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Original Article

Investigation of the Therapeutic Efficacy of Sepiolite in Neonatal Calf Diarrhea

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The objective of the present study was to determine the therapeutic effectiveness of orally administered sepiolite on neonatal calf with diarrhea. The study was conducted on fifty 1-30 days old public owned calves (40 animals for experiment and 10 animals for control groups), in Afyonkarahisar region, Turkey. Sepiolite was administered orally to all animals in the experiment group for 30 days, once daily at a rate equal to 2% of their total food intake weight. All studied animals were examined for their clinical, hematological, immunological and blood biochemical parameters, in addition to native examination of feces for Eimeria, Cryptosporidia, Giarda protozoa; rota and corona viruses and *E.coli* using SRID kits before and after sepiolite intake on days 1, 2, 3 and weeks 1 and 2. The treatment with sepiolite had a significant effect on the body weight and the treatment of diarrhea. No toxic effects were observed. The data obtained from this study indicate that oral intake of sepiolite in calves with diarrhea might contribute to the prevention of economic losses due to diarrhea.

Keywords: Sepiolite, calves, diarrhea, treatment

It has been reported that bacteria such as *E. coli, Salmonella* spp., *Cl. perfringens, Camphylobacter jejuni* and *Chlamydia* spp., viruses such as rotavirus, coronavirus, adenovirus, parvovirus, astrovirus, calicivirus, parasites such as coccidia, cryptosporidium, giardia, *Neoascaris vitulorium*, care and nutrition disorders, as well as enzyme deficiency, have a role in the etiology of the diarrhea in calves (1-3). As the use of antibiotics may cause bacterial resistance against antibiotics (4-6), therefore scientists has focused on new products and priming methods more effective against pathogenic factors and which may be alternatives for antibiotics and improve the health and productivity (7-10). The most accentuated product class for this purpose is idle clay minerals (11-12).

Sepiolite ($Mg_8Si_{12}O_{30}(OH)_4(OH_2)_4{\cdot}8H_2O$) is a natural clay mineral which belongs to paligorskite group. |It has an alkaline structure and anti-acid properties due to the presence of magnesium hydrosilicate, a gastrointestinal protector. It is also an antibacterial absorbent with antidiuretic effects, and can be used orally or topically (13-15). Sepiolite is a substance which has a high cation change capacity at 15 meq/100 g level (16) which increases the viscosity of mucus, preventing the degradation

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of the glycoproteins in the mucus and decreasing the pepsin effect (17-18).

The purpose of this study was to investigate the therapeutic effect of sepiolite which is a new and natural agent for the treatment and prophylaxis of calf diarrhea that causes losses in terms of productivity and economics.

Materials and methods

Animals

This study was conducted on 50 neonatal 1-30 days old public owned dysenteric calves (40 animals for experiment and 10 animals for control groups) in the province of Afyonkarahisar in Turkey. While the animals in the control group (C) were fed routinely, the animals in the study group (S) were given sepiolite at a rate of 2 % of the total daily nutritients in addition to the routine food intaken. This study was conducted within the frame of ethical rules of the Afyon Kocatepe University Animal Experiments Ethics Committee with AKUHADYEK 14-16 reference number.

Application of sepiolite

The calves were given sepiolite orally at a weight corresponding to 2 % of the total food given to them 1-2 times a day. For this purpose, the food was weighed and necessary amounts of sepiolite was mixed with water, and the calves drunk it from bottles. Catheters were used for the calves which did not drink from the bottle, to enable them to take sepiolite at the same rate.

Infection evaluation

All animal feces were examined for Eimeria, Cryptosporidia, Giarda protozoa; rota and corona viruses and *E.coli* using SRID kits before and after sepiolite intake on days 1, 2, 3 and weeks 1 and 2.

Hematological, biochemical and immunological evaluation

Hematologic and blood biochemical and immunological parameters were measured for all calves included in the study, using suitable methods on the 1st, 2nd and 3rd days and at the end of the 1st and 2nd weeks before and after the study. Values of measured variables are indicated as mean± standard deviation.

Statistical analyzes

Descriptive statistics (dispersion, average, standard deviation, standard error, etc...) were primarily included in the research. In addition, single direction variance analysis (one-way ANOVA) and Duncan test were applied in order to test the differences between the groups. Moreover, variance analysis (repeated measures ANOVA) was used for repeating measurements in the comparison of the measurements in different times for the same individuals. The significance level was determined as 0.05 in the analyzes made within the research and SPSS 18.0 for Windows package software was used for the analysis of the data.

Results

Clinic examination findings

Although the body heat of the practice group was within the reference limits on the repeating days, the study group's body heat (40.50± 3.40 ºC) was statistically higher than the control group (38.40± 0.40 ºC) before the application ($P< 0.05$). However, no significant difference was observed between them in the measurements on the 2nd day after the application and later on. Also, the heart beat frequency of the study group (119.50± 7.60) was significantly higher than the control group (88.40± 6.4) ($P< 0.05$), but this high level returned to normal by the third day following the application. Similarly, although the respiration frequency of the study group before the application (72.50± 6.30) was significantly higher than the control group (39.00± 2.80) ($P< 0.05$), these values approached normal rates in the advancing days.

Hematological indices

The statistical evaluation of the hematological indices obtained from this study is shown in Table 1. The white blood cells (WBC) numbers on the 1st, 2nd and 3rd days before and after the application were significantly higher than the control group (P< 0.05), but statistically important differences could not be determined between the study and control groups on the 1st and 2nd week after the application (P> 0.05). The red blood cells (RBC) numbers of the study group before the application (5.40± 1.00 m/mm3) was statistically lower than the control group (P< 0.05), but this low level increased with time and no statistically significant difference was observed (P> 0.05) between the study group's RBC (7.20± 2.00 m/mm^3) and the control group (7.40± 1.30 m/mm^3) by the 1st week after sepolite application. Moreover, RBC numbers showed significant differences (P< 0.05) in terms of time periods of measurements, with a continuous increase with time.

The hematocrit (HCT) level of the study group before the application (50.20± 4.40%) was statistically higher than the control group (36.60± 2.80%) (P<0.05). This high level was observed until the 3rd day post-application, after which the study group's HCT (37.00± 4.10%) approached the control group's values (36.20± 3.00%) and no statistically significant difference was observed between them (P> 0.05). Contrary to the HCT, the hemoglobin level of the study group before the application (6.40± 2.10 g/dl) was statistically lower than the control group (9.20± 1.30) (P<0.05).

Metabolic profiling

The statistical evaluation of the metabolic profile findings is shown in Table 2. The assessment of aspartate aminotransferase (AST) enzyme levels of the study group on the 1st and 2nd days before and after the application revealed statistically significant higher levels in comparison with the control group (P< 0.05). Similarly, statistically significant higher lactate dehydrogenase (LDH) enzyme levels were found in the study group in comparison with the control group (P< 0.05) during the 1st week before and after the application in all examined time

Table 1. Hematological indices of the control and study groups at different time periods

Time	Group	WBC (k/mm^3)	RBC (m/mm^3)	HB (g/dl)	HCT %	MCV(fl)	MCH (pg)	MCHC (g/dl)
Before the application	**C**	5.22±1.40^c	7.60±0.80^a	9.20±1.30^a	36.60±2.80^d	48.15±1.40^e	12.10±1.16^c	25.13±0.80^a
	S	7.40±2.20^a	5.40±1.00^c	6.40±2.10^d	50.20±4.40^a	92.96±4.30^a	11.87±2.20^c	12.74±0.56^d
After the application 1st day	**C**	5.60±0.80^c	7.30±1.4^a	9.50±2.30^a	35.90±2.80^d	49.17±2.18^e	13.01±0.98^b	26.46±1.40^a
	S	6.80±1.00^b	5.65±1.30^c	7.30±1.50^c	47.20±5.20^b	83.53±4.00^b	12.92±0.84^b	15.46±0.80^c
AA 2nd day	**C**	5.16±1.22^c	7.26±1.90^a	9.10±1.20^a	35.98±3.50^d	49.55±2.90^e	12.53±0.62^b	25.30±1.14^a
	S	6.20±1.10^b	6.00±1.40^b	8.90±2.04^b	42.40±3.81^c	70.66±3.20^c	14.83±1.08^a	20.99±1.20^b
AA 3rd day	**C**	5.50±1.730^c	7.50±1.80^a	9.40±1.08^a	36.20±3.00^d	48.26±2.05^e	12.53±1.20^b	25.98±1.06^a
	S	5.90±1.44^c	6.50±2.60^b	9.63±1.80^a	37.00±4.10^d	56.92±2.44^d	14.81±1.05^a	26.02±1.25^a
AA 1st week	**C**	6.00±1.20^b	7.40±1.30^a	10.00±2.30^a	36.90±3.60^d	49.86±3.10^e	13.51±0.79^b	27.10±0.60^a
	S	5.90±1.70^{bc}	7.20±2.00^a	9.70±2.20^a	37.00±3.40^d	51.38±1.07^e	13.47±0.30^b	26.21±0.82^a
AA 2nd week	**C**	5.80±1.90^c	7.30±1.20^a	9.70±2.20^a	36.40±3.10^d	49.86±2.24^e	13.28±0.49^b	26.64±0.60^a
	S	5.90±1.56^c	7.40±1.00^a	9.80±2.30^a	36.80±3.20^d	49.72±3.10^e	13.24±0.30^b	26.63±0.50^a

WBC: white blood cells; RBC: red blood cells; HB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; BA: before sepiolite application; AA: after sepiolite application; C: control; S: study. Data are expressed as mean± SD. ^{a,b,c,d,e} Different letters on the same column indicate that the difference between the indices measured at different time points in the control group was significant (P< 0.05).

Table 2. Metabolic profile of the control and study groups at different time periods

Time	BA		AA 1st day		AA 2nd day		AA 3rd day		AA 1st week		AA 2nd week	
Group	S	C	S	C	S	C	S	C	S	C	S	C
AST (IU/L)	110.30±8.10^{a}	60.42±6.50^{e}	94.28±10.20^{b}	60.45±27.00^{e}	88.64±8.40^{c}	59.60±6.80^{b}	80.40±8.50^{d}	61.03±5.20^{e}	62.30±6.44^{e}	59.08±4.20^{e}	60.75±5.80^{e}	59.65±4.40^{e}
LDH (IU/L)	764.30±106.50^{b}	518.27±44.00^{e}	818.20±100.46^{a}	540.74±60.00^{e}	740.98±76.00^{c}	550.60±40.73^{e}	614.30±82.44^{d}	534.20±34.00^{e}	560.30±55.50^{e}	541.68±43.20^{e}	560.20±36.40^{e}	543.76±33.40^{e}
GGT (IU/L)	348.90±80.45^{c}	270.30±45.40^{e}	415.10±96.90^{b}	280.20±40.15^{e}	444.48±89.24^{a}	369.40±68.50^{c}	415.80±72.94^{b}	337.60±50.00^{d}	418.64±72.63^{b}	324.50±66.34^{d}	400.66±74.18^{b}	323.54±60.48^{d}
GLU (mg/dl)	103.60±11.36	105.50±12.40	103.740±10.20	104.50±14.37	104.24±13.40	103.44±13.22	105.35±1436	105.64±12.00	106.45±16.41	106.50±15.44	106.32±13.14	105.50±13.21
TP (g/dl)	4.37±0.60	4.34±0.08	4.40±0.50	4.04±0.20	4.96±0.30	4.00±0.80	4.52±0.30	4.80±0.50	4.66±0.13	4.10±0.36	4.70±07.53	4.50±0.46
ALB (g/dl)	1.60±0.04^{b}	2.74±0.05^{a}	1.34±0.03^{b}	2.88±0.02^{a}	1.67±0.04^{b}	2.98±0.04^{a}	2.30±5.32^{a}	2.97±0.09^{a}	2.84±0.05^{a}	2.98±0.02^{a}	2.84±0.07^{a}	2.96±0.06^{a}
BILT (mg/dl)	0.66±0.04	0.66±0.03	0.68±0.05	0.65±0.04	0.66±0.02	0.68±0.03	0.68±0.04	0.69±0.02	0.68±0.03	0.66±0.04	0.69±0.04	0.65±0.04
TRIG (mg/dl)	29.62±4.86	29.40±3.20	29.04±5.20	28.60±4.45	29.03±5.46	28.88±5.04	29.16±6.02	28.76±5.20	29.89±4.72	28.90±5.64	29.03±5.34	28.98±5.74
URE (mg/dl)	27.60±4.72^{a}	20.68±3.70^{c}	27.52±6.45^{a}	20.16±4.50^{c}	25.40±4.06^{ab}	23.09±4.60^{b}	23.20±4.00^{b}	22.70±3.85^{b}	23.18±3.44^{b}	22.70±3.20^{b}	23.00±4.65^{b}	22.82±4.30^{b}
CREA (mg/dl)	2.70±0.06^{a}	1.22±0.01^{b}	2.45±0.04^{a}	1.29±0.05^{b}	2.07± 0.05^{a}	1.37± 0.02^{b}	2.00± 0.03^{ab}	1.21± 0.00^{b}	1.67± 0.04^{b}	1.37± 0.04^{b}	1.32± 0.04^{b}	1.27± 0.04^{b}

AST: aspartate aminotransferase; LDH: lactate dehydrogenase; GGT: gamma-glutamyl transferase; GLU: glucose; TP: total protein; ALB: albumin; BILT: bilirubin total; TRIG: triglyceride; URE: urea; CREA: creatinine; BA: before sepiolite application; AA: after sepiolite application; C: control; S: study. Data are expressed as mean± SD. ^{a,b,c} Different letters on the same column indicate that the difference between the indices measured at different time points in the control group was significant (P< 0.05).

periods. Regarding gamma-glutamyl transferase (GGT) enzyme, the control group showed a statistically significant higher level in comparison with the study group (P< 0.05), at all measurement time periods. The albumin (ALB), urea (URE) and creatinine (CREA) levels of the study group were significantly lower than the control group (P< 0.05) on the 1st and 2nd days before and after the application, but no significant differences were observed between the groups after the 3rd day post-application (P> 0.05). Concerning the levels of other biochemical indices such as total protein (TP), bilirubin total (BILT), and triglyceride (TRIG), no statistically significant difference was observed between the control and study groups at all time points (P> 0.05).

The IgG and IgA levels of the animals in the control and study groups are shown in Table 3. The IgG levels of the study group animals in the 1st, 2nd and 3rd days and in the 1st week before and after the sepiolite application were significFantly lower than the control group for the same time periods (P< 0.05). However, both groups showed similar IgG levels at the 2nd week post-application. On the other hand, significant differences in the 1st, 2nd and 3rd days and in the 1st week before and after sepiolite application (P<0.05) and no significant difference in the last week (2nd week) after sepiolite application (P> 0.05) were observed in the study group. Moreover, no difference in IgG levels was observed during the entire evaluation time period in the control group (P> 0.05).

Regarding IgA levels, no statistically significant difference was observed between the 2 groups at all time periods (P> 0.05) although a significant time dependent increase of IgA levels was observed (P< 0.05).

Infection profiling

Rotavirus, coronavirus, cryptosporidium, *E. coli,* and *Eimeria spp*. were found at the rates of 25% (10/40), 15% (6/40), 12.5% (5/40), 45% (18/40) and 10% (4/40), respectively in neonatal dyesenteric

Table 3. The IgG and IgA levels of the control and study groups at different time periods

Time	Group	IgG (U/L)	IgA (U/L)
BA	**C**	1609,14±100,00[a]	68,10±60,85[c]
	S	680,98±100,42[e]	68,30±60,04[c]
AA 1st day	**C**	1611,24±110,03[a]	67,47±60,34[c]
	S	690,02±90,76[e]	67,60±70,20[c]
AA 2nd day	**C**	1604,62±120,32[a]	68,02±60,70[c]
	S	970,07±80,00[d]	68,62±45,41[c]
AA 3rd day	**C**	1632,34±120,65[a]	69,13±67,05[c]
	S	1011,11±90,90[c]	70,50±72,58[c]
AA 1st week	**C**	1615,96±103,00[a]	82,64±68,97[b]
	S	1413,30±93,30[b]	81,36±76,19[b]
AA 2nd week	**C**	1615,06±138,08[a]	108,64±80,97[a]
	S	1614,80±145,56[a]	109,36±76,19[a]

BA: before sepiolite application; AA: after sepiolite application; C: control; S: study. Data are expressed as mean± SD. [a,b,c,d,e] Different letters on the same column indicate that the difference between the indices measured at different time points in the control group was significant (P< 0.05).

calves younger than 30 days. However, *Giardia spp*. could not be found in any of the tested animals.

Discussion

The neonatal calf diarrhea is commonly encounterd in cattle breeding and causes significant economical losses. Diarrhea is the most important mortality cause in the first 30 days during neonatal period in which calf losses are most excessive. Clay minerals may affect pathological factors responsible of calf diarrhea, and toxins produced by the infectious factors like *E. coli*, and influence the occurrence and severity of the illness (2, 12). Hence, it has been reported that sepiolite prevents the inflammation of the digestive system, and its addition to the food facilitates the absorption of the mycotoxins, heavy metals and other toxins present in the digestive system and makes important contributions to the recovery process (8, 19). Moreover, due to its its surface structure, sepiolite can exert protective effects by connecting the bacteria and toxins. It also has a high capacity of absorption due to its components, holding the water in the intestines because of its metal hydroxyl groups, and protecting the mucosa because of the alkaline minerals such as magnesium and aluminium it contains (13-14, 20). Furthermore, it can stick to the mucosa and increase the viscosity of mucus, preventing the degradation of the glycoproteins in the mucus and decreasing the pepsin effect (17-18, 21). It has been suggested that sepiolite can have effects in preventing gastric ulcer by absorbingthe H+ ions and decreasing the acidity of the stomach (10, 22). The absorption capacity of different clay types is directly related to the structure of their particules, their surface characteristics and their ion change capacity (23-25).

Sepiolite is an alternative substance which has antibacterial and antiprotozoal effects on pathogens beside its many uses (23). Hence, clinoptilolite which has metal oxide ability like sepiolite has antibacterial effects on *Escherichia coli* and *Staphylococcus aureus* and strong antiprotozoal effects on *Paramecium caudatum* and *Euplotes affinis* after a short time (1 h) (23). Similarly, Magana et al. (26) reported that sepiolite was quite effective in protozoa *Entamoeba histolytica* treatment and did not cause side effects. Silva et al. (19) proved that sepiolite had anti-inflammatory effects beside its antibacterial properties. In a study conducted on pigs, it was reported that the addition of sepiolite to their food decreased by 50% the diarrhea cases besides increasing the live weight productivity (22). Studies on chicken whose food was mixed with aflatoxine at µg/kg level and who were given sepiolite simultaneously, showed that toxicosis did not form, and their aflatoxine connecting capacity was ≥ 99 ± 1 % when compared to medical carbon. Moreover, sepiolite addition caused a significant increase of zootechnical parameters (16). Orally taken sepiolite has been reported to be dissolved in the intestines after a short time and has important effects on ion change besides its antibacterial, enzyme stabilizing, toxine and heavy metal absorption abilities, colloidal effects and productive characteristics (13-14, 20, 23-25, 27). Electrolyte losses propound vital importance with liquid losses in neonatal calf diarrhea. Hence, sepiolite is a substance which has high cation change capacity at 15 meq/100 g level (16).

In the present study, significant increases were observed in the live weight gains in the calves whose rations were mixed with sepiolite at a rate of 2 %. In the same way, it was reported that the sepiolite added to pig rations leaded to significant increase in carcase weight because it increased ammoniac absorption and protein production (28-29). In the present study, the analysis of hematological parameters showed that high WBC levels obtained in the beginning of the illness was mostly due to

etiological factors but no important difference remained between the study and control groups in terms of WBC, RBC, MCV, MCH and MCHC levels with the recovery of the diarrhea following the sepiolite administration (P> 0.05). These results indicate that sepiolite does not affect the hematological paramaeters negatively, on the contrary, it makes important contributions for these parameters to reach normal levels. These findings are similar to the study conducted by Duan et al. (30).

Although it was reported that 10 mg/mL sepiolite with large fibers may cause erythrocyte hemolysis in sheep (31), however, 150 µg/mL sepiolite with short fibers (90 % shorter than 2 µm) does not cause toxic effects in mice, and sepiolite with longer fibers (90 % > 4 µm) may cause an increase of peritoneal macrophages and LDH enzyme levels. The lenght of the sepiolite mined in Turkey and Spain was reported to be shorter than 6 µm, which is accepted as a nominal value and do not form any toxic effect (32-33). Hence, in the present study, no increase of the blood cells concentration was observed with phagocytosis ability. However, no significant increase of the level of enzymes and other parameters indicator of toxication was found. Contrarily, the use of sepiolite was accompanied by a normalization of blood serum enzymes and other high parameters and a gradual recovery of diarrhea. These data suggest that the Turkish sepiolite with 3 % carbon content does not cause toxication besides its important curative effects in calf diarrhea. However, researches conducted in rats which were given intraperitoneal Chinese sepiolite (fiber length between 1-100 µm with long fiber) and Turkish and Spanish sepiolite (fiber diameter shorter than 6 µm) indicated that mesotheliomas formed, and no peritoneal tumor incidence increase was observed (33-35).

In the present study, the evaluation of AST, LDH, ALB, TBIL and GGT which can indicate damage in liver and other organs in calves whose food was mixed with sepiolite at a rate of 2 %, showed no toxic effect of the orally given sepiolite. Other studies have shown that the composition of the compounds given as well as the particule length and the administration way were effective in the toxicity of the substances present in the structure of clay (36-37). Although there is no study reporting the carcinogenic effect of the orally given sepiolite, it has been reported that sepiolite shorter than 6 µm does not cause cancer when administrated through other ways (inhalation, intrapleural, subperitoneal). Only few cancer cases were observed upon intrapleural use of the sepiolite with a fiber structure longer than 6 µm (16). In a study conducted on mice, it was reported that the sepiolite from China (with 1-100 µm fiber length) caused peritoneal mesotheliomas increase, while sepiolite from Turkey did not have this feature or any carcinogenic effect (38). *In vitro* genotoxicity studies indicated that the fibers with 2 µm length in average did not alter DNA synthesis in rat hepatocytes (39), and the sepiolite containing long fibers (> 20 µm) did not cause any structural change in hamster lung cells chromosomes (40). Toxicity studies in rats showed that the glucose and ALP levels increased and the cholesterol level decreased in the male rats which were killed because of sepiolite given for 29 days as a mixture with other clays at a high dose of 5g/kg, and in the female rats sacrificed on the 15th day, the uterus/ovary weight increased, with important changes noticed on microscopical examinations, while it was not possible to conclude about the toxicogenic or carcinogenic effect of sepiolite. The common views accepted about sepiolite is that it should be administred orally, and should have < 6 µm fiber length (16, 41).

Rotavirus, coronavirus, cryptosporidium, *E. coli,* and *Eimeria spp*. were found in 25% to 10% of

dysenteric calves analyzed in the present study. In an etiological study made on neonatal dysenteric calves, *Cryptosporidium spp.*, *rotavirus*, *E. Coli*, *coronavirus*, and *Salmonella spp*. were present at a rate of 52.3 %, 42.7 %, 11.9 %, 7.3 %, and 0.9 %, respectively. It was reported that *Eimeria spp*. was present in 21.9 % to 89.8 % of cases of first month diarrhea in the calf (42).

IgG and IgA levels in the present study were relatively low in the beginning, but increased after sepiolite application. These increases support the findings of other researchers who reported that the clay added to the rations of the pigs caused immunoglobuline absorption increase by the intestines, enhanced passive immunity, and prevented diarrhea (43).

In conclusion, 2 % sepiolite added to the food did not cause any side effect in animals, enabled hydradation by holding water as a good absorbent, was quite effective in elimination of the etiological factors, and was effective in preventing diarrhea. It also made positive contribution to the zoothechnical parameters (such as live weight and productivity) increase. The present study investigated for the first time the effects of sepiolite, an important reserve in Turkey, on animal's health. Positive effects of sepiolite added to the food in both the treatment and prophylaxis of calf diarrhea which cause large losses in productivity and economics, were proved. Besides, the use of sepiolite may influence human health indirectly as it prevents antibiotic resistance.

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Conflict of interest

The authors declared no conflict of interest.

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