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SYNTHESIS, CHARACTERIZATION, PHARMACOLOGICAL, AND BIOCHEMICAL EVALUATION OF SOME 2-OXOETHOXYPHENYL ACETAMIDE DERIVATIVES AS POTENTIAL NOOTROPIC AGENTS

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ABSTRACT

Cognitive enhancers (nootropics) are drugs to treat cognition deficits in patients suffering from Alzheimer's disease, schizophrenia, stroke, attention deficit hyperactivity disorder, or aging. Cognition refers to a capacity for information processing, applying knowledge, and changing preferences. It involves memory, attention, executive functions, perception, language, and psychomotor functions. The term nootropics was coined in 1972 when memory enhancing properties of piracetam were observed in clinical trials.

INTRODUCTION

In today's life of stress and strain, there is a dire need for agents having neuroprotective and neuropharmacological activity enhancing learning and memory function of the brain. Stress is also known to interfere with cognitive functions, tending to retard the memory anagram rather than the acquisition of learning.[1] Cognitive dysfunction is one of the main symptoms accompanying ageing, stroke, head injury and neurodegenerative diseases like Alzheimer's disease.

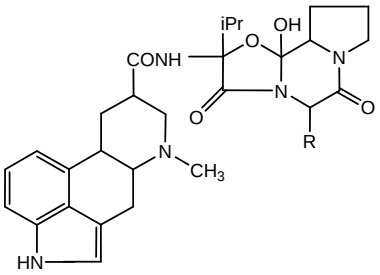
NOOTROPICS/BRAIN NUTRIENTS-Cognition enhancers, often referred to as nootropics, can be defined as drugs able to facilitate attentional abilities and acquisition, storage and retrieval of information, and to attenuate the impairment of cognitive functions associated with age and age-related pathologies. By definition, this class of drugs improves declining of cognitive functions but does not change the rate of progression of neurodegeneration.[37]

Main features in defining a nootropic are.

- The enhancement of learning acquisitions as well as the resistance of learned behaviors to agents that tend to impairs them.
- Partial enhancement of the general resistance of the brain and, particularly, its resistance to physical injuries induced by convulsion, hypoxia and chemical toxicosis.
- The facilitation of interhemispheric flow of information.
- The increase in the efficacy of the tonic cortico-subcortical control mechanisms.
- The display of above mentioned activities by selective functional impact on higher integrative telencephalic mechanisms.

CLASSIFICATION OF NOOTROPICS

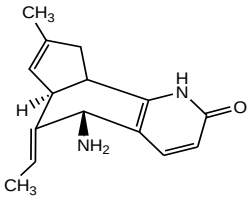
A. Metabolic Enhancers: Hydergine, Vinpocetine, and Bifemelane.



R = iPr **(1)**
R = CH_2Ph **(2)**
R = iBu **(3)**
R = sBu **(4)**

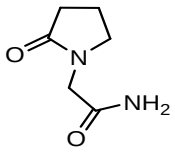
Hydergine is the name of a group of drugs known as ergoloidmesylates. It is a mixture of three hydrogenated ergot alkaloids i.e. dihydroergocornine**(1)**, dihydroergocristine**(2)**, dihydro-α-ergocryptine**(3)** and dihydro-β-ergocryptine**(4)**.

B. Cholinergic Activator: Huperzine A and DMAE.



Huperzine A

C. Cholinergic/ Metabolic Enhancers: Racetam derivatives such as Piracetam, Oxiracetam Etc.



Piracteam

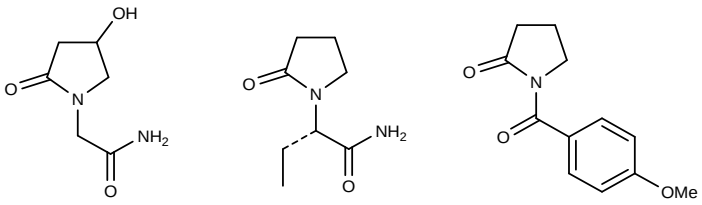
MECHANISM OF ACTION

Mechanism of action of nootropic agents at the molecular level is not completely delineated yet, but some of the pharmacological effects of these agents involve.

- Functional activation of the cholinergic network leading to an increase in ACh concentration.
- Enhancement of brain metabolism by stimulation of oxidative catabolism.
- Increase of ATP/ADP ratio and cAMP levels.
- Enhancement of phospholipid metabolism and protein biosynthesis.
- Increase in utilization of oxygen and glucose under conditions of decreased brain metabolism.
- Improvement in local perfusion.
- Modulation of ion fluxes.

CHEMICAL CATEGORIES WITH MEMORY ENHANCING POTENTIAL

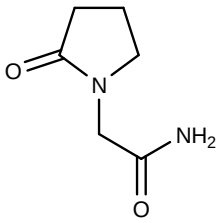
Pyrrolidone (2-oxopyrrolidine) derivatives: The first pyrrolidone to come to the attention of clinicians was piracetam (PA) in the early 1970's. About 1600 pyrrolidones have been synthesized and more than 300 of these have been taken to preclinical studies or further by more than ten different pharmaceutical companies. About a dozen, including piracetam and levetiracetam, are either licensed or at an advanced stage of clinical development.[47]



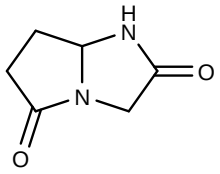
Oxiracetam **Levetiracetam** **Aniracetam**

The pyrrolidone derivative piracetam (PA) is one of the most widely used and best studied of these substances. PA promotes the formation and retention of escape reactions, changes the dynamics of adaptational and orientational responses, facilitates learning in mazes, has antiamnesiac properties, reduces the latent period of hippocampal post-discharges, and affects pain sensitivity.

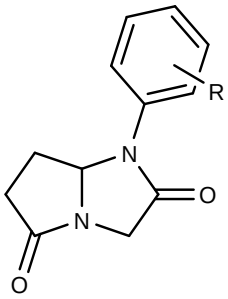
SAR -Taking, piracetam (PA)**,** as the model drug, new compounds endowed with increased potency and efficacy and with a similar profile in terms of safety are investigated. 2-pyrrolidinone nucleus is considered to mimic a dipeptide with a restricted conformation.[52]



Piracetam



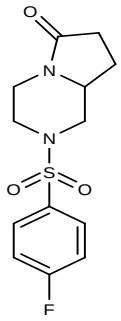
Dimiracetam



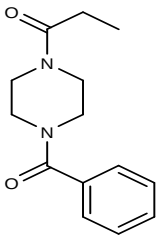
Derivative

Dimiracetam, a bicyclic pyrrolidinone analogue of piracetam, maintainsthe backbone of piracetam with the acetamide sidechain restricted in a folded conformation was 10–100times more potent than piracetam. Chemical modifications of dimiracetamwith substituted aromatic ring inserted into the dimiracetam structure afforded some novel derivatives having potential of nootropic activity. Derivatives with R = 4-Me, 4-CN, 3-CN, have displayed promising nootropic activity.

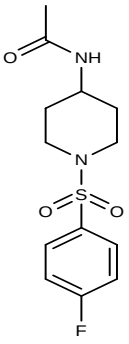
Piperazine derivatives: These compoundswere related to the family of piracetam-likenootropicsby the presence of the 2-pyrrolidinonering. Unifiram, as a novel cognition enhancer, strictly related to piracetam is found 1,000 times more potent than piracetam.



Unifiram



Sunifiram



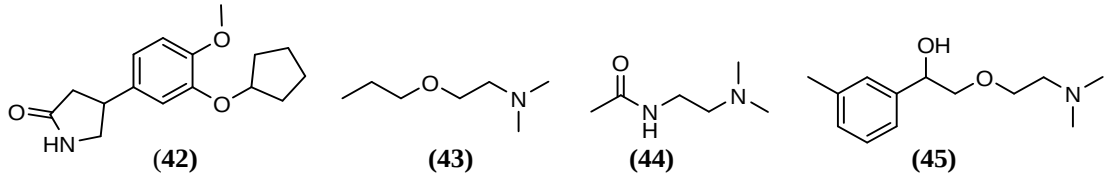
Sapunifiram

However, high activity was maintained when the2-pyrrolidinone ring was opened to give the correspondingpiperazine derivatives, exemplified by (sunifiram), suggestingthat in this class of compounds the 2-oxopyrrolidinemoiety is not critical for pharmacological action.

RESULT AND DISCUSSION

CHEMISTRY

The literature survey conducted in the search for safe and effective nootropic agents, enlisted 2-pyrrolidinone, 4-aminopyridine, arylaminoethoxyethanols etc. Recent literature of aryloxy derivatives with acyclic/heterocyclic ring systems e.g. rolipram**(42)**, dimethylaminoethyl ethers **(43)** and their carboxamide**(44)** derivatives and arylaminoethoxyethanols**(45)** as some of the series with antiamnesic activity and so far, compounds having dialkylaminoethanol moiety, carboxamide group and ethoxy/propoxy derivatives with amino ether function separated by two to three carbon alkyl chain have been designed and synthesized and shown to possess good pharmacological activity.

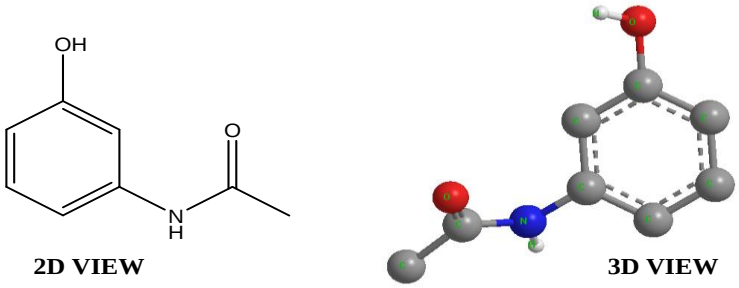


(42) **(43)** **(44)** **(45)**

On the basis of literature survey some common features can be deduced in all pharmacologically diverse classes of nootropic agents:

- Basic nitrogen which may be a part of the heteroaromatic ring or cyclic/acyclic system intended to form a cation – π interaction with Trp[86] and hydrophobic contacts with Tyr[337] and Phe[338] on the target[80].
- H-bond acceptor function to form a hydrogen bond with nitrogen of Tyr[124].
- An appropriate linker group giving optimal spacing to the H-bond acceptor function from the basic nitrogen, in order to align the basic nitrogen optimally in the target site for interaction.
- An acetamido group, which renders the compound to mimic the endogenous dipeptide substrates for the target enzyme.
- A supporting moiety, which renders the compound sufficiently lipophilic to penetrate into the brain.
- Correlation with the endogenous neurotransmitter acetylcholine.

In the light of literature survey, the proposed work was to synthesize some 2-oxoethoxyphenylacetamide derivatives as potential nootropic compounds taking *N*-(3-hydroxyphenyl)acetamide**(46)** as starting material. The –OH group at the 3 position allowed introducing various substituents at this position.

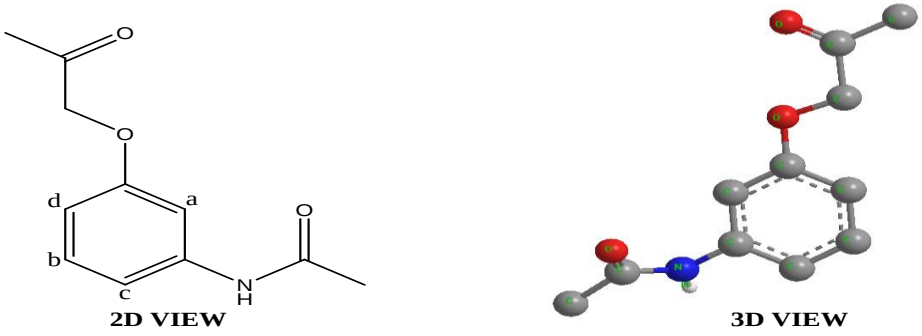


***N*-(3-Hydroxyphenyl)acetamide**

The synthesized compounds were characterized with the help of chromatographic (TLC), spectroscopic (FTIR, NMR, MS) techniques and elemental analysis.

Methyl 2-(3-acetamidophenoxy)acetate (RM-AM-01)(47)

For the synthesis of methyl 2-(3-acetamidophenoxy)acetate (RM-AM-01)**(47)**, *N*-(3-hydroxyphenyl)acetamide was refluxed with methyl-2-chloroacetate in ethyl methyl ketone in the presence of anhydrous potassium carbonate. Removal of excess of ethyl methyl ketone under vacuum gave a solid product which was crystallized from diethyl ether and ethyl acetate. Carbonyl C=O stretching vibration at 1760.89 cm^{-1}, C-O stretching vibration at 1186.14 cm^{-1} in the infrared spectrum showed the presence of an ester group, asymmetric and symmetric stretching vibrations of C-O-C bond at 1243.30 and 1000.99 cm^{-1} showed the presence of alkyl aryl ether function in the product.



Methyl 2-(3-acetamidophenoxy)acetate (RM-AM-01)

Table 2: Comparison of IR spectra of test compounds.

haracteristic bonds and Types of vibrations	Reported frequencies ranges (cm^{-1}) & References		Compound numbers & Observed frequencies (cm^{-1})								
			47	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**
N-H str. of sec. amide	~ 3300	Pavia-68	3315.41	3301.91	3319.26	3299.98	3319.26	3303.83	3371.34	3313.48	3313.48
Aromatic =C-H str.	3100-3000	Silversein-86	3074.32	3100.10	3074.32	3078.18	3100.18	3087.82	3087.82	3060.82	3091.68
Aliphatic C-H str.	3000-2840	Pavia-30,35	2947.03	2950.89	2925.81	2977.89	2923.88	2883.38	2968.24	2937.38	2881.45
C=O str. of aliphatic ester	1750-1735	Pavia-62	1760.89	Absent	Absent	Absent	absent	absent	Absent	absent	absent
C=O str. of amide	1680-1630	Pavia-68	1689.41	1666.38	1660.60	1681.81	1675.52	1685.67	1670.24	1666.38	1687.80
Aromatic C=====C ring str.	1600-1580 1500-1400	Silverstein-86	1590.60, 1500.14	1593.09, 1463.87	1594.45, 1494.73	1604.66, 1425.30	1600.14 1436.87	1604.66, 1463.87	1602.74, 1500.45	1581.52, 1500.52	1605.58, 1469.66
N-H def. of sec. amide	~ 1550	Pavia-69	1577.66	1593.09	1594.45	1554.52	1537.16	1548.73	1529.45	1581.52	1550.66
C-H def. of $-CH_3$	1450-1375	Pavia-26,30	1425.30	1379.01	1419.51	1350.08	1371.29	1348.15	1429.15	1352.01	1431.08
Asym. C-O-C str. (υas) of aryl alkyl ether	~ 1250	Pavia-49	1243.30	1245.93	1253.64	1255.57	1255.57	1247.86	1255.99	1271.00	1257.50
Sym. C-O-C str. (υs) of aryl alkyl ether	~ 1040	Pavia-50	1000.99	1062.70	1060.78	1037.63	1029.92	1035.70	1027.99	1024.13	1037.63
C-O str. of aliphatic ester	1300-1000	Pavia-63	1186.14	Absent	Absent	Absent	absent	absent	Absent	absent	absent
C-N str. of 3º amine	1350-1250	Pavia-74	Absent	1315.21	1253.64	1296.08	1294.15	1294.15	1255.99	1293.00	1294.15
Aromatic =C-H out of plane def. for disubstituted (meta)	690, 780, 880	Pavia-41,43	700.11, 806.19, 864.05	700.11, 788.83, 900.70	673.11, 777.26, 860.19	717.47, 784.97, 908.41	707.83, 790.76, 850.55	705.90, 786.90, 850.55	694.33, 775.33, 892.96	721.33, 786.90, 879.48	723.26, 784.97, 850.55

PHARMACOLOGICAL EVALUATION

All the synthesized compoundswere evaluated for their nootropic potential in two phases. At first, acute toxicity study was conducted to determine the LD_{50} dose for the synthesized compounds. After that, compounds were tested for their nootropic potential by using behavioral model (elevated plus maze test).

Out of all synthesized compounds, compound **47** was an intermediate compound for the synthesis of target compounds **48-55**. Compound **47** is not expected to exhibit nootropic potential as it is an ester intermediate which will hydrolyze in body to corresponding acid and compounds possessing acidic groups become too polar to cross CNS. But still, we carried out the studies on compound **47** to evaluate the in-vitro and in-vivo differences as expected.

Acute toxicity study

Compounds **(47-55)** were found to be toxic at 100 mg/kg and non-toxic safe up to 5 mg/kg body weight by oral route. The dose was fixed at 50 mg/kg as cutoff LD_{50}. So dose level i.e. 1, 3, 5 and 10 mg/kg were selected for present study. The toxicity data were represented by 'Irvin' table for acute toxicity.

Behavioral Study

Behavioral models for studying a drug or conditions that affect cognitive processes rely on stimuli to induce an aversive state within the organism. Once an animal has learned to escape from aversive events, the next favourable strategy is to try to avoid those aversive events totally.

Elevated plus maze test

Elevated plus maze[85] model is a very sensitive and extremely validated behavioral model for studying cognition processes. The test measures the transfer latency (TL) i.e. the time in which the mouse moves from open arm to the enclosed arm. Transfer latency (TL) on elevated plus maze was used as an index of learning and memory processes. The time taken by each mouse to move from the end of open arm to any enclosed arm of elevated plus maze was measured on 1st day and 2nd day of drug treatment[82]. The results are expressed as % retention (Mean ± S.E.M) calculated as:

$$\frac{\text{TL on } 1^{st} \text{ day - TL on } 2^{nd} \text{ day}}{\text{TL on } 1^{st} \text{ day}} \text{ X } 100$$

The mean ± S.E.M. value for control was 27.20 ± 1.37 (s). All the behavioral data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's test.

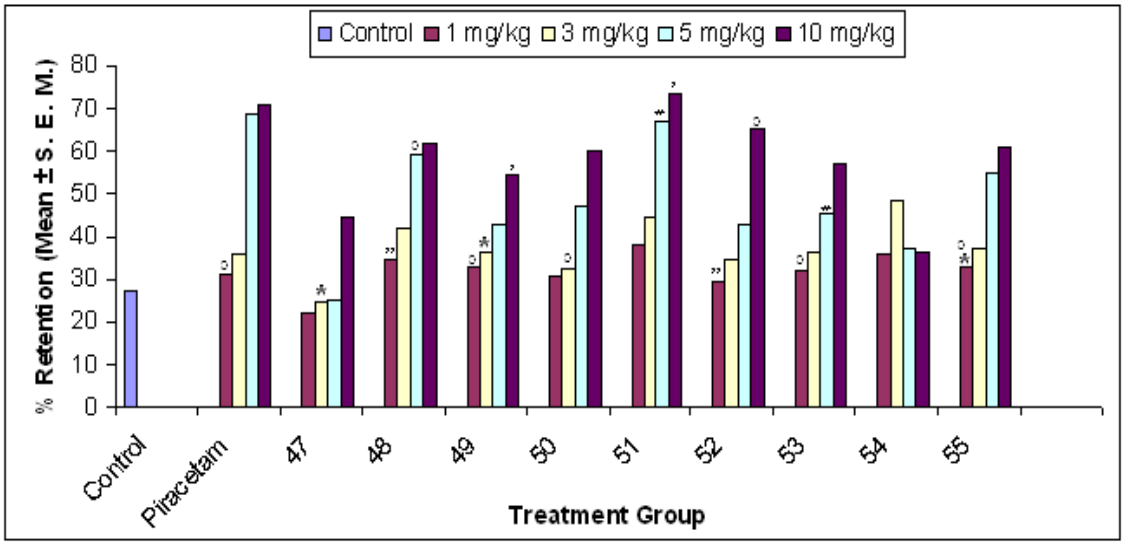


Figure 12: Effect of various compounds and reference drug (piracetam) (1, 3, 5 and 10 mg/kg, i.p.) on % retention measured on elevated plus maze in mice. "p ≤ 0.001 as compared to the reference drug (1 mg/kg) *p ≤ 0.001 as compared to the reference drug (3 mg/kg). $^{\#}$p ≤ 0.001 as compared to the reference drug (5 mg/kg). 'p ≤ 0.001 as compared to the reference drug (3 mg/kg) and $^{\circ}$p≤ 0.001 as compared to control.

BIOCHEMICAL EVALUATION

Biochemical studies of the synthesized compounds were carried out to study their acetylcholinestrase inhibiting profile.

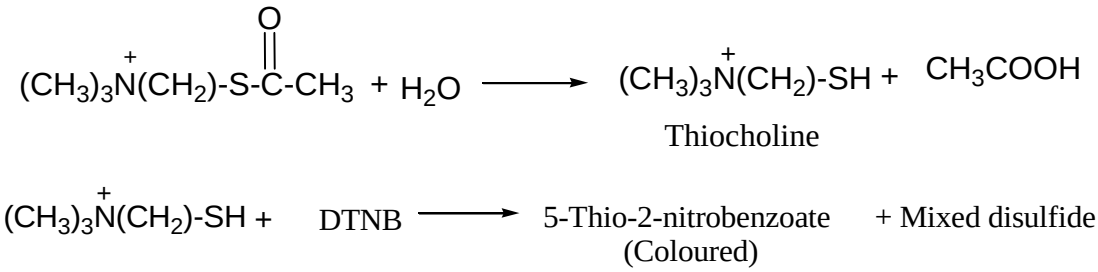
1 ASSAY OF ACETYLCHOLINESTERASE

The activity of acetylcholinesterase was determined in the brain homogenate according to the Ellman et al (1961) method.

Principle

The substrate used in the assay system is acetylthiocholine iodide, the ester of thiocholine and acetic acid. The substrate, acetylthiocholine is hydrolyzed into thiocholine and acetate by the enzyme AChE. Thiocholine forms mercaptan, which reacts with the oxidizing agent 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) to form 5-thio-2-nitrobenzoate, which has a maximum

absorption at 412 nm. Thus the activity of AChE can be measured by following an increase in absorbance at 412 nm.



Tissue homogenization

Theanimals were fasted overnight and sacrificed by decapitation. The brains were removed, rinsed in ice-cold isotonic saline (0.9% w/v NaCl), blotted dry, weighed separately. A 10% (w/v) tissue homogenate was prepared in 50 mM phosphate buffer saline (pH 7.4) using Potter-Elvehjem glass homogenizer.

Reagents

- 0.1 M Sodium phosphate buffer (pH 8.0)
- 10 mM DTNB (Ellman's reagent)
- 14.9 mMAcetylthiocholine iodide

Procedure

The reaction mixture consisted of 2.8 ml of 0.1 M phosphate buffer (pH 8.0), 0.1 ml of Ellman's reagent, 0.3 ml of drug and 10 µl of brain homogenate. The reaction was initiated by the addition of 0.1 ml acetylthiocholine iodide and the rate of change in absorbance was measured at 412 nm for 2 min. Neostigmine was used as positive control.

Calculations

AChE activity was calculated using molar extinction coefficient of 5-thio-2-nitrobenzoic acid (14.15×10^3 $M^{-1}cm^{-1}$). The results were expressed as nmols of acetylthiocholine iodide hydrolyzed/min/mg protein.

$$\frac{\text{Change in absorbance per minute}}{14150} \times \frac{\text{Volume of assay}}{\text{mg of protein by protein estimation}}$$

2 ESTIMATION OF PROTEIN CONTENT

The protein content was estimated according to the method of Lowry[90] *et al*, 1951.

Principle

This method is based on the colour reactions of amino acids tryptophan and tyrosine with the Folin's phenol reagent. These amino acids react with phosphomolybdic acid and phosphotungstic acid (present in Folin's reagent) to give blue colour, which is estimated colourimetrically. This colour is the result of reduction of phosphomolybdic acid and phosphotungstic acid and biuret reaction of proteins with Cu^{2+} ions in alkaline medium.

Reagents

- Reagent A: 1 % (w/v) Copper sulphate solution.
- Reagent B: 2 % (w/v) Sodium potassium tartarate.
- Reagent C: 2 % (w/v) Sodium carbonate in 0.1 N Sodium hydroxide.
- Lowry's reagent: It was prepared just before use by mixing reagents A, B and C in the ratio of 1:1:98.
- Folin-Ciocalteau reagent: It was prepared fresh by diluting the commercial 2N Folin's reagent with double distilled water (1:1, v/v).
- Standard bovine serum albumin (BSA) solution (1 mg/ml).

Procedure

0.1 ml of suitably diluted sample of brain homogenate, BSA standard and water (for blank) were taken separately in different test tubes and the final volume was made 1.0 ml with double distilled water. 3.0 ml of Lowry's reagent was added to all the tubes, vortexed and allowed to stand for 10 min at room temperature. Further, 0.5 ml of Folin-Phenol reagent was added to each of the tubes, vortexed for 30 s and allowed to stand for 30 min at room temperature. The absorbance was measured at 750 nm.

Calculations

The protein concentration was calculated from the standard curve made by taking different concentrations of the BSA standard.

$$\frac{\text{Volume of homogenate X sample OD}}{\text{0.372 (Standard)}} \times \frac{x}{1000} = \text{mg/ml protein}$$

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