



ANXIOLYTIC AND MEMORY BOOSTING EFFECT OF FRUIT COCKTAIL (CHERRY JUICE, PINEAPPLE JUICE AND COCONUT MILK)

Sidra Zubair^{1*}, Safila Naveed², Sana Sarfaraz³, Hira Afshan⁴, Subia Jamil⁵, Hira Raees⁶, Asma Eraj⁷, Mahrukh khurshid⁸

^{1*,6,7,8}PhD Scholar (Pharmacology) Lecturer, Department of Pharmacology, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

²PhD (Pharmaceutical-Chemistry) Professor, Department of Pharmaceutical-Chemistry, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan.

³PhD (Pharmacology) Assistant Professor, Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan.

⁴M.phil, Lecturer and Researcher, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan

⁵PhD (Pharmacology) Professor, Head of the Department, Department of Pharmacology, Faculty of Pharmacy, Jinnah University for Women. Karachi, Pakistan

***Corresponding Author: - Sidra Zubair**

*Pharm-D, M.Phil, PhD Scholar (Pharmacology) Lecturer ORCID: 0000-0001-7616-8203
Department of Pharmacology, Faculty of Pharmacy, Jinnah University for women, 5c Nazimabad, Karachi-74600 Pakistan. E-mail: phr.sidra@gmail.com ; contact no. +92333-2213891

Abstract:

Anxiety is an emotion of uneasiness, fear, or discomfort. It causes restlessness and difficulty in focus. Recently due to the high costs of allopathic medicines and increasing adverse effects, the trend has switched to exploring nutrients in normal diet that can be therapeutically beneficial. Cherry, pineapple, and coconut milk have a great impact on CNS activity because they contain different flavonoids and other phytochemicals. This study was designed to assess the anxiolytic and memory-boosting potential of cocktail of cherry juice, pineapple juice and coconut milk. This study was performed on albino mice weighing 18-22gm of both sexes. Mice were divided into 2 groups. Group I was taken as Control and was given normal saline 0.4ml. Group II was taken as treated and given a cocktail 0.4ml. The head dip, light and dark model, cage crossing, open field, and elevated plus maze were used to evaluate effects on anxiety. The stationary rod test was used to evaluate memory-boosting effects. Our study showed significant anxiolytic activities ($p \leq 0.05$) of cocktail in mice. Head dip activity, light and dark activity, cage crossing activity, and elevated plus maze show highly significant ($p \leq 0.001$) result on anxiety. Open field activity showed significant ($p \leq 0.05$) result on anxiety. Stationary rod activity showed highly significant ($p \leq 0.001$) result on memory boosting effect. It was concluded that the administration of the cocktail had an anxiolytic and positive effect on memory because it possesses antioxidant components such as multivitamins, minerals and flavonoids. It has less adverse effect as compare to allopathic drugs.

Keywords: Anxiolytic; coconut milk; pineapple juice; cherry juice

INTRODUCTION:

Anxiety is a reaction of uneasiness, fear, worry or discomfort. It interrupts the daily routine of the person because it causes restlessness and difficulty in focus. The general symptoms are palpitation, muscular tension, dry mouth, sweaty hands or feet, light headache, raised blood pressure, and churning in the stomach. The exact cause of anxiety is unknown. It may be hereditary or due to any underlying disease such as; cancer, Parkinson's disease, rheumatoid arthritis, or diabetes etc, Bipolar disorder or Major depressive disorder, and minimized levels of inhibitory neurotransmitter i.e. gamma amino butyric acid (GABA) also cause anxiety, it can also be due to history of any trauma, parenting factors and socioeconomics etc. It can also be due to prolonged use of drugs, misuse or withdrawal of many drugs including tobacco, alcohol or sedatives etc[1].

A balanced and healthy lifestyle plays a major role in treating the anxiety. Lack of sleep can exacerbate anxiety,[2, 3] caffeinated beverages also trigger anxiety. Caffeine is a CNS stimulant and triggers all types of jittery physiological effects like palpitation and trembling hands leading to agitation and restlessness[2]. Exercise reduces stress hormones, relieves tension, and increases the serotonin and endorphin neurotransmitters [3].

Antidepressants are prescribed as a first-line management for anxiety disorder. In antidepressants, Selective serotonin reuptake inhibitors (SSRIs) are newer options and recommended as first-line agents to treat long-term anxiety. Serotonin and norepinephrine reuptake inhibitors (SNRIs) and tricyclic anti-depressants (TCAs) are also effective treatments. Benzodiazepines are used as the second line of treatment. Beta-blockers are also used to treat anxiety. All drugs provide temporary relief, and have side effects and safety concerns [4, 5].

Recently due to increasing adverse effects of allopathic medicines, the trend has switched to exploring the nutrients in normal diet that can be therapeutically beneficial. It is currently extensively held that the antioxidants contained in fruits (berry family) and vegetables can provide protection against certain human degenerative conditions that are linked with oxygen free radical damage and improve memory in older adults[6]. Evidence stated that those foods which are rich in these three specific flavonoid such as, the flavanols, anthocyanins and/or flavanones, acquire the maximum capability to take action on the cognitive activity. Cherry, pineapple and coconut milk have a great impact on CNS activity because they contain different flavonoids[7].

The botanical name of cherry is *Prunus avium*. This species of cherry is native to Europe, Maghreb and Western Asia. It belongs to Rosaceae family. The main chemical constituents of cherry are mallic acid, glucose, flavanoids such as anthocyanins, flavonol and flavan-3-ols compounds. Anthocyanins are the most abundant flavonoids in sweet cherry. It reduces the growth of cancerous cell[8]. It is known fact that Alzheimer's disease, diabetes, atherosclerosis and cancer result in oxidative damage. It is also rich in phenolic acids[9].

The botanical name of pineapple is *Ananas comosus*. It belongs to the family Bromeliaceae. The main chemical constituents of pineapples are bromelain, phenols, flavonoids, polysaccharides, carotenoids, sugar and volatile compounds[10].

The botanical name of coconut is *Cocos nucifera* and it belongs to the family Arecaceae. It is rich in minerals, fiber and vitamins [11]. The objective of the study was to evaluate the impact of cocktail comprising of cherry juice, pineapple juice and coconut milk on the anxiety and memory of mice.

MATERIALS AND METHOD:

Procurement of Cocktail Ingredients:

Cherry, pineapple and coconut milk were bought from the local store and evaluated by the Department of Pharmacognosy, Faculty of Pharmacy, Jinnah University for Women and were allocated voucher # JUW | H-007 for cherry and JUW | H-008 for pineapple. Coconut milk had lot # ABF067 and expiry 08-2017.

Preparation of Fruit Cocktail:

Fresh juices were made from the cherry and pineapple in the juicer. We made the cocktail of cherry juice, pine apple juice and coconut milk in the ratio of 1:1:1 by blending together these by using Phillips juicer blender model number HR1845/33.

Selection of experimental animals:

Albino mice of both sexes weighing between 18-22 gm were used in this study. They were kept and bred under standard environment with 12 hours dark and 12 hours light cycle in animal house of Jinnah University for women. The animals were fed food and water ad libitum, and were kept under constant room temperature of 22 ± 2 °C. Lighting, temperature and noise level was kept constant for all rodents. After each experiment, feces and urine were removed and surfaces were wiped with 70% ethanol.

Animal grouping and dosing:

The mice were uniformly fractionated into 2 groups (6 animals in each group). Group I was given 0.4 ml of this cocktail, Group II was given 0.4 ml of distilled water as control. All groups were given measured quantity one time every day. The study was carried out for 45 days.

Neuropharmacological screening:

The following tests were carried out for evaluation of CNS activity.

Hole board test:

The hole board test consists of a grey square box arena 35 cm×45 cm × 45 cm. The walls of the box were 20 cm high. There were three holes having a diameter of 2.5 cm on all sides of box, so that rodents could poke their heads, which could be used to measure anxiety, stress and exploratory activity of rodents. Lower the number of head-dip, less will be the anxiety. The apparatus was located in testing room with dimmed lighting[12].

Procedure:

Before starting the test, mice were familiarized with environment. Mice were placed one at a time in the center of the hole board and allowed to explore freely in the apparatus for 5minutes. The number of holes explored by mice was the measure of the head dip test .

Cage crossing activity:

This test gives general locomotor movement, exploratory action and screening for anxiety-related behavior in mice. Lower the number of cage crossing by the mice less will be the anxiety. The apparatus of cage crossing activity consists of transparent perplex cage having an area of 26 × 26 × 26 cm, having the floor covered with sawdust.

Procedure:

A single albino mouse was placed in the centre of the apparatus gently with the help of tail. Rodent was allowed freely to explore and move in the arena for 5minutes. The number of times the mouse crossed the cage was observed[13].

Open field exploration test:

This test provides general locomotor activity, exploratory activity and screening for anxiety-related behavior in rat and mice. Apparatus consist of a large square chamber i.e. white Plexiglas box having 42 cm high walls and the floor being marked in 25 squares having a diameter of 15 cm each square. In the central arena of this apparatus, a central square of 15 × 15 was also present. Large square chamber can vary from 28 × 28 cm to 56 × 56 cm. Apparatus can be made up of plastic or wood, but normally prefer using transparent Plexiglas[14].

Procedure:

Single albino mouse was placed in the center of apparatus gently by the help of tail. Rodents were allowed freely to explore and move in the arena for 5 minutes. Observe the number of times the mice moved in peripheral and central squares. Each line crossed was scored as one unit of activity.

Light & dark test:

The light and dark exploration test was designed by Crawley and Goodwin (1980). This test might be useful to predict anxiolytic or anxiogenic activity of mice. Mice and rats naturally prefer dark protected compartment. This test was used to examine anxiety like behavior in rodents [15].

Procedure:

The mice were transferred individually in the center of the brightly illuminated compartment facing away from the separator. Mice were free to move in light and dark compartments for 5 minutes. Observe the time spent in each compartment by mice or record the transitions in the light compartments.

Elevated plus maze activity:

This test may be used to ensure anxiogenic and anxiolytic movement in mice. In this experiment, plus shaped apparatus was used, two enclosed arm with open roof (secure) and two open arms (insecure). The height of the apparatus from the floor must be 1m approximately. Mice were free to explore both arms, but mice avoid open areas especially when it was brightly lit. Mice favor dark spaces. Anxiolytic drug causes increased exploration of the open insecure arm, without affecting general locomotion and motivation[16].

Procedure:

Albino mice were placed gently by the tail in the centre of the plus shaped apparatus. Mice were allowed freely to move and explore the maze for 5 minutes. Observe the time spent in each arm and transition from open arm to enclosed arm.

Stationary rod test:

This test was used to evaluate locomotor activity and learning ability[17]. Stationary rod test was designed to determine co-ordination rather than strength of mice. The minimum time to cross the rod shows memory enhancement of the mice. The apparatus of stationary rod test consist of two parallel horizontal rod made up of stainless steel with the platform. Ability of mice to grip the bar was inversely proportional to rod's diameter (2mm) test[18].

Procedure:

Gently hold the mouse by the tail and place the mouse on the rod. Before starting the test, short training was given to albino mouse and allowed to balance on horizontal rod. During the experiment the mouse was allowed to walk separately on rod. Observe the time taken to cross the rod to another platform.

RESULTS:

Results have been analyzed by applying SPSS 20. By taking means of all values, the treated group was compared to control group. One-way Anova was applied and values of $P < 0.05$ were considered as significant, $P < 0.01$ was more significant and $P < 0.001$ was considered highly significant. We took a cocktail of cherry juice, pineapple juice and coconut milk to check different parameters of CNS activity.

Results of phytochemistry of red cherry juice, pineapple juice and coconut milk have been mentioned in Table 1.

Table 1: Phytochemistry of cherry juice, pineapple juice and coconut milk

PHYTO CHEMICAL CONSTITUENT	TYPE OF TEST	CHERRY JUICE	PINE-APPLE JUICE	COCONUT MILK
Alkaloids	Wagner's Test	+	+	+
	Dragendorff's test	+	+	+
Flavonoids	Pew's Test	+	+	+
Glycosides	Keller-Kiliani Test	+	+	+
Phenols	Ellagic Acid Test	+	+	+
Saponin	Foam Test	+	+	+
Sterols	Liebermann-Burchard Test	+	-	+
Tannins	Ferric Chloride Test	+	+	+
	Gelatin Test	+	+	+
Carbohydrates	Molisch Test	+	+	+
	Fehling's Test	+	+	+
Anthocyanins	Free Anthocyanins Test	+	-	-

(+ present; - absent)

Neuropharmacological screening:

Effects of Cocktail on Head dip Activity:

Post-hoc analysis by scheffe test showed that number of head dip was decreased highly significantly ($p < 0.001$) by cocktail of pineapple, red cherry and coconut milk throughout the period from 7th day to 45th day. **Table 2** shows the effect of cocktail of pineapple, red cherry and coconut milk on head dip activity of mice. Result indicate reduction in head dips as compared to control throughout treatment period.

Table 2: Effect of cocktail on head dip

MEANS (n) ± STANDARD DEVIATION				
GROUPS	BASELINE	DAY 15	DAY 30	DAY 45
CONTROL	21.50±0.707	26.60±1.074	25.40±0.516	23.30±1.636
TREATED	20.50±0.706	13.80±0.788***	6.70±0.948***	2.50±0.527***

n = number of head dips

P values are;

*** $p < 0.001$ is highly significant as compared to control

Effect of Cocktail on Cage Crossing Activity:

Previous result was further proved by looking into other test which was performed for evaluation of anxiety that was cage crossing activity. Post-hoc analysis by scheffe test showed that number of cage crossing was decreased highly significantly ($p < 0.001$) by cocktail of pineapple, red cherry and coconut milk throughout the period. **Table 3** showed the effect of cocktail of pine apple, red cherry and coconut milk on cage crossing activity of mice. Neuropharmacological screening of cocktail revealed that the locomotor and exploratory activities decreased after the administration of cocktail. Result was highly significant as the number of cage crossing was decreased day by day which showed the anxiolytic property of the cocktail.

Table 3: Effect of cocktail on cage crossing

MEANS (n) ± STANDARD DEVIATION				
GROUPS	BASELINE	DAY15	DAY 30	DAY 45
CONTROL	20.00±1.247	23.50±0.527	23.40±0.516	23.50±0.707
TREATED	21.20±0.632	15.00±0.816***	6.90±0.737***	2.90±0.737***

n = number of cage crossings

Values are Mean \pm SD

P values are;

*** p<0.001 is highly significant as compared to control

Effect of Cocktail on Open Field Activity:

Post-hoc analysis by scheffe test showed that number of open field activity in central region increased significantly (p<0.01) on day 15 and increased highly significantly (p<0.001) on day 21 to day 45 by cocktail of pineapple, red cherry and coconut milk. Open field activity in peripheral region was decreased highly significantly (p<0.001) by cocktail of pineapple, red cherry and coconut milk throughout the period. **Table 4** shows the effect of cocktail of pine apple, red cherry and coconut milk on open field activity of mice (central). **Table 5** shows the effect of cocktail on open field activity of mice (peripheral). Neuropharmacological screening of cocktail exposed that crossings in the central region in open field activity was increased and crossings in the peripheral region in open field activity was reduced in comparison to control throughout the treatment period.

Table 4: Effect of cocktail on open field activity (central)

MEANS (n) \pm STANDARD DEVIATION				
GROUPS	BASELINE	DAY 15	DAY 30	DAY 45
CONTROL	10.20 \pm 1.032	9.50 \pm 0.527	10.60 \pm 0.699	9.9 \pm 0.737
TREATED	8.30 \pm 0.674	10.80 \pm 0.788**	13.60 \pm 0.516***	16.10 \pm 0.994***

Table 5: Effect of cocktail on open field activity (peripheral)

MEANS (n) \pm STANDARD DEVIATION				
GROUPS	BASELINE	DAY15	DAY 30	DAY 45
CONTROL	64.50 \pm 1.080	63.70 \pm 0.823	63.20 \pm 1.032	63.30 \pm 0.674
TREATED	62.70 \pm 0.823	44.00 \pm 1.632***	23.80 \pm 2.740***	19.10 \pm 0.737***

n = number of squares

Values are Mean \pm SD

P values are;

*** p<0.001 is highly significant as compared to control

** p<0.01 is significant as compared to control

Effect of Cocktail on Light and Dark Activity:

Post-hoc analysis by scheffe test showed that in light and dark activity, time spent in light compartment was increased highly significantly (p<0.001) by cocktail throughout the period. **Table 6** showed the effect of cocktail of pine apple, red cherry and coconut milk on light and dark activity (time spent in light compartment). Neuropharmacological screening of cocktail exposed that time spend in light compartment in light and dark activity was increased in comparison to control throughout the treatment period.

Table 6: Effect of cocktail on light and dark activity (Time spent in light compartment)

MEANS (n) \pm STANDARD DEVIATION				
GROUPS	BASELINE	DAY 15	DAY 30	DAY 45
CONTROL	80.80 \pm 0.632	79.20 \pm 1.032	78.60 \pm 1.074	79.00 \pm 1.247
TREATED	75.20 \pm 1.032	86.70 \pm 1.337***	98.30 \pm 1.059***	105.30 \pm 1.418***

n= time (sec)

Values are Mean \pm SD

P values are;

*** p<0.001 is highly significant as compared to control

Effect of Cocktail on Elevated Plus Maze Activity:

Post-hoc analysis by scheffe test showed that in elevated plus maze activity (time spent in open arm) was increased highly significantly ($p < 0.001$) by cocktail throughout the treatment period. **Table 7** showed the effect of cocktail of pine apple, red cherry and coconut milk on elevated plus maze activity (time spent in open arm).

Table 7: Effect of cocktail on elevated plus maze activity (Time spent in open arm)

MEANS (n)± STANDARD DEVIATION				
GROUPS	BASELINE	DAY 15	DAY 30	DAY 45
CONTROL	85.70±1.159	86.10±1.100	86.70±1.159	86.60±1.074
TREATED	82.10±0.875	96.10±0.875***	124.70±2.750***	137.20±4.442***

n= time (sec)

Values are Mean ± SD

P values are;

*** $p < 0.001$ is highly significant as compared to control

Effect of Cocktail on Stationary Rod Activity:

Stationary rod activity was used to assess the memory and learning effect of compounds. Post-hoc analysis by scheffe test showed that time duration to cross stationary rod was decreased highly significantly ($p < 0.001$) by cocktail throughout the treatment period. **Table 8** showed the effect of cocktail of pineapple, red cherry and coconut milk on stationary rod activity of mice. Neuropharmacological screening of cocktail revealed that time duration to cross rod in stationary rod activity was reduced in comparison to control throughout the treatment period [19].

Table 8: Effect of cocktail on stationary rod activity

MEANS (n)± STANDARD DEVIATION				
GROUPS	BASELINE	DAY 15	DAY 28	DAY 45
CONTROL	55.00±2.981	53.00±1.414	51.90±0.737	51.10±0.737
TREATED	50.10±2.02	17.30±0.823***	10.70±0.823***	7.00±0.66***

n = time (min)

Values are Mean ± SD

P values are;

*** $p < 0.001$ is highly significant as compared to control

DISCUSSION:

A few responsible effects were linked with anxiety such as emotional, physical, cognitive effects. Deregulation of serotonergic, Gabaergic, adrenergic and dopaminergic neurotransmitters may cause anxiety [20]. The serotonin transporter i.e. 5-HTT regulates neurotransmission of serotonin by means of clearance of serotonin. Increase in the concentration of serotonin in extracellular would be likely to cause overload activity at postsynaptic receptors of serotonin, which in turn caused increased anxiety like behavior. So the abnormalities in serotonin transporter or its function were found to cause anxiety or mood disorders. Serotonin transporter was the main target for anxiolytics and antidepressants, these drugs block the serotonin transporter [21].

Phytochemical-rich foods such as flavonoids, anthocyanins have been shown to have a positive impact on memory and learning and cognitive function in both animals and humans [22]. Cherry, pineapple and coconut milk have a great impact on CNS activity because cherry contains anthocyanins which have a memory boosting effects. Pineapple contains mineral salts, iodine, magnesium, manganese, potassium, calcium & iron. Coconut milk contains phosphorous, calcium and potassium. All these minerals have memory enhancing effects [23]. Fruits are important source of many therapeutics effects including anti-cancer, memory boosting, anti-depressant, anxiolytic, anti-spasmodic and anti-microbial effect etc [24]. Phytochemical analysis of cherry juice, pineapple

juice and coconut milk revealed the presence of tannins, saponins, flavonoid, alkaloids, and carbohydrates. The chemical constituents present in juice have been reported to possess a lot of therapeutic value[25].

Recently carbohydrates were being used to produce polysaccharide immune-modulator with vaccine and therapeutic implications[26]. Glucose provides chief metabolic energy for the brain and for that reason it is necessary for intellectual performance[24]. Flavonoids are able to stimulate improvements in memory gaining, storage, consolidation and recovery. Flavonoids of numerous classes are inhibitors of monoamine oxidase A or B, in that way working as anti-depressants or to recover the conditions of Parkinson's patients. Flavanones, Flavanols and anthocyanidines have protective property preventing inflammatory processes leading to nerve damage. Anthocyanins, members of flavonoid group have an anti-oxidant property, it may provide protection from DNA cleavage, anti-inflammatory property, boosting production of cytokines, lipid peroxidation, cardiovascular disease protection which is linked to oxidative stress protection. Anti-mutagenic and anti-carcinogenic activity of tannins may be interlinked with their anti-oxidative property which is important in protection of cellular oxidative damage, as well as lipid peroxidation[7]. The anti-microbial properties of tannins are well documented. Saponins have an effect on immune system, so it protect against cancers, it also lower cholesterol level. Saponins decreases blood lipids lower the blood glucose response and lower risk of cancer [27]. Alkaloids have been reported for wide range of biological activities such as anti-tumor, anti-cholinergic, anti-hypertensive, anti-viral, anti-nociceptive, anti-microbial, myorelaxant and anti-inflammatory effects [28]. Minerals serve as nutritional sources for both animals and plants. Inorganic elements have also been reported to play an essential role in nutrition and they are important structural component of cellular processes. Elements such as potassium, sodium, calcium, manganese, magnesium and iron play important roles in human health and to cure different diseases[29].

Initially when mice were placed in the apparatus of head dip, they moved to the holes to try to escape, So due to state of fear after exposure of apparatus anxiety developed. Neophilia response decreased as the mice became familiar with the environment i.e. exploratory behavior of head dipping dropped as the hole board apparatus lost its novelty. This reduction in poking could also be associated with increased learning behavior that mice learnt not to poke as there is no chance to escape. The significant decline in head dip activity after administration of cocktail may show improved learning ability of mice[19]. So our observation proposed that cocktail reduced stress and improved memory. When mice were placed in apparatus, due to state of fear mice crossed the cage several times. Neophilic response decreased as the mice become familiar with the environment but after administration of cocktail, number of crossing decreased. Neuropharmacological screening of cocktail revealed that locomotor and exploratory activities were decreased after cocktail administration, it showed that cocktail possesses anxiolytic property. This reduction in cage crossing is also related with enlarged learning behavior that mice learn not to cross the cage again and again as there is no chance to escape[19]. The reduction in number of cage crosses is related with anxiolytic and calming profile of cocktail. 5HT enhances locomotor activity and increases anxiety, so this cocktail could decrease the level of 5HT. Behavior and exploratory activity can be varied in mice with the intensity of light and disturbances in the environment. The phytochemical screening of the cocktail exposed the presence of glycosides, steroids, alkaloids, saponins, phenol and tannins. So it might be possible that anxiolytic action of cocktail could be mediated by these phytochemicals.

Our result was highly significant. It showed that cocktail of cherry, pineapple and coconut milk possessed anxiolytic and calming property which reduced the exploratory and locomotor activity. Decrease in peripheral region in open field may also be linked to the improved learning behavior, as mice learnt that there is no possibility to run away[19]. Crossings in the central region was increased so we proposed that this cocktail reduced stress. It has been studied that the chemical constituent of plant containing alkaloids, flavonoids, essential oil, phenolic acid, tannins and saponins possess activity which is effective against many central nervous system disorders or it might be possible that cocktail could mediate the effects of GABA neurotransmitters by binding with GABA receptors, so opening of chloride ion occur which causes hyperpolarization, and muscle relaxation occur.

Our result in light and dark activity was highly significant. Due to the presence of flavonoids, alkaloids and other phytochemicals in cocktail, it produced anxiolytic like activity. Because flavonoids possess many beneficial effects such as anti-oxidants, anti-inflammatory, anti-proliferative and anti-depressants[7, 29].

Cocktail revealed anxiolytic activity since time spent in open arm was increased which was the main representative index of anxiolytic activity. In addition, environmental factors, genetic mutations, differences in background strain or pharmacological treatment can impact behavioral motivation, locomotor activity or exploratory behavior for novelty of mice. The cocktail could mediate the effects of GABA neurotransmitters by binding with GABA receptors, so opening of chloride ion occur which causes hyperpolarization.

The effect of cocktail on stationary rod test indicated that cherry, pineapple and coconut milk behaved as a very good memory booster. The effect of cocktail on stationary rod test might be due to its anti-oxidant property. Cherry, pineapple and coconut milk have a great impact on CNS activity because cherry contains anthocyanins which have a memory boosting effects. Pineapple contains mineral salts, iodine, magnesium, manganese, potassium, calcium & iron. And coconut milk contains phosphorous, calcium and potassium. All these minerals have memory enhancing effects [29].

CONCLUSION:

Traditional medicines have successful use when compared with synthetic drug due to fewer side effects. Natural remedies contain many phytochemicals and it is capable of treating diseases more effectively with less or no side effects in comparison with synthetic drugs. Due to these adverse effects of synthetic drugs, current study was carried out. This cocktail of cherry, pineapple and coconut milk contains many active compounds. Phytochemical studies have been done to check active compounds. A positive anxiolytic and memory boosting result was obtained. Further studies can be carried out to evaluate the exact active constituent responsible for these effects.

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