea)

After a pilot study, the challenge dose that chosed to induce infection (acute diarrhea) was 3.5×10^9 cfu/ml of E. Coil suspension, the inoculum preparation-standardized according to viable counting method-pour plate technique . E.coli O157 bacteria was inoculated in 10 ml of brain heart infusion broth (1) ml was given orally by modified warming gun method .then animals were watched for development symptoms of diarrhea in gaots in all treated groups after 24 hrs.of infection (4)(5).

Statistical analysis

Data that subjected to statistical analysis were represented as mean \pm standard error.Statistical analysis of data was performed by two-way ANOVA using Least significant difference (LSD) to compare means of collected data at level (P<0.05)

Result

1- Phytochemicals components in *D. innoxia* dry leaves Powder Extract

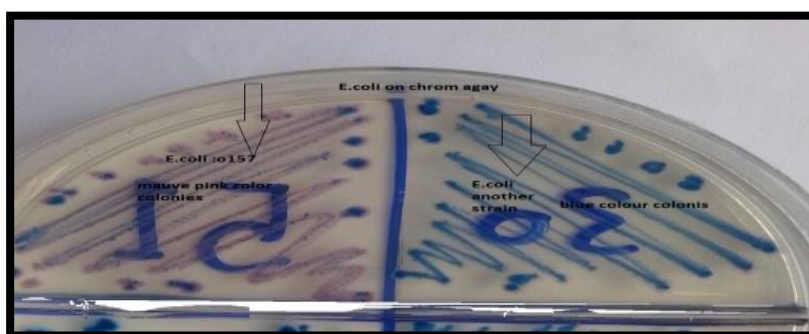
The plant leaves powder extract screening showed the

presence of the following phytochemicals; alkaloids, phenol, saponins, flavonoids, tannins and glycosides, Alkaloid, phenols, saponins and tannins seemed to be found in a high level in dry extract.These results were in agreement with that study conducted by (6)

2 Identification of pathogenic Escherichia coli O:157 isolate:

The results of biochemical tests have shown that these isolates gave positive results for catalase, insole and methyl red, while giving negative results for oxidase and Citrate utilization, and the bacterial isolates expressed gram-negative stain, this biochemical characteristic microscopic feature and this characted growth especially on chromagar that showed pink or mauve pink colour for E.coliO:157strain that different from other strains of E.coli ,because other strains of E.coli have blue color on chromagar(7) also growth on MacConky and sorbitol agar that showed specific straw to white or colourless colonies so these biochemical characteristics test indicating that the bacterial colonies belonged to *E. coli* O157. fig.(1)This result was similar to that reported by (7) who used the same tests to reach such identification result conclusion of pathogenic strain of E.coli:O:157

fig(1)different growth between E.coli :o157strain(left) from other strain of E.coli (right)on chrom agar



3. preparing infectious dose after counting colonies of pathogenic E.coli

The result of bacterial counting of the strain E.coli used in this study was 3.5×10^9 cfu/ml that confirmed by serial dilution count ,This dose was used to induce infection in goats orally by the aid of modified worming gun.

4. clinical observation signs

All the animals before infection were seem healthy with normal temperature and respiration fig (2-A) ,while after infection with E.coli O:157 isolate in all goats groups that exhibited clinical signs of enteritis .profuse diarrhoea with mucous and blood, pasty or loose faeces, anorexia, emaciation, rough body coat, poor body weight gain and reduced elasticity of skin,loss of hair indicating dehydration fig (2-B).

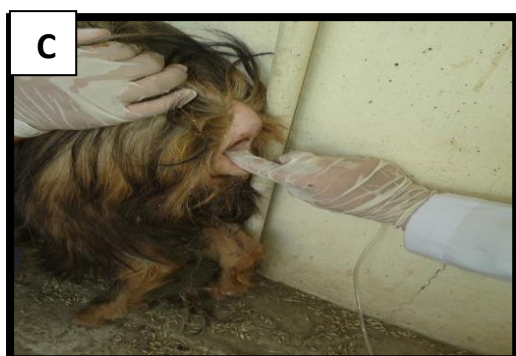
Efficacy of different treatment against induced diarrheal infection are listed in table (4-1) according to treated groups.**Group DMSO** untreated

diarrhoeic animals showed symptoms of severe dehydration, profuse to hemorrhagic diarrhea , tachycardia, anorexia, reluctance movement and dyspnoea,that observed throughout the experiemental period. Mortality in the treated positive infected control animal group by DMSO were found to be 100% after the end of treatmnt period.The animals of **Group T1** receiving D.innoxia 5% antidiarrhoeal formulation at dose of 25gm/kg for 14 days did not show complete recovery at the end of 14 days treatment since clinical signs were relatively mild. **Group T2** animals treated with 37.5gm/ kg D.innoxia extract formulae For 14 days exhibited faster recovery from day 7th treatment while complete recovery was attained on day14th. **Group cipro.** given antibiotics ciprofloxacin at dose 5mg/kg for 14 days showed recovery after 8th day onward, as listed in table (1).

Table(1): Responsive of treatment of E.coli O:157induced infection in goats according to improvement in clinical sings after treatment with D.innoxia extract formulae , ciprofloxacin and DMSO:

peroids Groups	After one week treatment	After two week treatment
T1 D.innoxia formulaeat dose 25 mg /kg	Profuse watery diarrhoea with body weight loss., fever ,and sever to moderat dehydration	mild diarrhoea with less weight loss,modeate dehydration ,rough coat with loss of hair, decrease in body temperture and respiratory rate. .
T2 D.innoxia formulae at does 37.5 mg/ kg	moderate dehydration and watery diarrhea with foul odor ,anaroxia,dry mucas mambarane of eyes, loss hair, anorexia,and rough body caot	Improvement in all clinical symptoms manifested by eatingt and drinking normally ,increase in body weight gain normal, movement inside the field with decrease to normal body temperature and respiration rate, , mild dehydration and stopped body weight loss with

		completely stopped diarrhea fig.(4-2-c)
Ciprofloxacin At dose 5 mg/kg	The behavior sign of depression, weight of loss , lethargy, moderate,dehydration,soft to watery diarrhea ,dry mucas mambarane of eyes, loss of hair, anorexia, rough body caot	Mild diarrhoea with e pale yellow colour mucous membrane,decrease in body temperature and respiratory rate ,with mild dehydration,stopped diarrhea and body weight loss.
DMSO vehicle 0.5 ml /kg	Green to blackish color ,watery profuse diahrrea ,severe dehydration ,loss of weight , anorexia, representing inability to grazing in the field. As well as paleness of eye mucous membrane , it can not on eating and drinking,rough skin and recombancy.	Profuse watery to bloody diarrhoea with progressive weight loss and sever emaciassion ,sever dehydration ,signs manifested by recombancy ,inability to eat ,fever,and rapid to shallow breathing rate with increase mortality occur at the end of treatment .fig (2-D)



Fig(2)-(A)-healthy normal goats before infection. (B) severe diarrhea in all groups during infection period. (C) stopped diarrhea after 2 week at 37.5 mg /kg D.innoxia formula treatment (d) dehydration and bloody diarrhea and mortality in DMSO treatment group goat.

Body weight

After infection and even after treatment periods there were significant decrease (P<0.05) in body weight after infection. While after treatment plant groups, showed some significant improvement (p<0.05) recorded in body weight gain in animal of T1 and T2 groups in comparison with ciprofloxacin group at the end of treatment or DMSO group that showed the signs of emaciation and sever dehydration during all course of treatment periods. Animals of DMSO group persisted in its significant decrease in body weight (P<0.05) during all treatment period in comparison with the other groups T1, T2 and cipro. (table 2)

Table(2): Effect of different treatment with D.innoxia plant formulae ,ciprofloxacin and DMSO on body weigh (Kg) of infected goats with E.coli O157 isolate

Group	Period		
	Pre infection	Infection with out treatment	End of treatment
T ₁	23.70 ± 0.32 BC a	20.30 ± 0.77 BC b	22.72 ± 0.24 A ab
T ₂	25.50 ± 0.37 A a	22.22 ± 0.67 A b	23.17 ± 0.65 A b
Ciprofloxacin	24.52 ± 0.43 AB a	21.17 ± 0.36 AB b	20.40 ± 0.43 B b
D.M.S.O (vehicle)	22.70 ± 0.28 C a	20.32 ± 0.60 C b	18.30 ± 1.00 C b
LSD value	1.106	1.922 *	1.996 *

T1 =group treated with *Datura innoxia formulae* at dose 25mg /kg bw T2=group treated with D.i. At dose 37.5 mg/kg bw Ciprofloxacin= group treated with antibiotic at dose 5 mg/kg bw Values represent mean ±S. E., Different small letters mean significant (P< 0.05) results between periods. Different capital letters mean significant (P< 0.05) results between groups

Body temperature

The result of body temprture change in goat groups dosed with D.innoxia formula T₁ , T₂ and ciprofloxacin . That showed signs of significantaly decrease in feverish temperature (P < 0.05) after treatment ranged between (38.30-38.75C⁰) when compared with infected group with DMSO that showed high temp. of 40.52C⁰ .The reduction in body temperature in T₁ and T₂ groups animals were lesser than that treated with ciprofloxacin. (Table3)

Table (3):Effect of different treatments with D.innoxia plant formulae ,ciprofloxacin and DMSO.on body temperature (C⁰) of infected goats with E.coli O157 isolate ;

Group	Period		
	Pre infection	Infection with out treatment	End of treatment
T ₁	38.42 ± 0.08 AB b	41.00 ± 0.22 AB a	38.32 ± 0.06 B b
T ₂	38.30 ± 0.10 B b	40.67 ± 0.32 B a	38.30 ± 0.08 B b

Ciprofloxacin	39.02 ± 0.33 A b	41.92 ± 0.31 A a	38.75 ± 0.20 B b
D.M.S.O (vehicle)	38.12 ± 0.24 B b	41.52 ± 0.45 AB a	40.52 ± 0.27 A a
LSD value	0.669	1.047 *	0.549 *

T1 =group treated with *Datura innoxia formulae* at dose 25mg /kg bw T2=group treated with D.i. At dose 37.5 mg/kg bw Ciprofloxacin= group treated with antibiotic at dose 5 mg/kg bw Values represent mean ±S. E., Different small letters mean significant (P< 0.05) results between periods.Different capital letters mean significant (P< 0.05) results between groups

Respiration rate

The results showed signifecant increase(p<0.05) of respiration rate after infection ranged between (32.67-35.00) in all groups ,but after treatment with different dose of extract in T1,T2 and with comparative drug of ciprofloxacin, these groups showed signs of decrease in respiration rate ranged between(29.00-31.00) in comparison with infected group,dosed with DMSO that showed high rate 33.25.as listed in table(4)

Table 4; Effect of different treatment with D.innoxia plant formulae ,ciprofloxacin and DMSO on(No. respiration per minute) of infected goats with E.coli O157 isolate

Group	Period		
	Pre infection	Infectionwithout treatment	End of treatment
T₁	25.67 ± 2.01 A b	33.32 ± 1.43 B a	31.00 ± 0.71 B a
T₂	24.67 ± 0.62 B b	35.00 ± 1.08 A a	29.32 ± 0.47 BC a
Ciprofloxacin	24.00 ± 0.41 AB b	32.67 ± 0.23 AB a	29.00 ± 0.41 C a
D.M.S.O (vehicle)	23.32 ± 0.94 BA b	34.32 ± 0.84 A a	33.25 ± 0.85 A a
LSD value	3.613	3.081 *	1.959 *

T1 =group treated with *Datura innoxia formulae* at dose 25mg /kg bw T2=group treated with D.i. At dose 37.5 mg/kg bw Ciprofloxacin= group treated with antibiotic at dose 5 mg/kg bw Values represent mean ±S. E., Different small letters mean significant (P< 0.05) results between periods.Different capital letters mean significant (P< 0.05) results between groups

Rectal bacterial count

There were a significant decrease (P<0.05) in the rectal bacterial count of all treated groups in comparison to its count after inducing infection . Rectal bacterial count significantly

reduced (P<0.05) after 14 days of treatment in comparison to its highly increasing count after inducing infection. Animal of T2 and ciprofloxacin groups possibly returned to normal bacterial count as before inducing infection after 14 days

of treatment ,indicating complete recovery while T1 showed signs of lesser antibacterial effect at the end of treatment recording (6.07×10^4) in comparison with (4.20×10^2 and 4.55×10^2)cfu/ml of fecal sample for T2 and ciprofloxacin groups

respectively indicacating not complete antibacterial effect than high formulae dose at 37.5 mg /ml or ciprofloxacin treatment .While group (DMSO)group showrd no change in bacterial count, table (5).

Table (5): Effect of different treatment with D.innoxia plant formulae ,ciprofloxacin and DMSO on Fecal bacterial count (cfu/1ml)of infected goats with E.coli O157 isolate

Group	Period	
	After infection with out treatment	End of treatment
T ₁	$10.84 \times 10^6 \pm 0.01$ A a	$6.07 \times 10^4 \pm 0.48$ B b
T ₂	$9.58 \times 10^7 \pm 0.64$ B a	$4.20 \times 10^2 \pm 0.51$ C b
Ciprofloxacin	$10.34 \times 10^6 \pm 0.29$ AB a	$4.55 \times 10^2 \pm 0.49$ C b
D.M.S.O (vehicale)	$10.60 \times 10^6 \pm 0.25$ AB a	$10.35 \times 10^6 \pm 0.29$ A a
LSD value	1.151 *	1.401 *

T1 =group treated with *Datura innoxia formulae* at dose 25mg /kg bw T2=group treated with D.i. At dose 37.5 mg/kg bw Ciprofloxacin= group treated with antibiotic at dose 5 mg/kg bw Values represent mean \pm S. E., Different small letters mean significant (P< 0.05) results between periods.Different capital letters mean significant (P< 0.05) results between groups

Discussion

Clinical observation signs/Gram-negative bacterial infection by *Escherichia coli* O:157 occur frequently in farm animals. Infection by this organisms result in release of endotoxin from outer bacterial membrane into the host’s internal environment which is responsible for some of the most common diseases in farm animals such as enteritis, endometritis, dehydration Animals treated with endotoxin exhibited different behavioral changes post-inoculation in comparison to the control animals as listed in table(1) .There were clear dose dependent effect in response to diarrheal therapy manifested at the end of therapeutic period .including complete recovery in T2 group even better than cipro. while T1 group showed better response but

not complete recovery because of very virulent E.coli inducing diarrhea in all experimental groups that led to 100% mortality in all DMSO group goats after few days from the end of treatment periods for 14 days because of deterioration of health due to profuse diarrhea and dehydration . Ciproflxacin is recommended antibiotic therapy in bacterial enteritis.chosen because fluoroquinolones, produced 99% reduction inE.coli bacterial count, also prevented the emergence of resistant organisms,(8) The results of T1&T2 could be attributed to D.innoxia extract composition of secondary metabolites like;alkaloids,flavonid,phenols,tannins, saponines,steroids, and glycosides, since these secondary metabolites have reported to have antibacterial

activity with different mechanisms of action (9). This is interesting results since one of the favorable character of anti-diarrhoeal drug its analgesic and anti-inflammatory effects (10), as well as astringents and antispasmodic effects (11).

Body weight/ Serial measurements of body weight, provide the best indication of hydration status, especially when fluid loss is acute, as in this study. This statement is similar to (12) that reported the percentage decrease in body weight was assumed to be equal to the degree of dehydration, because weight loss in this preruminant goat diarrhea model approximated to the decrease in extracellular fluid volume. Weight loss from under nutrition was considered minimal. Watery diarrhea lead to dehydration, and that cause weight loss initial body weight, within a 48-hour period. Goats had clinical evidence of severe dehydration at that time, as measured by extent of enophthalmos, and prolonged cervical and thoracic skin-tent duration. Most goats with severe dehydration and diarrhea have cold extremities because of poor peripheral perfusion.

Body temperature/The results of treatment in T1 and T2 showed more decrease in fever when compared with ciprofloxacin group, this may be due to the *D. innoxia* have antipyretic effect in its active secondary metabolites of *D. innoxia* extract plant, like flavonoid, saponine, tanine, alkaloids, and resin that in agreement with (13) who reported that secondary metabolites of plant like saponine and flavonoid were present with anti-inflammatory and antipyretic effects as well they have analgesic and anti-allergic activities. The superiority of *D. innoxia* extract formulae versus ciprofloxacin

treatment in reducing feverish body temperature after *E. coli* infection was due to the lack of ciprofloxacin of such antipyretic effect, of the multi active metabolites present in plant extract formulae. The presence of enough active ingredient in hydroalcoholic extract of *D. innoxia* leaves like flavonoids, phenols, Alkaloids and steroids that reported to have analgesic as well as anti-inflammatory effect. Probably other substances in relieving pain also have antipyretic effects. (15).

Respiration rate/The high ratio of alkaloid components in leaves of *Datura innoxia* when it compared with other parts of the plant. In addition to that, in this study extracted leaves were taken from vegetative, young leaves of plant which according to (14). Tropane alkaloids act as competitive blockers at muscarinic cholinergic receptors with different specificity for individual receptor subclasses. Symptoms associated with tropane alkaloids include dryness of the mucosa in the upper digestive and respiratory tract, alterations in the heart rate and central nervous effects such as irritability, ataxia, seizures and respiratory depression. So it is hydroalcoholic leaves extract contain some active ingredients with narcotic effect as well as other reported active ingredients with non-narcotic analgesic effect like flavonoids, tannin, alkaloid, phenols and glycosides. (15).

Rectal bacterial count/ The results of bacterial counts at the end of treatment were indicative of virulence of *E. coli* isolated that cause deterioration of healthy status in all DMSO group goats ended with their death after 2 week. pharmacological and therapeutically activity of *Datura innoxia* extract plant have antimuscarinic

effect causing clearly antispasmodic and antidiarrheal(16) .The body defense mechanism cannot overcome such virulent infection of E.coli O:157 isolate as reported with other bacteria. (7).The superiority of extract formulae over ciprofloxacin especially in high dose (37.5mg/kg) were possibly due to antispasmodic , anti-inflammatory ,antipyretic,as well as analgesic, astringent and antimicrobial effect of extract formulae of its active metabolite like ;flavenoids ,phenols .tannins ,saponines ,alkaloids and other phytochemical compounds.that were useful to over come other symptoms that act as stress for bacterial infection cause diarrhea ,colic,pains inflammation ,GIT,irritation as well as the antibacterial effect that shared with ciprofloxacin effect. (15).

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