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## Research Article

# Antioxidant Status in the Brain Cortex of Rabbits Exposed to a Mixture of Energy Drink and *Catha edulis*

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## Abstract

**Background and Objective:** Young and adolescents ingest the caffeinated energy drinks (ED) to enhance their mental and physical performances and improve alertness and concentration alone or by mixing them with other stimulants such as *Catha edulis* leaves. This study aimed to investigate the effect of mixing caffeine and *Catha edulis* on the antioxidants of brain cortices of rabbits. **Materials and Methods:** Three groups of rabbits received *Catha edulis* and caffeine for 35 days at doses of 5 mL kg<sup>-1</sup> and 1.1 mg, respectively, alone or together. The fourth group served as control. At the end of the study rabbits brain cortices were removed and different antioxidant and histopathological tests were performed. **Results:** Reduced activity of catalase and thiol contents were observed following exposure to caffeine and/or *Catha edulis* with changing in the levels of protein, albumin, glucose, cholesterol urea and UA as compared to control group. **Conclusion:** It is concluded that consumption of caffeinated ED and/or *Catha edulis* are responsible of biochemical changes as well as histopathology of the brain cortices of rabbits.

**Key words:** Antioxidant, caffeine, *Catha edulis*, energy drinks

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Despite the raised concern regarding the consumption of energy drinks (EDs) by youth, suggesting no place in the diet of children and adolescents<sup>1</sup>, there is a significant growth of EDs production worth expected to reach USD 61707.5 million by 2021 compared to USD 39760.8 million<sup>2</sup> in 2013. Caffeine is one of the major functional constituents in EDs that provide a stimulating effect and enhancing physical and mental performances<sup>3,4</sup>. The caffeine intoxication includes; restlessness, headaches, nervousness, irritability, anxiety, nausea, vomiting after consumption of as higher doses as 500-600 mg per day<sup>5</sup>. The effect of caffeine on brain physiology is not fully understood where it is reported to act on a number of neurotransmission systems such as dopamine<sup>6-8</sup>. However, Volkow *et al.*<sup>9</sup> showed that caffeine does not act directly on the neurotransmitters and instead acts through increasing their receptors levels or changing the receptors affinity.

Premixing EDs with other stimulants and drinks becomes a habit widespread among adolescent and young adult worldwide<sup>10</sup>. People of east horn of Africa and Yemen widely chew the leave of *Catha edulis* to improve performance, stay alert and increases work capacity. This habit of chewing *Catha edulis* becomes of major concern in countries where the plant is cultivated (East African Horn and Yemen) or in countries where the immigrants from those countries are still having the habit of chewing *Catha edulis* leaves<sup>11</sup>. Cathinone found in *Catha edulis* leaves, is the alkaloid that has stimulating effects upon the central nervous system resulting in mood elevation and euphoria. It has an amphetamine like action and is the main constituent of *Catha edulis* along with cathine<sup>12</sup>. One of the mechanisms reported in *Catha edulis* chewing is production of highly cells-damaging molecules, reactive oxygen species (ROS), affecting the cells biomolecules including DNA, proteins and lipids<sup>13</sup>. Those ROS are associated with many diseases including cancer and neurodegenerative disorders, they are produced normally during metabolic processes or in response to external sources such as environmental or industrial toxins<sup>14</sup>. The presence of free radicals and hence oxidative stress in the serum of *Catha edulis* abusers has been reported<sup>15</sup>. It has been reported that *Catha edulis* consumption responsible for reduction of antioxidant capacities in red blood cells<sup>16</sup>, plasma<sup>17</sup> and saliva<sup>18</sup>. Recently Masoud *et al.*<sup>19</sup> found that mixing both *Catha edulis* and EDs might affect the antioxidant system in the plasma and kidney of rabbits exposed to *Catha edulis* and ED. The habit of mixing both *Catha edulis* and ED has increased among

people in Yemen, this lead to design the present study with the aim to investigate the short term effect of consumption of both EDs and *Catha edulis* leaves in the brain cortices.

## MATERIALS AND METHODS

**Experimental animals:** Male rabbits (0.700-1.350 kg) were purchased and housed in cages in the facility of Faculty of Applied Science, Thamar University, Yemen. They were randomly segregated into four groups each having 6 animals as follows:

- **Control group:** Animals were received food and water *ad libitum*
- ***Catha edulis* group:** Animals were given *Catha edulis* extract (5 mL kg<sup>-1</sup>) orally for 35 days
- **Energy drink (ED) group:** Animals were received ED (5 mL kg<sup>-1</sup>, 1.1 mg of caffeine) for 35 days
- ***Catha edulis*-Energy drink (*Catha edulis*+ED) group:** Animals were received both *Catha edulis* and ED as in groups 2 and 3

A survey distributed to adult *Catha edulis* chewers in Thamar city (the body weight, approximate *Catha edulis* consumption period and quantity and ED type and amount consumed during the *Catha edulis* session have been questioned) to select the doses of both *Catha edulis* and ED. The study carried out between January and May 2014 and was approved by ethical Committee at Chemistry Department, Thamar University, in their meeting number 1 for the academic year 2014 and followed the guidance of animal use and care. At the end of the study, animals were sacrificed, their brain were removed and the brain cortices were rinsed, homogenized in phosphate buffered saline (10% w/v, pH 7.4) and used for measuring biochemical assays. Some brain cortices were kept in formalin (40%) and used for histopathological studies.

### Biochemical assays

**Catalase activity:** Catalase (CAT) was measured according to Luck method<sup>20</sup>. Briefly, the reaction mixture containing of 12.5 mM H<sub>2</sub>O<sub>2</sub> in 0.067 M phosphate buffer (pH 7.0) and supernatant was read at 240 nm for 2 min. Results were expressed as  $\mu\text{moles of H}_2\text{O}_2 \text{ decomposed min}^{-1} \text{ g}^{-1} \text{ protein}$  (molar extinction coefficient of H<sub>2</sub>O<sub>2</sub> is 71 M<sup>-1</sup> cm<sup>-1</sup>).

**Total thiol (T-SH) content:** T-SH levels were quantified in the homogenate according to the method of Ellman<sup>21</sup> as modified

by Sedlak and Lindsay<sup>22</sup>. The reaction mixture of homogenate with phosphate buffer 0.02 M and EDTA (pH 8.2) together with 100  $\mu$ L of 0.01 M DTNB was incubated for 15 min at room temperature and then centrifuged at 1,200 rpm for 5 min. The absorbance was measured at 412 nm and results were expressed as nmoles of T-SH/g protein (molar extension coefficient of DTNB is 13,600  $M^{-1} cm^{-1}$ ).

**Glutathione (GSH) levels:** The GSH level was measured in the in the homogenate according to the method of Ellman<sup>21</sup>. Trichloroacetic acid was added to the homogenate sample and centrifuged at 3000 rpm for 5 min. The supernatant was added to DTNB in 0.1 M phosphate buffer (pH 8.0) and the absorbance was read spectrophotometrically at 412 nm after 2 min. Results were expressed as nmoles of GSH/g protein (molar extension coefficient of DTNB is 13,600  $M^{-1} cm^{-1}$ ).

**Protein thiol contents (P-SH):** P-SH contents were measured by subtracting GSH from T-SH and the results were expressed as nmoles of P-SH/g protein (molar extension coefficient of DTNB is 13,600  $M^{-1} cm^{-1}$ ).

**Other biochemical assays:** Measurement of uric acid, urea, total protein, albumin and glucose concentrations were done using commercial kits (Spinreact, Spain), whereas, cholesterol assay was measured using the kit purchased from Enzopack (India), the Cvs% range between 0.21-1.64.

**Histopathological changes:** Histopathological studies were carried out by performing routine hematoxylin and eosin staining to evaluate the morphological and structural changes in the brain.

**Statistical analysis:** Data were represented as Mean  $\pm$  SD and were analyzed using one way analysis of variance ANOVA test followed by Newman-Keuls *post hoc* test. Differences between groups were considered significant when  $p < 0.05$ . All analysis were performed using the sigma-stat software (version 3.5).

## RESULTS

**CAT activity:** Figure 1 shows the effect of mixing *Catha edulis* with ED on CAT activity of rabbit brain cortices. Sharp decrease in the CAT activity was seen following short term exposure to *Catha edulis* extract and/or ED in the brain cortex of rabbits as compared to control animals. The reduction was more in *Catha edulis* group as compared to other treated animals ( $p < 0.05$ ).

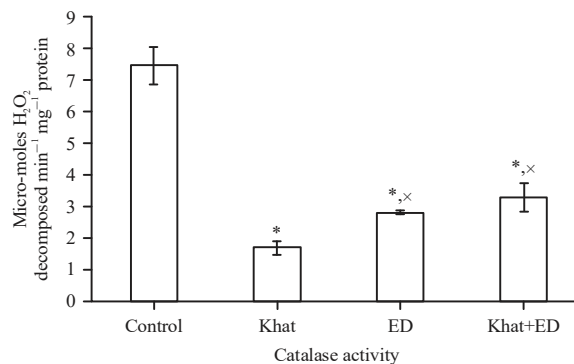


Fig. 1: CAT activity in the rabbit cortex of control, *Catha edulis*, ED and *Catha edulis*+ED groups

Results expressed as mean  $\pm$  SD, \*\* $p < 0.05$  considered significant compared to control (n = 6)

Table 1: Thiol contents in the brain cortex of control, Khat, ED and Khat+ED in rabbits (nmoles/g protein)

Groups/tests	GSH	P-SH	T-SH
Control	2.36 $\pm$ 0.02 <sup>a</sup>	22.24 $\pm$ 5.78 <sup>a</sup>	24.60 $\pm$ 5.8 <sup>a</sup>
Khat	1.83 $\pm$ 0.14 <sup>b</sup>	13.13 $\pm$ 2.14 <sup>b</sup>	14.96 $\pm$ 2.28 <sup>b</sup>
ED	1.06 $\pm$ 0.28 <sup>c</sup>	3.44 $\pm$ 0.17 <sup>c</sup>	4.50 $\pm$ 0.45 <sup>c</sup>
Khat+ED	1.61 $\pm$ 0.14 <sup>b</sup>	6.01 $\pm$ 0.03 <sup>d</sup>	7.62 $\pm$ 0.17 <sup>d</sup>

Results are expressed as Mean  $\pm$  SD, n = 6. Superscript alphabets are significantly different from their corresponding group ( $p < 0.05$ )

**Thiol contents:** Table 1 describes the changes in thiol contents of rabbit brain cortices following 35 days administration of both *Catha edulis* extract and/or ED. Similar pattern of results was seen in T-SH and P-SH contents as in CAT activity where, administration of *Catha edulis* and/or ED showed a significant decrease, with no significant changes between ED and *Catha edulis*+ED groups. However, GSH levels were significantly reduced in all groups as compared with control animals with no significant changes between *Catha edulis* group and ED group (Table 1).

**Protein and albumin levels:** Figure 2 shows the alterations in the protein and albumin levels of rabbit brain cortices following administration of *Catha edulis* extract and/or ED. The protein level was increased significantly following either exposure to ED or to both ED and *Catha edulis* as compared to control animals (Fig. 2a,  $p < 0.05$ ) in the brain cortices of rabbits. A significant reduction in albumin levels was seen in the brain cortices of *Catha edulis*+ED group as compared to control (Fig. 2b,  $p < 0.05$ ).

**Uric acid and urea:** Changes in uric acid (UA) and urea contents of brain cortices of rabbits in response to consumption of *Catha edulis* extract and/or ED were shown in Fig. 3. The UA levels were reduced in ED group only as

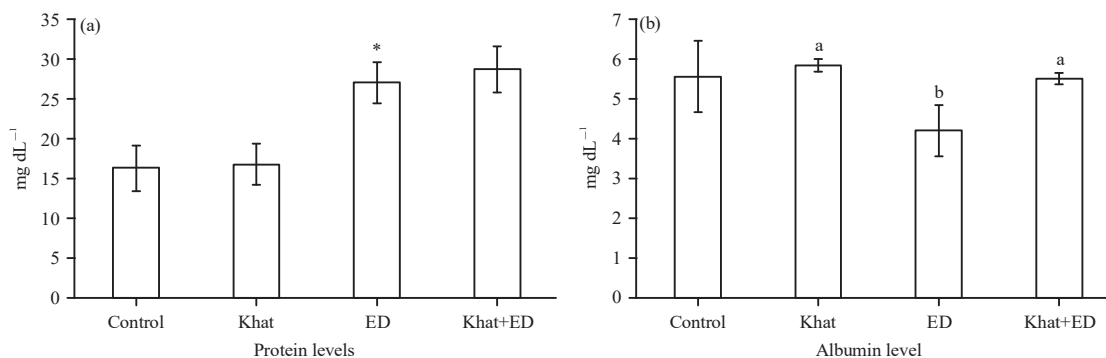


Fig. 2(a-b): (a) Protein level and (b) Albumin level in the rabbit cortex of control, *Catha edulis*, ED and *Catha edulis*+ED groups Results expressed as Mean±SD, superscript alphabets are significantly different from their corresponding group (p<0.05) considered significant compared to control (n = 6)

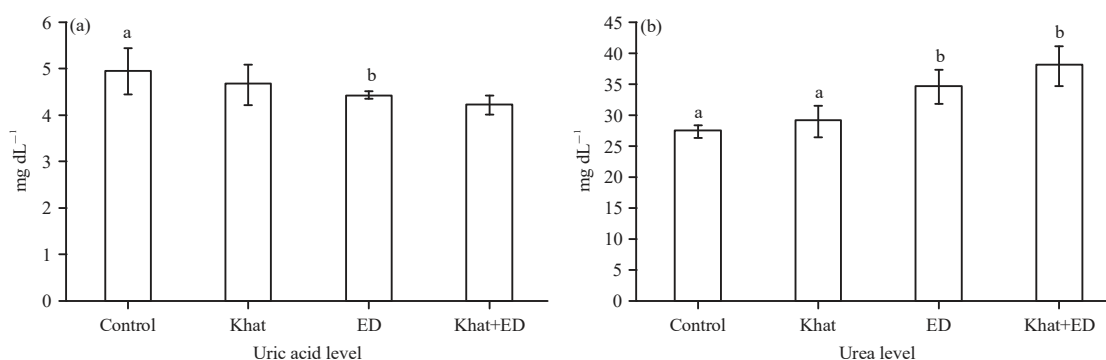


Fig. 3(a-b): (a) Uric acid level and (b) Urea level in the rabbit cortex of control, *Catha edulis*, ED and *Catha edulis*+ED groups Results expressed as Mean±SD, Superscript alphabets are significantly different from their corresponding group p<0.05 considered significant compared to control (n = 6)

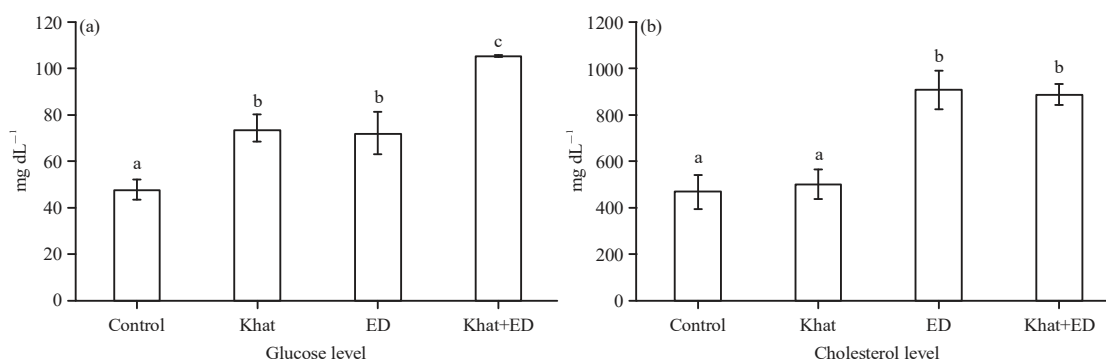


Fig. 4(a-b): (a) Glucose level and (b) Cholesterol level in the rabbit cortex of control, *Catha edulis*, ED and *Catha edulis*+ED groups Results expressed as Mean±SD, Superscript alphabets are significantly different from their corresponding group p<0.05 considered significant compared to control (n = 6)

compared with other groups (Fig. 3a, p<0.05). However, urea levels in the brain cortices showed significant changes following exposure to ED alone or if mixed with *Catha edulis* compared with control animals (Fig. 3b, p<0.05).

**Glucose and cholesterol levels:** In Fig. 4, the levels of glucose and cholesterol in the brain cortices of rabbits were shown following consumption of *Catha edulis* extract and/or ED. Higher glucose concentrations were seen following

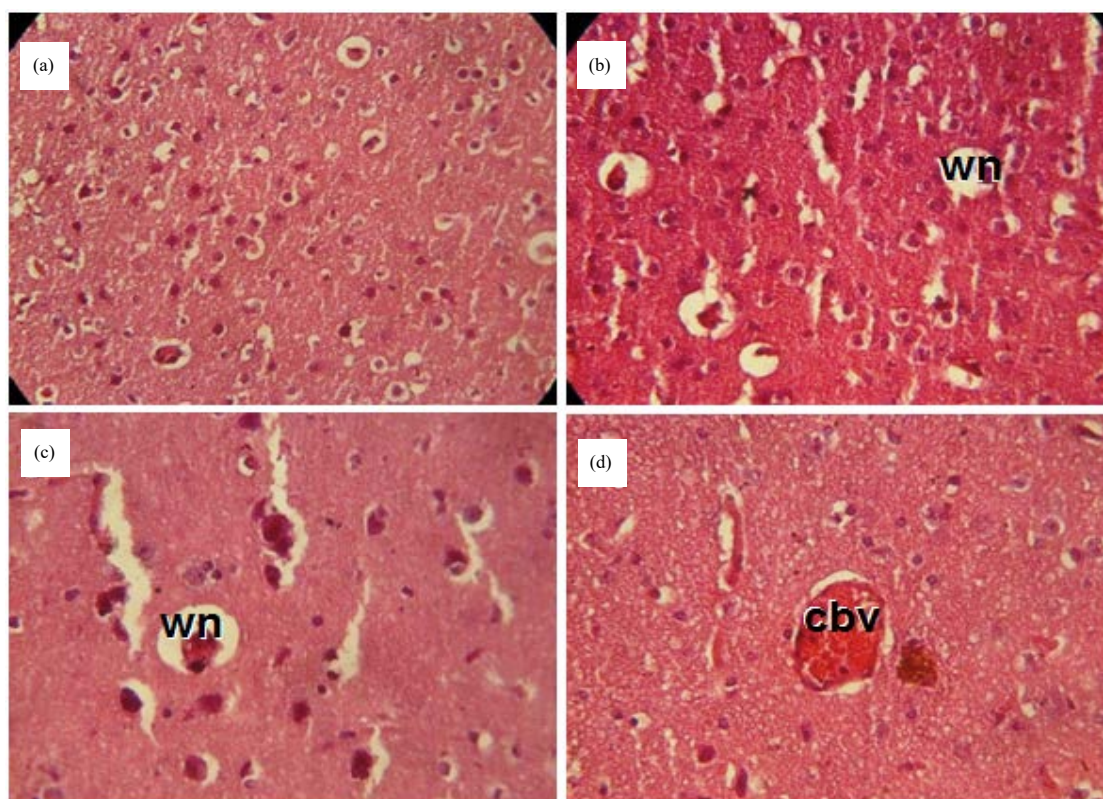


Fig. 5(a-d): Histopathological changes in the brain cortex of (a) Control, (b) *Catha edulis*, (c) ED and (d) *Catha edulis*+ED groups. (A) Normal cell morphology, (B) Congested blood vein, shrinking, necrotic neuron cell body and widening spaces around neuron cells, (C) Necrotic aeria and widening spaces around neuron cells and (D) Congested brain vein, enlarged neuron cell body, necrotic neuron cell body and widening spaces around neuron cells

administrations of *Catha edulis* and ED either alone or mixed in the brain cortices of rabbits compared to control (Fig. 4a), the high increase was seen in ED group compared with other groups. Cholesterol level was increased in the ED group and *Catha edulis*+ED group as compared to control or *Catha edulis* groups (Fig. 4b,  $p < 0.05$ ).

**Histopathological changes:** Histopathological changes of the cortex stained with hematoxylin and eosin of the rabbits exposed to *Catha edulis* extract and/or ED for 35 days were shown in Fig. 5. The control group showed normal cell morphology. However administration of ED resulted in congested blood vein, shrinking, necrotic neuron cell body and widening spaces around neuron cells, while necrotic aeria and widening spaces around neuron cells have been observed in cortex of *Catha edulis* group. Both *Catha edulis* and ED administration showed congested brain vein, enlarged neuron cell body, necrotic neuron cell body and widening spaces around neuron cells (Fig. 5).

## DISCUSSION

The antioxidant system of rabbit's brain cortices has been affected following 35 days of administration of *Catha edulis* and/or ED in the present study. Reduced activity of CAT and thiol contents were observed following exposure to caffeine and/or *Catha edulis* with changing in the levels of protein, albumin, glucose, cholesterol urea and UA. The present study reported for the first time these changes in the antioxidant levels of rabbit's brain cortices following a short term of mixing both ED with *Catha edulis*, the main stimulant found in the leaves of *Catha edulis* and chewed in many countries.

One of the habits spread among young and adolescents is ingestion the caffeinated ED to enhance their mental and physical performances and improve alertness and concentration. They also mix these EDs with other stimulants for maximum effect raising concerns of using these stimulants individually or together<sup>10</sup>.

Caffeine affects different neurotransmitters either by increasing the levels of their receptors or changing their

affinity<sup>6-9</sup>. On the other hand, *Catha edulis* has amphetamine like structure and function and act on catecholaminergic synapses by increasing levels of dopamine, serotonin and noradrenalin<sup>23</sup>. Neurotransmitters are factory of ROS and serve as sources of oxidative stress in the brain<sup>24,25</sup>.

Masoud *et al.*<sup>19</sup> reported that despite the low dose of caffeine used (0.22 mg mL<sup>-1</sup>, 1.1 mg of caffeine) daily for 35 days might results in alteration the antioxidants in plasma and kidney of rabbits exposed to both caffeine and/or *Catha edulis*. The same dose and duration have more effect in the brain cortices of rabbits suggesting the tissue specific action of these two stimulants. Present findings are in agreement with those of Zeidan-Chulia *et al.*<sup>26</sup>, who reported that CAT activity decreases in a concentration-dependent manner in human neuronal SH-SY5Y cells. This change in CAT activity attributed to increase ROS in the brain following exposure to caffeine and/or *Catha edulis*, which was concomitant with reduction in the levels of thiol groups including GSH, P-SH and T-SH.

Present observation also in agreement with those of Svatikova *et al.*<sup>27</sup> where both reported that the consumption of ED increasing glucose levels in young healthy adults or in the brain cortices of rabbits in this study. *Catha edulis* present in the leaves of *Catha edulis* showed a significant decrease in the anti-oxidants of red blood cells<sup>16</sup> plasma<sup>17</sup> and saliva<sup>18</sup> which is also in consistent with the present data. One of the main actions of the presence of ROS in the cells is affecting different biomolecules including proteins and lipids<sup>13</sup>, in the present study, protein, albumin and cholesterol levels have been changed following ED ingestion either alone or when mixed with *Catha edulis* but no changes were seen in *Catha edulis* group. This might showed that caffeine has effect on brain despite the low dose and short term but not *Catha edulis*.

The UA levels in the brain cortices of rabbits showed reverse pattern as compared with previous finding in the plasma<sup>19</sup> or with those of Wahlqvist<sup>28</sup> where UA has been reported to be increased after consumption of drinks or alcohol and decreased only in the brain cortices of ED group.

As the mixing of ED and *Catha edulis* becomes a daily habit among people who chewing *Catha edulis* for long period, more investigations need to be carried out to study the effect of this mixing in long term in both animal and human subjects.

## CONCLUSION

It is concluded that mixing both ED and *Catha edulis* during chewing session or ingestion them alone

results in alteration of antioxidant system in the brain cortices despite the low doses and short term.

## SIGNIFICANCE STATEMENT

This study discovered the effect of mixing ED and/or *Catha edulis* on brain cortex of rabbits exposed to short term of ED and/or *Catha edulis* that can be beneficial for population used to ingest these two chemicals as stimulants. This study will help the researchers to uncover the critical areas of the relation of the use of these two stimulants on the CNS that many researchers were not able to explore. Thus a new theory on effect of ED and/or *Catha edulis* consumption on CNS may be arrived at.

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