KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.

17BCU612A DRUG BIOCHEMISTRY PRACTICAL Semester VI

1. Handling of small experimental animals

2. Route of drug admistration – Oral and parenteral

3. Behavioural Changes upon drug Adminstration

4. Liver toxicity Studies

   SGOT

   SGPT

   GGT

5. Renal toxicity studies

   Urea

   Uric acid

   Creatinine

6. LD_{50} Determination

REFERENCE BOOKS

1. Hamilton, D., Philips, R.J., and Scott, D., (2004). Occupational, Industrial and Environmental Toxicology, Moshy Inc Publishers.

2. Berg, G.M.I., Hendrickson R.G., and Morocco, A., (2005). Medical Toxicology Review. McGraw Hill Mical Publishing Company**.**

3. Foye, W., (2012). Principles of Medicinal Chemistry, 7th edition, B.I. Wanerly Pvt. Ltd, New Delhi.

4.Grahame-Smith, D.G., and Aronson, J.K., (2002). Oxford textbook of ClinicalPharmacology and Drug Therapy: 3rd edition. Oxford University Press.

5. Tripathy, K.D., (2009). Essentials of Medical Pharmacology, Jaypee brothers medical publishers, New Delhi.

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DEPARTMENT OF BIOCHEMISTRY

PRACTICAL PLAN

SUBJECT NAME: DRUG BIOCHEMISTRY PRACTICAL

SUB.CODE: 17BCU612-A

SEMESTER: VI **CLASS: III B.Sc., BIOCHEMISTRY**

S.No	Topics to be Covered	Supporting material with Page No.
1.	Handling of small experimental animals	T3:517 - 525
2.	Route of drug administration –oral and parental	T1: 100 – 101
3.	Behavioral Changes upon drug Administration	T1: 100 – 101
4	Liver toxicity studies: assay the activity of SGOT in serum	T1: 293 - 295
5.	Assay the activity of serum in SGPT	
6.	Assay the activity of GGT serum	
7.	Renal toxicity studies : Assay 1:Estimation of urea in blood	T1: 161 - 162
8.	Renal toxicity studies : Assay 2:Estimation of uric acid in blood	T1: 204 - 295
9.	Renal toxicity studies : Assay 3:Estimation of creatinine in blood	T1: 199 - 201
10.	LD_{50} Determination	T2: 140
11.	Model practical examination	

REFERENCE BOOKS

1. Hamilton, D., Philips, R.J., and Scott, D., (2004). Occupational, Industrial and Environmental Toxicology, Moshy Inc Publishers.

2. Berg, G.M.I., Hendrickson R.G., and Morocco, A., (2005). Medical Toxicology Review. McGraw Hill Mical Publishing Company.

3. Handling and restraints by Thomas Burege and Tilla waiss

4. Foye, W., (2012). Principles of Medicinal Chemistry, 7th edition, B.I. Wanerly Pvt. Ltd, New Delhi.

5. Grahame-Smith, D.G., and Aronson, J.K., (2002). Oxford textbook of ClinicalPharmacology and Drug Therapy: 3rd edition. Oxford University Press.

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(For the candidates admitted from 2017 onwards)

DEPARTMENT OF BIOCHEMISTRY

COURSE MATERIAL

STAFF NAME : Dr.K.Poornima
SUBJECT : DRUG BIOCHEMISTRY PRACTICAL
SUBJECT CODE : 17BCU612-A
SEMESTER : VI **CLASS : III B.Sc. Biochemistry**

PRACTICAL SYLLABUS

1. Behavioural Changes upon drug Adminstration.
2. Liver toxicity Studies
   - SGOT
   - SGPT
   - GGT

3. Renal toxicity studies
   - Urea
   - Uric acid
   - Creatinine

4. LD_{50} Determination

1. ASSAY THE ACTIVITY OF SGOT (OR) ASPARTATE TRANSAMINASE

AIM

To determine the activity of aspartate transaminase in the given serum sample.

Principle

Aspartate transaminase catalyses the reverse inter conversion between aspartate and glutamate and their 2 oxo analogues.

L-Aspartate +α-oxoglutarate $\xrightarrow{SGOT}$ Oxaloacetate + L-glutamate

The oxalo acetate is measured by colorimeter by a reaction with 2, 4 – dinitro phenyl hyrdrazine giving a brown coloured hydrazone derivative after the addition of 0.4N sodium hydroxide. The colour developed is read at 520 nm.

Reagents

1. Aspartic acid
2. α-keto glutaric acid
3. Pyruvic acid (sodium salt)
4. Sodium hydroxide
5. Disodium hydrogen phosphate
6. Dinitrophenyl hydrazine
7. Chloroform
8. Potassium hydrogen phosphate
9. Potassium dihydrogen phosphate
10. Phosphate buffer

Phosphate buffer (0.1M, pH 7.4)

Solution A

Disodium hydrogen phosphate – 14.2g/L

Solution B

Potassium dihydrogen phosphate – 13.6g/L

The buffer is prepared by mixing 420ml of Sol.A and 80 ml of sol.B.

SGOT Buffered substrate C:

Dissolve 2.66g of aspartic acid and 29.2mg of α keto glutaric acid in 1N NaOH and made up to 100 ml with phosphate buffer. The pH is adjusted to 7.4.

Dinitro phenyl hydrazine

20mg of dinitro phenyl hydrazine in 100 ml of 1N hydro chloric acid.

Stock pyruvate (20 mmol)

220mg of sodium pyruvate dissolved in 100 ml of phosphate buffer and stored in Refrigerator. The concentration is 2.2mg.

Working standard

10 ml of stock standard is diluted to 100 ml with phosphate buffer. The concentration is 220µg/ml which is 20µm/ml.

PROCEDURE

Into a series of test tubes pipette out 0.1 to 0.5ml of working standard pyruvate solution corresponding to the concentration range 02 to 1.0 µg/ml and made up the volume in all the test tubes to 1.0 ml by adding buffered substrate. 1ml of buffered substrate is taken in different test tubes labelled as control and test. 0.2 ml of serum is added to the test alone.

0.2ml of phosphate buffer is added to all the test tube and the std tubes are incubated at 37^0 C for 30 minutes and the control and test are incubated for 1hrs.

At the end of the incubation 1ml of DNPH is added to all the test tubes and 0.2 ml of serum is added to control, tubes were incubated at 37^0 C for 15mins.

Then 10ml of sodium hydroxide is added to all the tubes and the brown colour development is read at 540nm.

From the graph obtained by plotting concentration range on x axis and the corresponding optical density values on y axis. The enzyme activity is calculated.

Normal range – 3-16IU/Litre.

RESULT

The serum aspartate transaminase activity was found to be ----------

CALCULATION

Colorimeter reading____corresponds to_____µm of pyruvate

That is 0.2 ml of serum contains____µm of pyruvate

Therefore 1000 ml of serum liberate $\frac{0.1 \times 1000}{0.2}$

=_____µm of pyruvate

Activity of SGOT is given by $\frac{=}{60}$

=_____IU/litre

REAGENTS	B	S1	S2	S3	S4	S5	C	T1	T2
Volume of working standard (ml)	-	0.1	0.2	0.3	0.4	0.5	-	-	-
Concentration of working standard (µg)	-	0.2	0.4	0.6	0.8	1.0	-	-	-
Volume of buffered substrate (ml)	1.0	0.9	0.8	0.7	0.6	0.5	1.9	1.0	1.0
Volume of phosphate buffer (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Volume of serum (ml)	-	-	-	-	-	-	-	0.2	0.2
Incubate at 37ºC for one hour									
Volume of DNPH (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Volume of serum (ml)	-	-	-	-	-	-	0.2	-	-
Incubate at 37ºC for 15 minutes									
Volume of sodium hydroxide (ml)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Optical density at 540 nm									

2. ASSAY THE ACTIVITY OF SGPT (OR) ALANINE TRANSAMINASE

Aim

To assay the activity of alanine amino trasferase in serum.

Principle

When serum is incubated with substance containing α keto glutarate and L-alanine L-glutamate and pyruvate are formed.

The enzyme catalyses the following reaction:

L-Alanine + α-oxoglutarate ⟶ Pyruvate + L-glutamate

Pyruvate so formed is coupled with 2, 4- dinitro phenylhydrazine to give the corresponding hydrazone which gives a brown colour which is measured at 540 nm.

Reagents

11. L alanine
12. α-keto glutaric acid
13. Pyruvic acid (sodium salt)
14. Sodium hydroxide
15. Disodium hydrogen phosphate
16. Potassium dihydrogen phosphate
17. Di nitro phenyl hydrogen

1. Phosphate buffer (0.1M, pH 7.4)

   Add 11.3g dry anhydrous disodium hydrogen phosphate and 2.7g dry anhydrous potassium dihydrogen phosphate in one litre volumetric flask and make up to the mark with water. Check the pH and store at 4ºC.

2. Buffered substrate:

   Dissolve 1.78g of alanine and 29.2mg of α keto glutaric acid in 1N NaOH and made up to 100 ml with phosphate buffer. The pH is adjusted to 7.4.

3. Dinitro phenyl hydrazine

   20mg of dinitro phenyl hydrazine in 100 ml of 1N hydro chloric acid.

4. Stock pyruvate (20 mmol)

   220mg of sodium pyruvate dissolved in 100 ml of phosphate buffer and stored in refrigerator. The concentration is 2.2mg.

5. Working standard

   10 ml of stock standard is diluted to 100 ml with phosphate buffer. The concentration is 220µg/ml which is 20µm/ml.

6. 0.4 N NaOH: Dissolved 16 g of NaOH in 1000 ml water

Procedure

0.1 – 0.5 ml of pyruvate standard of concentration range 0.2 – 1µm is pipette out in to a series of test tubes. The volume is made up to 1.0 ml with buffered substrate. 1.0 ml of buffered substrate serves as blank.

Added 1.0 ml of buffered substrate and 0.2 ml of phosphate buffer to the test and control tubes. Added0.2 ml of serum to the test alone. The standards, test and control were incubated along with the blank at 37^{o}C for 30 minutes. At the end of incubation period added 1.0 ml of 2, 4 dinitro phenyl hydrazine to all the tubes. To the control tube added 0.2 ml of serum after arresting the reaction with 2, 4 dinitro phenyl hydrazine. All the tubes are incubated at 37^{o}C for 15 minutes. At the end of incubation period added 10.0 ml of 0.4N NaOH to all the tubes. The brown colour developed was read at 540 nm after 10 minutes.

From the standard graph obtained by plotting the concentration of pyruvate on x axis and optical density value on y axis the enzyme activity is measured.

Normal value: 4 – 17IU/litre.

Result

The activity of SGPT in serum is found to be_____IU/litre.

REAGENTS	B	S1	S2	S3	S4	S5	C	T1	T2
Volume of working standard (ml)	-	0.1	0.2	0.3	0.4	0.5	-	-	-
Concentration of working standard (µg)	-	0.2	0.4	0.6	0.8	1.0	-	-	-
Volume of buffered substrate (ml)	1.0	0.9	0.8	0.7	0.6	0.5	1.9	1.0	1.0
Volume of phosphate buffer (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Volume of serum (ml)	-	-	-	-	-	-	-	0.2	0.2
Incubate at 37ºC for about 30 minutes									
Volume of DNPH (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Volume of serum (ml)	-	-	-	-	-	-	0.2	-	-
Incubate at 37ºC for 15 minutes									
Volume of sodium hydroxide (ml)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

Optical density at 540 nm									

CALCULATION

Colorimeter reading____corresponds to_____µm of pyruvate

That is 0.2 ml of serum contains____µm of pyruvate

Therefore 1000 ml of serum liberate $\frac{0.1 \times 1000}{0.2}$

=_____µm of pyruvate

Activity of SGOT is given by $\frac{=}{30}$

=_____IU/litre

2. Estimation of Urea
DAM TSC Method

Aim

To estimate the amount of urea present in the given unknown and serum sample.

Principle

Urea directly reacts with diacetyl monoxime in the presence of thiosemicarbazide to form a red coloured product which is measured colorimetrically at 540nm.

Reagents

1. Diacetyl monoxime:

   1.56 g of diacetyl monoxime in 250 ml of distilled water.

2. Thiosemicarbazide:

   41 mg of thiosemicarbazide in 250 ml of distilled water (stored in brown bottle)

3. Acid reagent:

   (a) Ferric chloride reagent:

   324 mg of ferric chloride is dissolved in 10ml of 56% ortho phosphoric acid (Stored in brown bottle).

   (b) 20% sulphuric acid:

   20ml of sulphuric acid in 80ml of water.

   In 1 litre of 20% sulphuric acid added 1ml of ferric chloride reagent.

4. Stock standard urea solution:

   Dissolved 100mg of urea in 100ml of distilled water. 1.0ml of this solution contains 1mg of urea.

5. Working standard urea solution:

   2.0ml of stock standard solution was made upto 100ml with distilled water. 1.0ml of this solution contains 20 µg of urea.

Procedure

Into a series of test tubes pipetted out 0.5, 1.0, 1.5, 2.0 and 2.5ml of working standard urea solution corresponding to 10, 20, 30, 40 and 50 µg of urea respectively. The given unknown solution was made up to 100ml with distilled water and from this 1.0ml was taken for the experiment. To 0.2 ml of serum added 1.8 ml 0f 10% tri chloro acetic acid and centrifuged for 10 minutes at 3000 rpm and from this 0.5 ml was taken for the experiment. The volumes in all the test tubes were made-up to 3.0ml with distilled water. Then added 1.0ml of diacetyl monoxime, 1.0 ml of thiosemi carbazide and 3.0ml of acid reagent to all the test tubes. Mixed well and heated vigorously in a boiling water bath for 20 minutes. Along with this a blank was also treated in a similar way. Remove the tubes, cooled and read against the reagent blank at 540nm in a colorimeter.

A standard graph was drawn by plotting the concentration of urea on x axis and colorimeter reading on y axis. From the standard graph the concentration of urea in the given unknown solution was calculated.

Result

The amount of urea present in the given unknown solution is---------- mg/dl

The amount of urea present in 100 ml of the given serum sample is_____mg/dl

Estimation of Uric Acid

S.No	SOLUTION		Volume of distilled water(ml)	Volume of sodium carbonate (ml)	Volume of Uric acid reagent (ml)	Allowed to stand for 15 minutes	Optical density at 640nm
	Volume in ml	Concentration in µg					
1	Blank	-	3.0	1.0	1.0		
	Standard						
2	0.5	10	2.5	1.0	1.0		
3	1.0	20	2.0	1.0	1.0		
4	1.5	30	1.5	1.0	1.0		
5	2.0	40	1.0	1.0	1.0		
6	2.5	50	0.5	1.0	1.0		
	Unknown						
7	1.0	-	2.0	1.0	1.0		
8	1.0	-	2.0	1.0	1.0		

Calculation:

Optical density of_x_ corresponds to_y_ µg of uric acid

1.0 ml of the given unknown solution contains _y_ µg of uric acid.

∴100 ml of the given unknown solution contains $\frac{y \times 100}{1.0 \times 1000}$

= __z__mg

3. Estimation of Uric Acid
Caraway Method

Aim

To estimate the amount of uric acid present in the given unknown solution.

Principle

Uric acid reduces sodium phosphotungstic acid in the given alkaline medium to give a blue colour which is measured colorimetrically using a red filter at 640nm.

Reagents

1. 14% Sodium carbonate solution
2. Uric acid Reagent:

   Dissolved 5g of sodium tungstate in 400 ml of distilled water. Added 40ml of 84% phosphoric acid and refluxed gently for 2 hours. Cooled, transferred to 500ml standard flask and made upto the mark with distilled water. Stored the reagent in a brown bottle. Diluted 1ml to 10ml before use.

3. Stock standard:

   Weighed about 100mg of uric acid in a small beaker. Also weighed 60mg of lithium carnbonate into the beaker. Added 15-20ml of water. Heated the solution to about 60^{o}C and poured into 100ml standard flask and made upto the mark with distilled water.

4. Working standard solution:

   Diluted 2ml of stock standard to 100ml with distilled water. 1ml of the working standard solution contains 20µg of uric acid.

Procedure

Into a series of test tubes pipetted out 0.5, 1.0, 1.5, 2.0 and 2.5ml of working standard solution corresponding to the µg values 10, 20, 30, 40 and 50 respectively. The given unknown solution was made upto 100ml with distilled water. From that 1.0ml was taken for the experiment. The volume of all the test tubes were made upto 3.0ml with

distilled water. To all the test tubes added 1ml of uric acid reagent follower by 1.0ml of 14% sodium carbonate solution and allowed it to stand for 15minutes and the blue colour developed was read in the colorimeter at 640nm against a reagent blank in a colorimeter.

A standard graph was drawn by plotting the concentration of uric acid on x-axis and the colorimeter reading on y-axis. From this the concentration of uric acid present in the unknown solution was calculated.

Result

The amount of uric acid present in 100ml of the given unknown solution is found to be ---------- mg.

S.No	SOLUTION		Volume of distilled water(ml)	Volume of DAM (ml)	Volume of TSC	Volume of acid reagent (ml)	Allowed to stand for 15 minutes	Optical density at 540nm
	Volume in ml	Concentration in µg						
1	Blank	-	3.0	1.0	1.0	3.0		
	Standard				1.0	3.0		
2	0.5	10	2.5	1.0	1.0	3.0		
3	1.0	20	2.0	1.0	1.0	3.0		
4	1.5	30	1.5	1.0	1.0	3.0		
5	2.0	40	1.0	1.0	1.0	3.0		
6	2.5	50	0.5	1.0	1.0	3.0		
	Unknown				1.0	3.0		
7	1.0	-	2.0	1.0	1.0	3.0		
8	1.0	-	2.0	1.0	1.0	3.0		

5. Estimation of creatinine - Caraway method

Aim:

To estimate the amount of creatinine present in the given blood sample.

Principle:

Creatinine is estimated by making use of jaffe s reagent in which the creatinine gives an orange yellow color with alkaline picrate solution whose value is measured calorimetrically at 540nm**.**

Reagents:

1. **Stock standard solution**

   100mg of creatinine in 100ml of 0.1N HCL. Coc.is 100g/ml

2. Working standard solution

   4.0 ml of the stock is made upto 100ml with distilled water. Conc. Is 0.04mg/ml.

3. 10% NaOH
4. 1% Picric acid solution
5. 5% sodium tungstate
6. 2/3N sulphuric acid

Preparation of serum sample:

Dilute 2ml of serum/plasma with 2ml of distilled water and precipitate the protein by adding 2ml of sodium tungstate and 2ml of 2/3N sulphuric acid. Added the reagent dropwise with constant shaking, allow to stand for 10mins and differed. Collect 3ml of supernatant and 1ml of picric acid and 1ml of sodium hydroxide.

Procedure:

0.4, 0.8, 1.2, 1.6 and 2.0ml of working standard creatinine solution corresponding to µg respectively 16,32,48,64 & 80 µg values are pipetted out into a series of test tubes.

3ml of supernatant collected from serum sample pipetted out into another test tube.

The volume of the tubes are made upto 8ml with distilled water. A blank is also made upto 8ml with distilled water. 1ml of 10% sodium hydroxide and 1ml of 1% picric acid to all the test tubes and mixed well.

The orange yellow color was developed after 15min was read at 540nm against the reagent blank. A standard graph was drawn by plotting creatinine concentration on x axis and optical density an y axis through which the amount of creatinine was calculated.

Result:

The amount of creatinine present in the given blood sample is found to be-------

The amount of creatinine present in the 100ml of unknown solution is found to be -------

S.No	SOLUTION		Volume of distilled water(ml)	Volume of Na2CO3 (ml)	Volume of Picric acid	Allowed to stand for 15 minutes	Optical density at 540nm
	Volume in ml	Concentration in µg					
1	Blank	-	8.0	1.0	1.0		
	Standard				1.0		
2	0.4	16	7.6	1.0	1.0		
3	0.8	22	7.2	1.0	1.0		
4	1.2	48	6.8	1.0	1.0		
5	1.6	64	6.4	1.0	1.0		
6	2.0	80	6.0	1.0	1.0		
	Unknown				1.0		
7	3.0	-	5.0	1.0	1.0		
8	3.0	-	5.0	1.0	1.0		

Calculation:

Optical density of_x_ corresponds to_y_ µg of creatinine

1.0 ml of the given unknown solution contains _y_ µg of creatinine.

∴100 ml of the given unknown solution contains $\frac{y \times 100}{1.0 \times 1000}$

=___z___mg