

EVALUATION OF EFFECT OF GREEN TEA AND SAFFRON ON LEARNING PROCESS
AND MEMORY IN HEALTHY HUMAN VOLUNTEERS

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Summary

The study was conducted in healthy human volunteers adhering to good clinical practices and institutional human ethical norms. All the volunteers have completed the study. There were no dropouts and no signs and symptoms of any adverse effect after consumption of the beverages. This indicates the positive compliance in the study.

The overall effect observed in the study that the consumption of beverages (especially Green tea) increases alertness in the volunteers and it helps to facilitate the performance and thus enhance the memory. This is confirmed by the level of confidence development in the volunteers to perform the psychopharmacological parameters. The parameters selected in the study are the functional characteristics of thought process, learning and memory. The reduction in reaction time after the consumption of green tea signifies the effect on learning process. The improvisation of this facilitates the memory in the form of retention and its implementation in developing skills. Clinically these results are very promising and can be extrapolated for the treatment of neurodegenerative disorders like Alzheimer's disease, dementia where at least partial effect on the process of learning and recent memory.

Green tea and saffron are consumed many times by the individuals in the situations like appearing for examinations where the level of performance is of significance to have optimum outcome. These may be recommended for short time use. However, the additional effects may be confirmed in larger studies in controlled manner.

Key words: Green Tea, Saffron, Memory, Learning, Physiological Performance

Introduction

Learning process and memory are important physiological parameters during our growth and play important role at various circumstances. These could be in situations like increased demand and needs as occurs during examinations and stressful conditions. Tea and saffron are two such plants which have shown great potential in medical practice like prevention of cancer, cardiovascular diseases, diabetes, neurodegenerative disorders etc. Both these plants are shown to improve learning behavior also by many research scientists. Tea is the second-most commonly consumed beverage, is virtually calorie-free, and helps maintain proper fluid balance. Green tea has been used in oriental medicine because of its beneficial effects. All varieties of tea come from leaves of the *Camellia sinensis* plant. Saffron *Crocus sativus* used to flavor and color foods. Saffron has many nonvolatile active components, many of which are carotenoids, including zeaxanthin, lycopene, and various α - and β -carotenes. In studies in mice, saffron extract improved ethanol-induced impairments of learning behaviors and prevented ethanol-induced inhibition of hippocampus long-term potentiation related to learning and memory. Researchers believe that crocin may attribute this effect. In a study of hyperlipemic rats, crocin decreased cholesterol, triglyceride and density lipoprotein levels, and increased the content of high density lipoprotein. Researchers believe that crocin prevents atherosclerosis in hyperlipemia, via inhibition of both proliferation of smooth muscle cells and activation of p38MAPK. In our day to day life we consume different types of beverages without knowing what effect they can have on our physiology and psychology. Hence, this study was conducted to identify any effect of Green Tea and Saffron on functional parameters especially the learning and memory process in healthy human volunteers. These characteristics are evaluated using various psychopharmacological tests commonly indicating the process of learning and memory

Methods

Green Tea and Saffron were purchased from ready market. Both were consumed by volunteers as routine conventional dose that is tea as one cup and saffron in one cup of milk. For the preparation of tea the decoction was prepared in one cup of freshly boiled water with one teaspoon of tea leaves, filtered and added one teaspoon of sugar and served to all the volunteers. For preparation of Saffron milk, Saffron stigmas were added to freshly boiled milk for few minutes, one teaspoon of sugar was also added before serving to volunteers.

This was a human study in healthy volunteers between the age of 22 and 15 years, approval of clinical protocol was obtained from institutional ethics committee for the conduct of the study. The study was conducted adhering to norms of good clinical practices and good laboratory practices. No blood samples or any other body fluids were withdrawn from the volunteers during the study. The volunteers were divided in two groups each consisting of six volunteers. On detailed discussions with them regarding the nature and objective of the study, written informed consent was obtained and enrolled in the study after complete physical and systemic examination including general examination, pulse, blood pressure and routine systemic examination. The evaluation parameters included as the following:

Evaluation Parameters for learning and memory:

Mathematical Calculation test: In this test, the volunteers were asked to solve a mathematical summation problem. The mathematical chart consisted of 100 small squares in 10 row and 10 columns in which two digit numbers between 1 and 100 were incorporated (Chart – 1). The volunteers were asked to perform vertical summations and following points were taken for measurement of performance.

- 1) Total time taken to complete summations
- 2) Total correct answers
- 3) Total incorrect answers.

Chart 1: Mathematical Calculation Test

	35	40	40	60	40	55	35	30	35	12
	25	50	50	90	30	60	60	20	95	45
	50	30	30	25	25	40	45	64	15	55
	40	20	50	60	55	30	30	35	34	35
	60	50	40	75	15	50	20	95	56	53
	55	40	30	30	50	40	55	35	43	17
	25	55	20	55	80	45	25	65	17	37
	20	60	80	35	40	45	15	30	50	13
	30	15	50	60	45	35	65	20	38	27
	25	75	80	20	25	20	30	25	12	20
Total										

Number Cancellation Test: In this test, the volunteers were given a table consisting of 10X10 (100 small squares), one number is randomly repeated at least seven to eight times, and were asked to cancel a particular number (Chart – 2). The following points were considered for performance evaluation.

- 1) Total time taken to complete to cancel the given number
- 2) Total number of correct cancellation.
- 3) Total number of incorrect cancellation

Chart 2: Number Cancellation

6	1	5	7	9	4	3	7	1	2
5	2	4	8	5	1	3	5	9	7
7	6	9	1	2	6	9	4	6	1
9	5	3	8	9	4	2	9	3	5
2	9	2	1	3	9	1	7	2	4
3	4	4	7	4	8	3	8	1	9
5	4	3	5	6	2	8	1	3	7
8	5	9	4	1	3	6	7	6	3
6	1	7	3	4	1	7	5	4	9
4	5	5	2	5	9	4	6	2	4

Alphabet Cancellation Test: In this test, the volunteers were given a table containing alphabets in 100 small squares, each alphabet repeated 7 to 8 times randomly and were asked to cancel a particular alphabet (Chart – 3).

Following criteria were considered for performance:

- 1) Total time taken to cancel the given alphabet
- 2) Total correct alphabets cancelled
- 3) Total number of incorrect alphabet

Chart 3: Alphabet cancellation Test

A	D	C	B	F	G	H	M	B	V
Q	G	S	X	S	B	Z	X	A	D
E	T	F	C	F	G	H	B	X	Z
S	L	A	G	J	K	A	S	N	Z
K	J	H	D	A	G	S	X	V	K
J	E	H	J	D	D	A	D	C	S
K	J	H	D	A	G	S	X	V	K
F	S	B	L	K	A	G	D	X	A
H	E	S	A	G	J	C	R	Y	U
P	O	Y	E	A	P	O	A	W	R

Upward Digit Scale Test: In this test, the volunteers were verbally informed eight numbers (between 10 and 99) in upward scale and were asked to memorize the numbers in the same manner. The verbal information was repeated three times consecutively (Chart -4).

Following parameters were considered for performance evaluation:

- 1) Synchrony of numbers
- 2) Missing numbers
- 3) Incorrect numbers

Chart 4: Upward digit scale

12
21
29
47
53
62
77
83

Fixed Digit Test: In this test the volunteers were given a fixed number with two digits and were asked to apply mathematical calculation as add or deduct certain numbers from the fixed digit. Each time the evaluator would give exercise the sum as add or cancel. The answer within 10 seconds would be given the score of 2, later than 10 second 1 and incorrect and missing answer as 0. The total score is measured at evaluation each time. Every volunteer may have the minimum score of zero and maximum could be 20. This was compared before and after the consumption of tea or milk (Chart – 5).

Chart 5: Fixed Digit Test

Sr. No.	Number	Result	Score
1	25 – 15		
2	25 + 45		
3	25 ÷ 5		
4	25 + 40		
5	25 + 7		
6	25 – 11		
7	25 + 50		
8	25 – 13		
	Total Score		

Four Words Test: In this test the volunteers were given a card mentioned with four words with no interlink among them and were asked to memorize them in a particular sequence they want and to recall the word when asked (Chart – 6).

The following points were taken for performance evaluation.

- 1) Synchrony of words
- 2) Missing Word
- 3) Incorrect Word(s)

Chart 6: Four Words Test

Aircraft	Desert
Needle	Lunch

The performance was evaluated three times. A) Basal Reading: Before administering the beverages. B) 30 Minutes: Volunteers were evaluated for above mentioned tests after 30 minutes of ingestion of beverage. C) 90 Minutes: Volunteers were evaluated for above mentioned tests after 90 minutes. The comparative analysis was made using Prism software. The values are compared with basal and 30 and 90 minutes. The level of significance was defined as $p < 0.05$.

Results

All the volunteers have completed the study. There were no dropouts from the study volunteers.

Mathematical Calculation Test: The basal time taken for solving mathematical calculation test was 426.3 and 317.4 seconds in the groups of green tea and saffron milk respectively. This time was reduced to 248.5 & 209.2 seconds in green tea and saffron milk respectively after 30 minutes. The reduction in time taken to solve the mathematical calculations indicates the influence of tea and saffron milk on performance. This was also associated with increasing alertness and to improve physiological stamina. This effect is maintained at 90 minutes evaluation. The number of correct summations in this test has been 9.667 and 9.2 at 30 minutes in tea and Saffron groups as compared to basal which was 9.167 & 9. Similarly incorrect numbers were reduced (Table-1).

Table 1: Analysis of Mathematical Calculation Test

Beverage		Total Time Taken (Sec.)			Correct Answer			Incorrect Answer		
		Basal	30 min	90 min	Basal	30 min.	90 min	Basal	30 min	90 min
Green Tea	Mean	428.3	*248.5	*280.7	9.167	*9.667	8.5	0.8333	0.333	1.5
Saffron Milk	Mean	317.4	*209.2	*228.6	9	9.2	9.2	1	0.8	0.8

The level of significance was defined as $p < 0.05$

Number Cancellation Test: The time taken to cancel the given number is decreased after green tea consumption. However, there was no significant change in case of saffron milk consumption. The total number of correct cancellation before and after 30 minutes of consumption of beverage remained unchanged, however, time required to complete the test is reduced after the consumption of tea (Table – 2).

Table 2: Analysis of Number cancellation Test

Beverage		Total Time Taken (Sec.)			Total Number Cancelled			Incorrect Number Cancelled		
		Basal	30 min	90 min	Basal	30 min.	90 min	Basal	30 min	90 min
Green Tea	Mean	20.17	*15.33	*15	97.22	96.67	98.15	0	0	0
Saffron Milk	Mean	17.8	19.4	20.2	98.46	96.92	100	0	0	0

* The level of significance was defined as $p < 0.05$

Alphabet Cancellation Test: The time taken to cancel the given alphabet in random manner is decreased after green tea consumption; however, there was no significant change after saffron milk consumption. The results were uniform at 30 minutes and 90 minutes (Table- 3).

Table 3: Analysis of Alphabet cancellation Test

Beverage		Total Time Taken (Sec.)	Total Alphabet Cancelled	Incorrect Alphabet Cancelled
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		Basal	30 min	90 min	Basal	30 min.	90 min	Basal	30 min	90 min
Green Tea	Mean	16.67	16.17	16.33	95.83	98.15	97.92	0	0	0
Saffron Milk	Mean	16.8	18	19.4	100	100	98.33	0	0	0

Upward Digit Scale Test: The analysis of upward digit test did not indicate any significant difference in memorizing synchronically the upward digits, missing numbers and incorrect numbers (Table- 4).

Table 4: Analysis of Upward Digit Scale Test

Beverage		Synchrony			Missing Number			Incorrect Number		
		Basal	30 min	90 min	Basal	30 min.	90 min	Basal	30 min	90 min
Green Tea	Mean	1.667	1	*1.833	0.8333	0.3333	0.3333	0.8333	0.6667	0
Saffron Milk	Mean	0.8	1.8	0.4	0	0.8	0.2	0.8	1	0

* The level of significance was defined as $p < 0.05$

Fixed Digit Test: The total score obtained in evaluation of fixed digit test showed trend towards improvisation of memory. The effects were uniform after 30 minutes and 90 minutes evaluation. The correct answers were maintained in most volunteers (Table- 5).

Table 5: Analysis of Fixed Digit Test

Beverage		Total Score			Correct Answer			Incorrect Answer		
		Basal	30 min	90 min	Basal	30 min.	90 min	Basal	30 min	90 min
Green Tea	Mean	90.63	*96.88	*97.92	7.5	*7.833	*7.833	0.5	0.1667	0
Saffron Milk	Mean	0.8	*1.8	0.4	0	0.8	0.2	0.8	1	0

* The level of significance was defined as $p < 0.05$

Four Words Test: There was ease of memorizing four words each time at 30 minutes and 90 minutes after the consumption of beverages. It was observed that memorizing words were easier than mathematical numbers. Although, there was no distinctive difference between tea and saffron group, both had shown alertness, increasing concentration and indirectly improved the physiological and psychopharmacological performance (Table-6).

Table 6: Analysis of Four Words Test

Beverage		Synchrony			Missing Word			Incorrect Word		
		Basal	30 min	90 min	Basal	30 min.	90 min	Basal	30 min	90 min
Green Tea	Mean	100	*100	*100	0	0	0	0	0	0
Saffron Milk	Mean	100	*100	*100	0	0	0	0	0	0

* The level of significance was defined as $p < 0.05$

The level of significance is determined by using students paired T-test (Table - 7).

Table 7: Statistical Analysis

Beverage	Mathematical Calculation Test					
	t -value					
	Total Time Taken (Sec.)		Correct Answer		Incorrect Answer	
	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min
Green Tea	2.803	2.026	2.236	0.6742	2.236	0.6742
Flavored Milk	2.424	1.367	0.2182	0.4082	0.2182	0.4082
Beverage	Number Cancellation Test					
	t -value					
	Total Time Taken (Sec.)		Total Number Cancelled		Incorrect Number Cancelled	
	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min

Green Tea	2.351	1.759	1.000	0.2545	0	0
Flavored Milk	0.5258	1.136	1.000	1.000	0	0
Beverage	Alphabet Cancellation Test					
	t -value					
	Total Time Taken (Sec.)		Total Alphabet Cancelled		Incorrect Alphabet Cancelled	
	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min
Green Tea	0.4251	0.4152	0.473	0.4152	0	0
Flavored Milk	0.5632	2.316	0	0	0	0
Beverage	Upward Digit Test					
	t -value					
	Synchrony		Missing Number		Incorrect Number	
	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min
Green Tea	1.581	0.2104	1.464	1.464	1.000	1.195
Flavored Milk	1.291	0.5898	1.372	1.000	1.000	1.177
Beverage	Fixed Digit Test					
	t -value					
	Total Score		Correct Answer		Incorrect Answer	
	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min	Basal vs 30 Min	Basal vs 90 Min
Green Tea	0.9684	1.151	0	0	0.791	1.581
Flavored Milk	0.000	1.000	0	0	0.000	1.000

Discussion

The study protocol, volunteer screening format, and informed consent forms were analyzed and approved by institutional ethics committee. It was observed in this study that compliance with both tea and saffron milk had been excellent. There was no drop out of volunteers at any stage of the study indicates the positive compliance. All volunteers were co-operative and there were no evidences of adverse effects during the study. The time taken to solve the mathematical

summation and other two tests like cancellation of random number and random alphabet from the chart is reduced, indicating that green tea and saffron helps to improve alertness and facilitates the learning process. During the phase of alertness the physiological performance increases which reflects in facilitating solving mathematical summations, ability to perform faster and better in arithmetic number cancellation and random alphabet cancellation. The influence on performance ability suggests improvement or facilitation of learning process. Although, analysis of results shows only marginal level of statistical significance, this can be improved by increasing the number of volunteers and using strict adherence to selection criterion of volunteers. The large and broad variation performance between volunteers could be one of the factors for marginal statistical significance as compared to clinical significance.

The experiment conducted on rats have shown that extract of green tea significantly improves the learning and memory in terms of increased retention latency to enter difference in passive avoidance test in the elevated maze test and more number of entries in the enclosed arm constituents of green tea prevent obstructive sleep apnea which causes cognitive deficits (1). Green tea polyphenols possesses anti-oxidant properties and act as free radical scavengers. Experimental studies conducted in rats demonstrated that green tea polyphenols had better memory and performed better in the maze test.

In another set of experimental studies the improvement of long term memory has been seen in rats. The rats were able to find and perform faster after treatment with caffeine; increase in memory retention was also present (2).

In human study it was shown that caffeine possesses cognition enhancing property. The result in our study on volunteers with green tea consumption shows improvement on functional cognitive performance. This could be the effect of transient stimulation of higher functions related to physiological and psychological performance similar to that of caffeine (3).

The beneficial effects of Green Tea are attributed to polyphenols, particularly catechins. The catechins have potential antioxidant properties which scavenge reactive oxygen species. This could be beneficial in prevention of neuronal degeneration (4).

Recent studies have shown the memory enhancing properties of saffron extract. The saffron extract or its active constituents, Crocetin and Crocin may benefit the people suffering with neurodegenerative disorders accompanying memory impairment (5 and 6) In a study conducted in hemorrhagic rats. Crocin had improved the cerebral oxygenation. (7).

Kaur Tranum and his colleagues studied the effects of green tea extract administration on age-related cognition in young and old male Wistar rats. Young and old rats were orally administered 0.5% green tea extract for a period of eight weeks and were evaluated by passive avoidance, elevated maze plus paradigm and changes in acetyl cholinesterase activity. Treatment of young and old rats with the extract resulted in no significant difference in performance on the rota rod treadmill test/righting reflex time. Green tea extracts significantly improved learning and memory in older rats, with increased retention latency to enter difference in passive avoidance test. In the elevated maze test, green tea treatment resulted in significantly more number of entries in the enclosed arm by the young and old rats. Decline in acetyl cholinesterase activity was observed in the cerebrum of green tea treated old rats in comparison to the green tea treated young rats. Green tea extract administration is effective in enhancing learning and memory in aged rats, and hence, may serve useful in reversing age-related deficits (1).

The benefits of tea in memory improvement has been reviewed and quoted by many scientists. It has been found that both green and black tea inhibited the activity of enzymes associated with the development of Alzheimer's disease, but coffee had no significant effect. Both teas inhibited the activity of the enzyme acetyl cholinesterase (AChE), which breaks down the chemical messenger, or neurotransmitter, acetylcholine; thus can be improve the memory in Alzheimer's disease which is characterized by a drop in acetylcholine. Green tea went one step further in that it obstructed the activity of beta-secretase, which plays a role in the production of protein deposits in the brain which are associated with Alzheimer's disease. Scientists also found that it continued to have its inhibitive effect for a week, whereas black tea's enzyme-inhibiting properties lasted for only one day. It also decreases neuronal loss due to polyphenols (-)-epigallocatechin-3-gallate (MPTP), which is a potent neurotoxin that specifically affects dopamine production and receptors. Green tea only exhibits antioxidative properties at relatively low concentrations and at high concentrations can actually prove to be pro-oxidative. (8 and 9) studied the effect of *Crocus sativus* in rats by using the object recognition and the step-through passive avoidance task. The post- training administration of CSE (*Crocus sativus* extract) (30 and 60 g/kg) successfully counteracted extinction of recognition memory in the normal rat, suggesting that CSE modulates storage and/or retrieval of information. In a subsequent study, pre-training treatment with CSE (30 and 60 mg/kg) significantly antagonized the scopolamine (0.75 mg/kg)-induced performance deficits in the step-through passive avoidance test. These results support findings about the implication of CSE in learning and memory mechanisms. (*Crocus sativus* L. extracts antagonize

memory impairments in different behavioral tasks in the rat. investigated the effects of saffron extract and its ingredients on learning behaviors and long-term potentiation (LTP). Oral administration of *Crocus sativus* L. extract (CSE) antagonized both ethanol-induced memory impairment and ethanol-induced suppression of LTP generation. It was concluded that Crocin was the main active component in the CSE, with regards to both learning performance and LTP generation, and its pharmacological action was triggered via modulation of the central NMDA receptors. The results suggested that crocin improved learning deficits by attenuating the inhibitory effect of ethanol on LTP generation, and highlighted the possibility that crocin is a candidate for an ethanol-induced central nervous system disorders. The researchers proposed saffron constituents' involvement in learning and memory processes. They investigated the effects of Crocin on recognition and spatial memory in the rat (10). For this aim, they had used: the object recognition task which evaluates non-spatial working memory and a novel version of the radial water maze which assesses spatial reference and spatial working memory. Crocin counteracted delay-dependent recognition memory deficits in the normal rat, suggesting that these carotenoids modulate storage and/or retrieval of information. In a subsequent study, treatment with Crocins attenuated scopolamine induced performance deficits in the radial water maze test. The results support and extend the enhancing effects 45 of Crocin on memory and, then, to our knowledge, for the first time, demonstrate its implication in the mechanisms underlying recognition and spatial memory

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