

Effect of ethanolic extraction of *croton zambesicus* fruit on some biochemical parameters

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ABSTRACT:

This current study aimed to evaluate the *C. zambesicus* extracts were harvested in Sudan, on biochemical parameters (Glucose, total protein, albumin, triglyceride, cholesterol, urea, and creatinine, ALT, AST and ALP) and toxic effects in albino male rats compared to representing control.

Thirty five male albino rats with initial weights ranging from 100-150 g were used in this study. Ethanol 70% for 72 hours at room temperature with occasional shaking and prepared extract was used for the various tests. Rats' plasma were tested for biochemical parameters. The data were subjected to one –way analysis of variance (ANOVA).

Different groups were treated with ethanolic extract.

Glucose, T. protein, AST and ALT they were increased in experimental group B 1 while the remainders they were decreased. In experimental group C 1 Glucose, T. protein, Albumin and AST they were increased, the remainders were decreased. In experimental group D 1 Glucose, T. protein, Albumin, AST, ALT, and ALP they were increased, while the remainders they were decreased.

In experimental group B2 all parameters they were decreased, except AST it was increased. Albumin, triglycerides, creatinine, ALT and ALP in the group C2 were decreased, while Glucose, T. protein, Urea and AST were increased. Cholesterol, AST, ALT and ALP in the group D2 were increased, while Glucose, T. protein, triglycerides, urea and creatinine were decreased.

Key words: *Croton zambesicus*, T. protein, triglycerides, urea, Sudanese, Ethanolic extract.

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Introduction

Croton zambesicus Muell. Arg. (syn. *C. amabilis* Muell. Arg.; *C. gratissimus* Burch) (Euphorbiaceae) and commonly known as “Um Glegla” in Sudan. Is a plant native in the Africa including Sudan and it is one of the plants that have wide application in African folkloric medicine. The plant is used by the Sudanese local people to treat fever associated with malaria, cough, and to relieve menstrual pain (1), the GC/MS chromatogram of the

C. zambesicus essential oil revealed the presence of 58.86% monoterpenes and 23.28% sesquiterpenes. Predominant mono terpenoids were m-cymene (21.56%), linalool (7.21%) and p-mentha -1-en-8-ol (5.28%) while the predominant sesquiterpenoids were alpha-guaiene (3.55%) and C15H24 (3.23%) (1). the lack of records of previous chemical and toxicological studies of the

fruit extracts stimulated us to initiate them through the present research work

Medicinal uses of *C.zambesicus* in Traditional medicine the leaf decoction is used in Benin as anti-hypertensive and anti-microbial and in parts of Nigeria as antidiabetic and malarial remedy (2). The roots are used as antimalarial, febrifuge and anti-diabetic in Niger Delta region of Nigeria (2). The root is also used in Sudan for menstrual pain (3). The plant fruit is used by the Sudanese local people to treat fever associated with malaria, cough and to relieve menstrual pain. (1)

Pharmacological activity and bioactive compounds of Croton: Numerous research works have proven its uses of *C. zambesicus* beyond the ethno medicinal ones in experimental animals. Studies have revealed its were as cytotoxic, vasorelaxant, antiulcer genic, anti-inflammatory, antipyretic, antimicrobial, antiulcer and, anti-convulsion, plasmodial and anti-diabetic activities.

Chemical constituents of *C.zambesicus* extracts the survey of literature revealed that *C.zambesicus* is enriched with many phytochemicals like (triterpenes, steroids, essential oils, fatty acids, etc.) The literature about the *C. zambesicus* indicates a high variability in the chemical composition of the essential oils of leaves, stem bark, flowering tops, fruits within the same plant species from different collections. Usman et al., reported that *C.zambesicus* leaves of the plants growing in north central Nigeria consists mainly of limonene, gamma-terpinene, beta-pinene, neral and geranial (4). Menut et al., have investigated the leaf oil of central African grown *C.zambesicus*

(5). The principal constituent of the oil were p-cymene, linalool and beta-caryophyllene. Caryophyllene oxide, beta-caryophyllene, alpha-copanene, linalool and beta-pinene have been identified as the major constituents of Benin grown *C.zambesicus* (6). Fekam et al., investigated the leaf and root and stem bark oils of Cameroonian grown *C. zambesicus*, their report showed that the oils from the leaves and stem bark oil consists mainly of monoterpenes, while the root bark oil contained sesquiterpenes (7). The root and stem bark oils contain spathulenol and linalool as the major components, respectively. It is worth mentioning that there was a report on the analysis of *C.zambesicus* flowering tops from Sudan by Mekkawi which reported the major constituents found in the flowering tops oil are pinene, limonene, menthol, carvone, thymol, alpha-humulene and ceisnerolidol (8). The essential oil composition of *C.zambesicus* fruits is described for the first time in the work of EL-Kamali et al., (1). The *C.zambesicus* fruit contain 2-Naphthalenemethanol, decahydro-alpha (6.45%), longifolene – (V4) (6.32%), n-hexadecanoid acid (5.57%) and benzo furan 2, 3-dihydro- (5.05%) as major components in ethanolic extract. A number of diterpenoids from the stem bark of *C.zambesicus* have been reported (Ngadjui et al., Ngadjui et al., Block et al., reported that ent-trachylobane diterpene isolated from dichloromethane extract of the leaves has cytotoxic activity on Hela cells (9, 10 ,11) . Antidiabetic activity of ethanolic leaf extract of *C.zambesicus* was evaluated using alloxan-induced hyperglycemic rats (2). The root extract

has been reported to possess antimalarial and anticonvulsant and ulcer activities (2). The aqueous leaf extract of *C.zambesicus* has been reported to possess anticonvulsant activity in mice and rats (12). The root extract and its fractions exhibit antispasmodial activity (2).

Animals and experimental set-up: Thirty five male albino rats with initial weights ranging from 100-150 g were used in this study .They brought from National Center for Research (NCR), Medical and Aromatic Research Unit, Khartoum, Sudan. All animal housed and reared at the premises of the (NCR) in metal cages (stain less steel wire mesh cage) they will be maintained for one week as an acclimatization period, and maintained under controlled conditions. The temperature of the room was thermostatically controlled at 22-24°C and a 12-hour light/dark cycle was maintained.

Protocol:

Thirty –five animals were randomly divided in to seven groups with each group five rats. The seven groups of rats were subjected to the following oral treatments once a day for **14 and 28days:**

Group (A): Served as normal control was received distilled water

Group (B1): rats were received 1000mg/kg of *C. zambesicus* ethanolic extract **14 days**.

Group (C1): rats were received 500mg/kg of *C. zambesicus* ethanolic extract **14 days**.

Group (D1): rats were received 250 mg/kg of *C.zambesicus* ethanolic extract. **14 days**

Group (B2): rats were received 1000mg/kg of *C. zambesicus* ethanolic extract **28 days**.

Group (C2): rats were received 500mg/kg of *C. zambesicus* ethanolic extract. **28 days**

Group (D2): rats were received 250 mg/kg of *C.zambesicus* ethanolic extract. **28 days**

The experiment was conducted for 28 days during the experiment, readings was taken in 14 days and after 28 days. After 14 days' blood samples were collected from all rats through retro orbital puncture. Rats were anaesthetized in chloroform-saturated chamber. Animal in test and control group were sacrificed on the 28 days. Five micro liters' blood was taken from animal eyes to determine biochemical parameters. The samples were collected into two different containers. The first container was a plain bottle containing no anticoagulant with the sample used for biochemical determination whiles the second container containing. Is an automatic multi-pair blood cell counter for in vitro diagnostic use in clinical laboratory? It performs speedy and accurate analysis of blood parameters and detects the abnormal samples

The effect of ethanolic extract of *C.zambesicus* fruit on biochemical parameters after treatment of rats for 14 days At 1000 mg /kg:

The rats of Group (B1) treated with 1000 mg /kg of fruit ethanolic extract after 14 days, showed no significant change at ($P<0.05$ and $P<0.01$) in Glucose, total protein, albumin, urea, Creatinine , AST and ALP. There was significant change at ($P<0.05$) in Triglyceride and cholesterol, whereas showed significant ($P<0.05$ and $P<0.01$) in ALT .
(Table 1)

At 500 mg/kg: The rats of Group (C1) treated with 500 mg /kg of fruit ethanoic extract after 14 days,

showed significant change at ($P<0.05$ and $P<0.01$) in urea, and showed no significant change at ($P<0.05$ and $P<0.01$) in other biochemical parameters tested except in glucose, triglyceride and ALT whereas showed significant change at ($P<0.05$) to comparison with control. **(Table 2)**

At 250 mg/kg: The rats of Group (D1) treated with 250 mg /kg of fruit ethanoic extract after 14 days, showed no significant change at ($P<0.05$ and $P<0.01$) in all biochemical parameters tested relative to their respective control. **(Table 3)**

The effect of ethanoic extract of *C.zambesicus* fruit on biochemical parameters after 28 days at 1000 mg/kg: Treatment of rats Group (B2) with 1000 mg/kg of fruit ethanoic extract after 28 days, did not produce any significant change at ($P<0.05$ and $P<0.01$) in glucose, total protein, urea, ALT, AST and ALP whereas was exhibit significant change at ($P<0.05$ and $P<0.01$) in albumin, and

were showed significant change at ($P<0.05$) in cholesterol, triglyceride and creatinine compared to control **(Table 4).**

At 500 mg/kg: Treatment of rats Group (C2) with 500 mg/kg of fruit ethanoic extract after 28 days, did not produce any significant difference in all biochemical parameters tested at ($P<0.05$ and $P<0.01$) except were showed significant difference at ($P<0.05$) in triglyceride and urea compared to control **(Table 5)**

At 250 mg/kg: -Treatment of rats Group (D2) with 250 mg/kg of fruit ethanolic extract after 28 days, did not produce any significant difference at ($P<0.05$ and $P<0.01$) in glucose, total protein, albumin, cholesterol, urea, AST, and ALP, and there was significant difference at ($P<0.05$) in triglycerides, creatinine and ALT relative to their respective control. **(Table 6)**

RESULTS

Table 1: Control 1000 mg / kg s of ethanolic extract of *C.zambesicu* after 14days for biochemical parameters in rats serum blood: -

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	Sat p<0.05	Change	Change %
Glucose	47.4± 9.34	56.6 ±10.36	1.47469	0.178528	Ns	Ns	increment	19.41 %
T. protein	70.0 ±6.20	71.2 ± 2.86	0.39265	0.704831	Ns	Ns	increment	1.71 %
Albumin	20.2 ±2.77	9.8 ± 1.30	-0.29173	0.777915	Ns	Ns	decrement	1.98 %
Triglyceride	105.2±8.6	87.6 ± 9.53	-3.06377	0.015494	Ns	S	decrement	16.73 %
Cholesterol	73.2±8.56	54.4 ±14.15	-2.54193	0.03606	Ns	S	decrement	25.68 %
Urea	37.6±6.35	36.4 ± 4.34	-0.05745	0.95597	Ns	Ns	decrement	3.19 %
Createnine	0.64±0.15	0.56 ±0.15	-1.04257	0.327625	Ns	Ns	decrement	12.5 %
ALT	8.9±2.21	5.0 ±0.55	-3.45119	0.00868	S	S	increment	43.82 %
AST	4.26±0.94	4.52 ±0.41	0.44201	0.670185	Ns	Ns	increment	6.10 %
ALP	107.8±27.67	103.6 ±10.14	-0.2625	0.799569	Ns	Ns	decrement	3.90 %

Table 2: Control 500 mg / kg of ethanolic of *C.zambesicu* after 14 days for biochemical parameters in Rats' serum blood: -

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	47.4 ±9.34	63.2 ±4.97	3.33836	0.010256	Ns	S	increment	33.33%
T. protein	70.0 ±6.20	76.0 ±5.87	1.57027	0.154993	Ns	Ns	increment	8.57%
Albumin	20.2 ±2.77	22.8 ±1.92	1.72189	0.123391	Ns	Ns	increment	12.87%
Cholesterol	73.2 ±8.56	65.6±6.66	-1.56776	0.155573	Ns	Ns	decrement	10.38%
Triglyceride	105.2 ±8.61	95.0±4.85	-2.30748	0.049885	Ns	S	decrement	9.70%
Urea	37.6 ±6.35	25.6 ±2.13	-3.62266	0.006757	S	S	decrement	31.92 %
Createnine	0.64 ±0.15	0.66 ±0.09	0	1	Ns	Ns	increment	3.13%
ALT	8.9 ±2.21	5.58 ±1.70	-2.66549	0.028562	Ns	S	decrement	37.30 %
AST	4.26 ±0.94	5.16 ±1.53	1.1222	0.294327	Ns	Ns	increment	21.13%
ALP	107.8±27.67	83.0 ±33.76	-1.27031	0.239672	Ns	Ns	decrement	23.01 %

Table 3: Control 250 mg / kg of ethanolic extract of *C.zambesicus* after 14 days for biochemical parameters in rats' serum blood:

Parameters	Control	Treatment	T.value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	47.4 ±9.34	54.4 ±6.19	1.39665	0.200047	Ns	Ns	increment	14.77 %
T. protein	70.0 ±6.20	73.6 ±5.81	0.94671	0.371499	Ns	Ns	increment	5.14 %
Albumin	20.2 ±2.77	22.6 ± 1.52	1.69706	0.128133	Ns	Ns	increment	1.19 %
Cholesterol	73.2 ±8.56	66.2 ±7.12	-1.4062	0.197297	Ns	Ns	decrement	9.56 %
Triglyceride	105.2 ± 8.61	99.4±2.3	-1.45455	0.183876	Ns	Ns	decrement	5.51 %
Urea	37.6 ±6.35	31.8 ± 6.26	-1.19257	0.267207	Ns	Ns	decrement	15.43 %
Creatinine	0.64 ±0.15	0.64 ± 0.17	-0.19803	0.847961	Ns	Ns		0 %
ALT	8.9 ± 2.21	11.54 ± .70	1.69266	0.128978	Ns	Ns	increment	29.66 %
AST	4.26 ±0.94	4.5 ±0.49	0.37006	0.72094	Ns	Ns	Increment	5.63 %
ALP	107.8 ±27.67	123±14.16	1.09344	0.306029	Ns	Ns	Increment	14.1 %

Table 4: Control 1000 mg / kg fruit of ethanolic extract of *C.zambesicu* after 28 days for biochemical parameters in rats serum blood:

Parameters	Control	Treatment	T.value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	53.2 ±6.41	44.6 ±5.11	-1.05699	0.321388	Ns	Ns	decrement	16.17 %
T. protein	67.8 ±2.29	64.8 ±1.77	-1.03633	0.330354	Ns	Ns	decrement	4.42 %
Albumin	37.6 ± 0.68	31.8±0.8	-3.80699	0.005185	S	S	decrement	15.43 %
Cholesterol	75.6 ±3.08	65.2 ±2.60	-2.5839	0.032419	Ns	S	decrement	13.76 %
Triglyceride	61.4 ±1.33	54.4 ±2.29	-2.64198	0.029622	Ns	S	decrement	11.40 %
Urea	36.8±1.50	34.8 ± 2.15	-0.76249	0.46765	Ns	Ns	decrement	5.43 %
Creatinine	1.36±0.07	1.04 ± 0.09	-2.59554	0.031838	Ns	S	decrement	23.53 %
ALT	9.6 ±1.29	7.8 ±1.14	-1.04605	0.326111	Ns	Ns	decrement	18.75 %
AST	3.36 ±0.22	6.04 ±0.47	1.29624	0.231035	Ns	Ns	increment	79.76 %
ALP	114.6 ± 12.61	101.6 ± 8.28	-0.86166	0.413952	Ns	Ns	decrement	11.34 %

Table 5: Control 500 mg / kg fruit of ethanolic extract of *C.zambesicus* after 28 days for biochemical parameters in rats serum blood:

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	53.2 ± 6.41	59.8 ± 5.37	0.78896	0.452878	Ns	Ns	increment	12.41 %
T. protein	67.8 ±2.29	69.0 ± 0.89	0.48827	0.638458	Ns	Ns	increment	1.77 %
Albumin	37.6 ± 0.68	36.2 ± 0.49	-1.67332	0.132801	Ns	Ns	decrement	3.72 %
Cholesterol	75.6 ±3.08	74.4 ± 2.71	-0.2926	0.77276	Ns	Ns	decrement	1.59 %
Triglyceride	61.4 ±1.33	53.6 ± 2.25	-2.98677	0.017419	Ns	S	decrement	12.70 %
Urea	36.8±1.50	44.2 ± 2.65	2.42017	0.41256	Ns	S	increment	20.11 %
Creatinine	1.36±0.07	1.08 ± 0.11	-2.087	0.070346	Ns	Ns	decrement	20.59 %
ALT	9.6 ±1.29	7.6 ±1.01	-1.22375	0.255863	Ns	Ns	decrement	20.83 %
AST	3.36 ±0.22	5.48 ±0.32	0.30779	0.7661	Ns	Ns	increment	2.24 %
ALP	114.6 ±12.61	89.8 ±14.78	-1.27642	0.237613	Ns	Ns	decrement	21.64 %

Table 6): Control 250 mg / kg fruit of ethanolic extract of *C.zambesicus* after 28 days for biochemical parameters in rats serum blood:

Parameters	Control	Treatment	T. value	P-value	S at P<0.01	S at P<0.05	Change	Change %
Glucose	53.2 ±6.41	49.2 ±2.60*	-0.57807	0.579119	Ns	Ns	decrement	7.52 %
T. protein	67.8 ±2.29	66.0 ±1.90	-0.60541	0.561687	Ns	Ns	decrement	2.65 %
Albumin	37.6 ± 0.68	37.4 ±0.6	-0.22086	0.830732	Ns	Ns	decrement	0.53 %
Cholesterol	75.6 ±3.08	76.4 ± 3.36	0.17575	0.864858	Ns	Ns	increment	1.06 %
Triglyceride	61.4 ±1.33	54.4 ±1.69	-3.2567	0.011584	Ns	S	decrement	11.40 %
Urea	36.8±1.50	35.6 ± 2.48	-0.41404	0.689723	Ns	Ns	decrement	3.26 %
Creatinine	1.36±0.07	1.08 ± 0.04	-3.34664	0.010131	Ns	S	decrement	20.59 %
ALT	9.6 ±1.29	14.4 ±1.44	-2.49136	0.037443	Ns	S	increment	50 %
AST	3.36 ±0.22	5.36 ±0.58	0	1	Ns	Ns		0 %
ALP	114.6 ±12.61	12.28 ±7.59	0.65229	0.532503	Ns	Ns	increment	8.38 %

Discussion

In general, folk medicines uses plant extracts in tradition without taking into account the toxicity aspect. *C.zambesicus* is uses as medicinal plant in West and Central Sudan to treat malaria, cough, dysentery, fever , convulsions, tonic , antihypertensive and antimicrobial (urinary infections) (1). However, toxicity and phytochemical studies about the fruits/seeds of this plant are rare. Assessment of biochemical parameters can be used to determines the extent of deleterious effect of plant .In the rats that were received 1000mg/kg of *C. zambesicus* ethanolic extract with in 14 and 28 days (B1 and B2) , Triglycerides and cholesterol in this experiment treated with ethanolic extract were decreased to 16.73%, 25.68%, (after 14 days), and decreased to 13.76%, 11.40 % , ,(after 28 days) and they were showed significant result change at ($P<0.05$), respectively, while ALT in group B1 was increased to 43.82% , and was showed significant result change at ($P<0.01$ and $P<0.05$) but it was increased to 37.30 % and showed significant result change at ($P<0.05$). In group B1, Glucose, T. protein were increased to 19.41 % , 1.71%, and decreased to 16.17 % , 4.42 % , (after 28 days) and they were showed no significant result change at ($P<0.01$ and $P<0.05$). Albumin was decreased to 1.98 % with no significant result change at ($P<0.01$ and $P<0.05$) in group B1 , and it was decreased 15.43 % with significant change at ($P<0.01$ and $P<0.05$) (table 4). Urea and creatinine in the experimental treated with ethanolic extract were decreased in groups B1 and B2 to 3.19 % , 5.43 % , 3.19 % and 23.53%, respectively with

significant change. In group C1, Glucose, T. protein were increased to 33.33% , 8.57%, (with significant change at $P<0.05$ for glucose) and increased to 12.41 % , 1.77 % , (in group C2) and they were showed no significant result change at ($P<0.01$ and $P<0.05$). Albumin was increased to 12.87% with no significant result change at ($P<0.01$ and $P<0.05$) in group C1 , and it was decreased 3.72 % with no significant change . (table 5). Urea in the experimental treated with ethanolic extract was decreased in group C1 to 31.92 % with significant change, but was increased in C2 to 20.11 % with significant change at ($P<0.05$) , and creatinine was increased to 3.13% with no significant change,(Table 2) and it was decreased in C2 to 20.59 % with no significant change . In the last group D1 , glucose , T . protein , Albumin, ALT ,AST, and , ALP they were increased to 14.77 % , 5.41 % , 1.19 % , 29.66, 5.63 and 14.1%, respectively with no significant change, but in group D2 , cholesterol , ALT ,AST, and ALP they were increased to 1.06%, 50 % , 59.5 % , and 8.38%, respectively with no significant change for cholesterol , ALT ,AST, and ALP, except for ALT there was a significant change at($P<0.05$)

There are many enzymes such as phosphatases and transferases that are found in the serum which did not originate from the extracellular fluid. During tissue damage, some of these enzymes find their ways into the serum by leakage through disrupted cell membranes (13). This serum enzymes measurement provides a marker in toxicity studies as well as in clinical diagnosis, alkaline phosphatase (ALP) is a marker co-enzyme for the

plasma membrane and often used to assess the integrity of the plasma membrane . Liver damages/infections are characterized by elevated ALP concentration in serum as in experiment , and these may be associated with inflammation and / or injury to hepatic cell (14). Generally, enzymes such as ALT, AST and ALP are marker enzymes for function and integrity of organs such as liver. The enzymes found within organs and tissues are released into the blood stream following cellular necrosis and cell membrane permeability and are used as diagnostic measure of liver damage (15). Furthermore, serum ALT/AST has been used as an index to monitor liver pathology (16). Treatment of rats with 250 mg/kg of fruit ethanoic extract after 28 days, did not produce any significant difference in lipid profile that mean high dose of *C.zambesicus* fruit can cause lipid lower; there was no significant effect on glycemic, and kidney and there was no toxicity all liver enzyme were normal .The administration of *C.zambesicus* fruit caused an obvious alteration in the cholesterol compared to control. This reduction was found to be significant ($P<0.05$). Increase in HDL (high density lipoprotein) ratio is one of desirable of an ideal hypocholestromic agent since the higher the ratio that lowers the atherosclerotic risk. The decrease in cholesterol balance is indicate the total changes in body pools of cholesterol and may be due to the compensatory mechanism such as a decrease in resorption of endogenous cholesterol (17) or an increase in the rate of secretion into intestinal tract. The changes in the lipid profile with *C.zambesicus* fruit treatment may contribute

to decreased incidence of atherosclerosis. *C.zambesicus* fruit may activate the enzyme lipoprotein lipase which uptake cholesterol from blood vessels or enhance lecithin cholesterol acyl transferase in HDL, which has a role in scavenging of cholesterol (18).

Cholesterol is a biochemical parameter that is affected by many factors such as age, sex diet and genetic factors. One of such factors may provide an explanation to the pattern observed in this study, other factors could be attributed to the active components in *C.zambesicus* fruit under question. Coumarin has a role in antihyper lipidemic activity .Potent anti-hyper- lipidemic activity and was found to decrease the plasma Triacylglycerol (TAG) by 55% total cholesterol (TC) by 2% accompanied by increase in HDL. HDL /TG ratio 42% in hyper lipedemic rate to greater degree than some the references statin drug (19). Polyphenol inhibit fat cell formation and increase lipolysis by inhibition of digestion by lipase (in vitro) resulting in decreased lipolysis of Triacylglycerol which may be translated to reduced fat digestion in human and weight loss. Polyphenols inhibit pancreatic lipase and triacyl glycerol absorption (20) and inhibit apoprotein β synthesis in intestine mucosa and inhibit of cholesterol esterification and intestinal lipoprotein production .And inhibit protein in lipoprotein like apo protein then cholesterol level was decreased which prevent cholesterol absorption

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