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Effects of methanol extract of *Salacia senegalensis* leaves on some biochemical and haematological indices of albino mice.

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Abstract

Background and Aims: Herbal medicine is one of the crucial regime disease treatment and management in African. Nevertheless, *Salacia senegalensis* among many of the plants used are yet to be authenticated scientifically. Therefore the effects of the leaves of methanol extract of *Salacia senegalensis* on some biochemical and haematological parameters were assayed.

Method: The albino mice (six per group) weighing 18-22 g used for the haematology and biochemical studies were grouped into A (control) given 5 mL normal saline/kg/body weight per day, B (given 1000 mg), C (given 1200 mg) and D given 1400 mg extracts respectively/kg body weight per day for one week. Spectrophotometry and colourimetry methods (Haematology Autoanalyzer) were used in the assay**.** Biochemical parameters assayed were serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein, while haemoglobin (Hb), platelets (Plat), total white blood cell (WBC), lymphocyte (Lymp) and neutrophil (Neut) levels were the haematological indices measured.

Results: The haematological results showed that the extract produced a dose-dependent increase in haemoglobin concentration in all the animal groups which were significantly higher than that of mice in the control group ($P < 0.05$). The platelets levels were significantly reduced compared to the control group, while the white blood cells, lymphocytes and neutrophils levels of the animals in groups B-D were significantly increased compared to the control ($P < 0.05$). The biochemical results showed that the serum aspartate aminotransferase and alanine aminotransferase levels were significantly increased compared to the control ($P < 0.05$), while a decrease was observed in protein levels compared to the control ($P > 0.05$). The weights of the animals were not significantly altered compared to the control group. Photomicrographs of the extract-treated albino mice livers showed a moderate inflammatory response at the extract doses used.

Conclusion: The results suggest a mild hepatotoxic effects after one week (prolonged) treatment with the methanol extract of *Salacia senegalensis* leaves at various doses used.

Keywords: *Salacia senegalensis*, haematology, biochemical, herbal, indices

Introduction

The use of plants as medicines is known by man in time immemorial. Such plants are called medicinal plants or herbal plants. A Medicinal plant refers to any plant which, in one part or more of its organ, contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. Medicinal plants constitute one of the main sources of new pharmaceuticals and healthcare products (Zhao, 2007). World Health Organization (WHO) estimates that 80 % of the population living in developing countries depends on herbal medicines for their primary health care (PHC) needs (WHO, 2001).
The evaluation of all medicinal plant is based on phytochemical and pharmacological approaches which lead to drug discovery and it is referred to as "natural product screening" (Foye, *et al.*, 2008). According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards the identity and the degree of purity of plant materials and should be carried out at first before any tests are undertaken. Any part of the plant may contain active components like bark, leaves, flowers, roots, fruits, seeds *etc* (Gordon and David, 2001). Secondary products from the plants are responsible for its action or pharmacological activity.

Studies have showed that haematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals (Sexena, *et al*., 2011; Ohaeri and Eluwa 2011). Haematological parameters are used to determine the extent of deleterious effect of plant extracts on blood of an animal. Straus, (1998), Onyeyilli, *et al.*, (1998) and Adedapo, *et al*., (2007) reported that reduction in RBC, Hb and PCV is an indication of either the destruction of RBC or their decreased production, which may lead to anaemia. The measurement of the activities of various enzymes in tissues and body fluids plays a very significant role in disease investigation and diagnosis (Malomo, 2000). Increase in the serum levels of AST and ALT (especially ALT) are reported to be associated with liver injury or damage (Mukherjere, 2003).

Many plants of Nigeria origin including *Salacia senegalensis* have been traditionally claimed to be medicinal. It is therefore very necessary that such plants used by the local people be scientifically investigated to prove their ethnotherapeutic activity or performance. Therefore the effects of the leaves of methanol extract of *Salacia senegalensis* on some biochemical and haematological parameters were assayed.

Materials and Methods

Determination of haematology indices

(Automated method used- impedance cell counts)

Erma PCE 210N Haematology Autoanalyzer used

Procedure

Reagent Installation

The reagents used are known as lysing (haemolysing reagent) and diluents (Isotonic diluents). Both reagents were placed at the instrument level and the electronic sensors, yellow for lysing reagent and red for the diluents were checked to know the level of the reagents.

Start-up Sequence

The analyser was then powered on and left to complete background check. Once this was completed, the analyzer was then ready for the analysis.

Sample preparation

Twenty seven pre-diluted tubes were filled with dilute reagent from the Haematology Analyser using the dispense function. The diluents reagent was discarded. Then one after another the tubes were filled again. One millilitre of each sample was added to each tube containing diluents using a micropipette. The tubes were titled for a while (20 seconds each) to ensure proper mixture of the diluents and sample one after the other before identification and aspiration.

Sample Identification

The new sample button on the main screen of the Haematology Analyser was pushed to begin sample identification. Numerical values were then displayed on the screen for sample identification number to be entered. The sample identification number was then entered. Then the "ok" button was pushed to save the sample identity (ID) and begin sample aspiration.

Sample Analysis

Aspiration of the sample was carried out by gentle inserting the aspiration needle into the sample tube. The whole blood start plate was "powered on" and aspiration immediately started. At the sound of a beep, the sample was removed. Then 45 seconds later, results were displayed on the sample menu. The displayed results were recorded. When the new sample button turned green, the procedure for sample identification and analysis was repeated for the remaining samples.

Determination of biochemical indices

Assay of serum alanine transaminase activity

The serum alanine transaminase (ALT) or alanine aminotransferase activity was determined by Reitman and Frankel, (1957) using Randox Text Kits (Randox Laboratories Ltd., Crumlin, England, United Kingdom).

Principle

Alanine transaminase activity determined by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophyenylhydrazine.

$$\alpha\text{-Oxoglutarate} + \text{L-Alanine} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

Procedure

Two test tubes were set up labeled T_1 (reagent blank) and T_2 (test sample). T_1 contained 0.10 mL of distilled water and 0.50 mL of Randox buffer solution, while T_2 contained 0.10 mL of serum and 0.50 mL of Randox buffer solution. The contents were mixed and incubated for 30 min at 37 ^{o}C. To each tube was added 0.50 mL of Randox 4-dinitrophenylhydrazine solution and the contents were mixed and allowed to stand for 20 min at 25 ^{o}C in a water bath. Then 5 mL of sodium hydroxide solution was added to each of the tubes. The contents were mixed, and after 5 min their absorbances were read at 546 nm against the reagent blank in the spectrophotometer.

Calculation

The activity was obtained by intrapolation from table provided in kit's leaflet, reproduced in Table A1 at the appendices.

Assay of serum aspartate transaminase activity

The serum aspartate transaminase (AST) or aspartate aminotransferase activity was determined by Reitman and Frankel, (1957) using Randox Text Kits (Randox Laboratories Ltd, Crumlin, England, United Kingdom).

Principle

Aspartate transaminase was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

$$\alpha\text{-Oxoglutarate} + \text{L-Apartate} \xrightarrow{\text{AST}} \text{L-Glutamate} + \text{Oxaloacetate}$$

Procedure

Two test tubes were set up labeled T_1 (reagent bank) and T_2 (test sample). T_1 contained 0.10 mL of distilled water and 0.50 mL of Randox buffer solution, while T_2 contained 0.10 mL of plasma and 0.50 mL of Randox buffer solution. The contents were mixed and incubated for 30 min at 37 ^{o}C. To each tube was added 0.50 mL of Randox 4-dinitrophenylhydrazine solution and the contents were mixed and allowed to stand for 20 min at 25 ^{o}C. Then 5 mL of sodium hydroxide solution was added to each of the tubes. The contents were mixed, and after 5 min their

absorbance were read at 546 nm against the reagent blank in the Spectrophotometer.

Calculation

The activity was obtained by intrapolation from Table provided in kit's leaflet, reproduced in Table A2 at the appendices.

Determination of serum protein concentration

Estimation of serum Total Protein Concentration

This was carried out by the Biuret Method (Tietz, 1999), using Randox Test Kits (Randox Laboratories Ltd. Crumlin, England, United Kingdom).

Principle

Alkaline copper solutions react with peptide bonds in protein to produce a violet colour whose intensity is directly proportional to the amount of protein present.

Materials

These include (i) the samples (serum). (ii) Randox standard protein solution (58.48 g/L or 5.85 g/dL); Randox Biuret Reagent (R1) [contains 100 mMNaOH, 16 mM Na-K-tartrate, 15 mM potassium iodide and 6 mM cupric sulphate] (iii) Spectrophotometer (model 752S (Spectrumlab)], timer, water bath, test tubes, test tube rack and pipettes.

Procedure

Three tubes were set up labeled T_1 (blank), T_2 (standard) and T_3 (test sample). T_1 contained 0.04 mL of distilled water and 2.00 mL Biuret Reagent, T_2 contained 0.04 mL of standard protein solution and 2.00 mL of Biuret Reagent, while T_3 contained 0.04 mL of serum and 2.00 mL of Biuret Reagent. The contents were mixed, incubated at 25 ^{o}C in a water bath for 30 min, before cooling and reading of absorbance at 560 nm against the blank, in a spectrophotometer.

Calculation

Total protein concentration (g/L)

$$= \frac{Absorbance_{\ sample} * concentration\ of\ standard\ (g/L)}{Absorbance_{\ standard}}$$

Histopathology

Liver samples were carefully excised from sacrificed animals and introduced into separate specimen bottles filled with 40 % formaldehyde prior to histological assay. The liver tissues were subsequently processed and tissues blocks were sectioned and stained via haematoxylin and eosin (H & E) technique. The photomicrographs of the stained tissue block sections were taken via an Olympus microscope with in-built camera.

Data analysis

Statistical Package for Social Sciences (SPSS) version 20.0 was used in the statistical analyses and the means compared at 95 % levels of confidence using one way analysis of variance (ANOVA).

Results

Table 1, showed the effect of methanol extract of *Salacia senegalensis* leaves on haemoglobin (Hb) levels (g/dl) in albino mice. The Hb levels were not significantly affected ($p<0.05$) at the various doses of the extract administered, but differed significantly compared to the control group given normal saline at ($p<0.05$). The Hb levels of the groups treated with various doses of the extract were higher than that of the control group administered normal saline.

Table 1. Effect of methanol extract of *Salacia senegalensis* leaves on some Haematological indices of albino mice

Treatment	Hb (g/dl)	Plat (/mm^3)	tWBC (/mm^3)	Lymp (%)	Neut (%)
Normal saline (5ml/kg b.w)	9.33 ±0.48^b	276500.00 ±32004.69^a	4900.00 ±470.11^c	82.17 ±2.56^b	15.00 ±2.83^b
Extract (1000mg/kg/b.w)	12.30 ±0.27^a	110833.33 ±1329.16^b	6700.00 ±1080.74^{bc}	77.83 ±1.47^b	18.83 ±4.40^b
Extract (1200mg/kg/b.w)	13.70 ±2.67^a	84233.33 ±56328.38^c	10133.33 ±2761.64^a	88.67 ±7.87^a	16.83 ±14.14^b
Extract (1400mg/kg b.w)	13.18 ±0.63^a	247500.00 ±66488.34^d	7525.00 ±871.64^b	70.00 ±4.10^c	29.33 ±4.03^a

Values are means ± standard deviation, n= 6.Values with same superscripts in the same column are not significantly different from each other (P>0.05).

Platelet counts (/mm^3)in the group of albino mice administered various doses of the extract were significantly lower than that of the control group given normal saline as shown in Table 1. A decrease in platelet levels (/mm^3) were observed at 1000 mg and 1200 mg of the extracts which were significantly lower (p>0.05) compared to the control group as shown in Table 1. However, a slight increase in platelet counts of mice given 1400 mg extract was observed which was still lower than the control group administered normal saline.

White blood cell counts (/mm^3) in albino mice administered various doses of the extract were significantly higher than that of the control group given normal saline as shown in Table 1. White blood cell counts (/mm^3) in albino mice given 1000 mg and 1200 mg showed a significant dose-dependent increase compared to the control group at (P<0.05).

A dose-dependent decrease in lymphocyte levels (%) was observed at 1000 mg and 1400 mg of the extract compared to the control group as shown in Table 1, while a significant increase (P<0.05) in lymphocyte count was noticed at 1200 mg extract compared to the control group administered normal saline.

The highest extract dose (1400 mg) produced a significant increase (P<0.05) in neutrophil levels (%) compared to the control, while there were no marked significant changes in neutrophil counts at extract doses of 1000 mg and 1200 mg respectively.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (iu/l) in extract (at various doses) treated mice were significantly higher (P<0.05) than that of the control group administered normal saline as shown in Table 2. While significant decrease (P>0.05) was observed in protein levels (g/dl) in extract treated albino mice compared to the control group as. This increase was not dose-dependent.

Table 2: Effect of methanol extract of *Salacia senegalensis* leaves on some Biochemical indices of albino mice

Treatment	AST (iu/l)	ALT (iu/l)	Protein (g/dl)
Normal saline (5ml/kg b.w)	28.83 ± 4.31^{c}	24.50 ± 4.32^{c}	10.00 ± 2.28^{a}
Extract (1000mg/kg/b.w)	60.17 ± 12.06^{ab}	44.33 ± 7.74^{b}	5.42 ±0.31^{b}
Extract (1200mg/kg/b.w)	39.50 ± 10.27^{bc}	39.33 ± 14.00^{bc}	5.12 ± 0.57^{b}
Extract (1400mg/kg b.w)	77.83 ± 17.99^{a}	66.83 ± 8.86^{a}	5.00 ± 0.49^{b}

Values are means ± standard deviation, n= 6.Values with similar superscripts in the same column are not significantly different from each other (P>0.05).

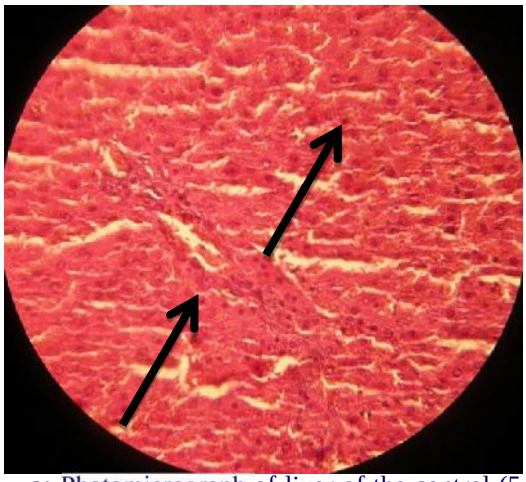
The effects of the extract on the albino mice body weights (g) are shown in Table 3. Results showed that there were no marked significant changes (P<0.05) in the mice body weight at various doses of the extract within the period of the study.

Table 3: Effect of methanol extract of *Salacia senegalensis* leaves on the albino mice body weights

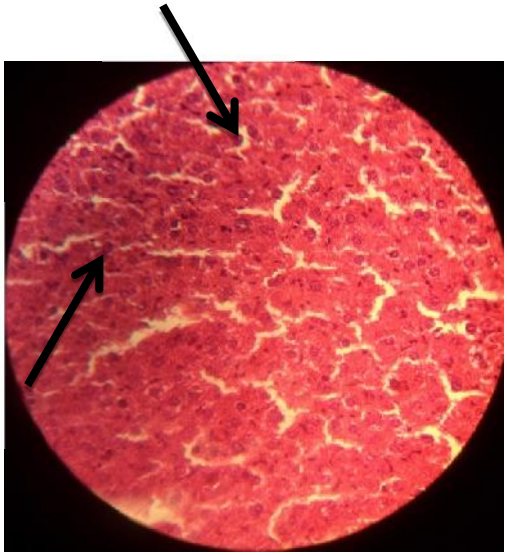
Treatments	Weights(g) Day 0	Weights(g) Day 8
Normal saline (5ml/kg b.w)	19.97±1.24^{a}	20.13 ± 0.98^{b}
Extract (1000mg/kg b.w)	20.48±0.62^{a}	20.73 ±0.78^{b}
Extract (1200mg/kg b.w)	20.28±0.68^{a}	19.95 ±0.69^{b}
Extract (1400mg/kg b.w)	20.18±1.15^{a}	20.00±1.10^{b}

Values are means ± standard deviation, n= 6. Values in the same column with same superscript are not significantly different from each other (P>0.05).

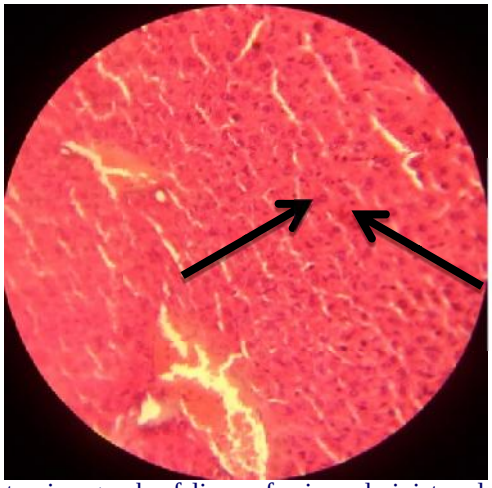
The photomicrograph of liver cells of the extract treated mice showed a moderate dose-dependent inflammatory response compared to the normal saline treated group (control) with normal hepatocytes as presented in Figure 1(a-d).



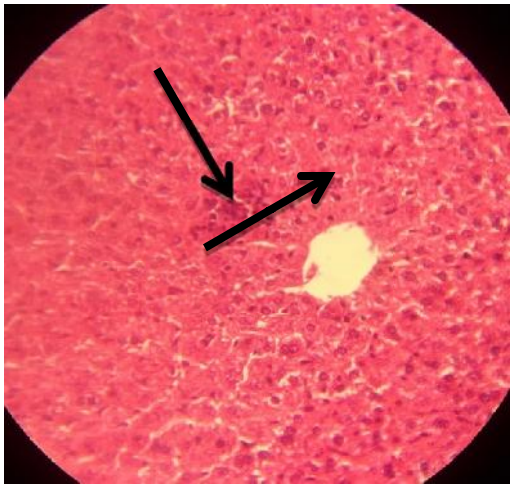
a: Photomicrograph of liver of the control (5ml /kg b.w. normal saline) showing(arrows) preserved hepatocytes (H&E x40)



b: Photomicrograph of liver of mice administered 1000 mg /kg b.w of the extract showing(arrows) inflammatory response amidst balanced nucleo-cytoplasmic ratio



c: Photomicrograph of liver of mice administered 1200 mg /kg b.w of the extract showing(arrows) inflammatory response amidst balanced nucleo-cytoplasmic ratio



d: Photomicrograph of liver of mice administered 1400 mg /kg b.w of the extract showing(arrows) inflammatory response amidst balanced nucleo-cytoplasmic ratio

Figure 1: Liver histology of albino mice treated with various doses of methanol extract of *Salacia senegalensis* leaves

Discussion

Studies have showed that haematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals (Sexena, *et al*., 2011; Ohaeri and Eluwa 2011).

Haematological parameters are used to determine the extent of deleterious effect of plant extracts on blood of an animal. Straus, (1998), Onyeyilli, *et al.,* (1998) and Adedapo, *et al*., (2007) reported that reduction in RBC, Hb and PCV is an indication of either the destruction of RBC or their decreased production, which may lead to anaemia.

A dose-dependent increase observed in Hb levels (Table 1) indicates that the extract had positive effects on haemopoiesis (Iranloye, 2012; Mansi and Lahham, 2008; Kuppast, *et al*., 2009, Okpuzor, *et al*., 2009). This may be attributed to the presence of iron (Adumanya, *et al.*, 2015) and quercetin (Adumanya, *et al.*, In press). Iron is associated in haemopoiesis (Adeyeye and Otoketi, 1999) and quercetin has an established anti-anaemic activity (Sen, *et al*., 2005). This highlights the potential of the plant leaves in the management of anaemia.

Adedapo *et al*., (2008) and Adeniyi *et al*., (2010) have reported that reduced blood platelets affects the viscosity of blood, which is correlated positively to blood pressure. As shown in Table 1, the *Salacia senegalensis* leaves extract significantly reduced the blood platelet counts which may produce negative effect on the blood viscosity. This also suggests that the extract has a negative effect an megakaryocyte which regulates platelets production and release (Arise, *et al*., 2013). Platelets plays major role in mediating blood clotting, modulating inflammatory and immune responses. This is achieved by the regulated expression of adhesive and immune receptors on the platelet surface and by the release of a multitude of secretary products including inflammatory mediators and cytokines, which can mediate the interrelation with leukocytes and enhance the recruitment (von Hundelshausen and Weber, 2007).

The observed significant increase in total WBC in extract treated mice (Table 1) suggests a possible stimulation of immune defense system (Evans, 2008; Mohajeri, *et al*., 2007). This may have been elicited by the immune-sitmulatory activity of saponins (Soetan, 2008; European Food and Safety Authority, 2009) and tannic acid (Chung, *et al*., 1998), which were abundantly present in the leaves of *Salacia senegalensis* (Adumanya *et al*., In press). Stress and poisoning from drugs are among the main causes of raised WBC count (leukocytosis).

Lymphocytes are the main effectors cells of the immune system (Saliu, *et al*., 2012). Significantly increase in lymphocyte count at 1200 mg extract may be an indication of immune-stimulation. While significant reduction in lymphocyte level at 1400 mg of the extract could suggest otherwise.

Neutrophil counts were significantly increased only at 1400 mg extract, compared to the control (administered normal saline). The most important role of neutrophil is phagocytosis (cellular of an offending agent) by the process of opsonization (Guyton and Hall, 2006).

The measurement of the activities of various enzymes in tissues and body fluids plays a very significant role in disease investigation and diagnosis (Malomo, 2000).

Table 2 showed that serum AST and ALT levels were significantly increased in the extract treated mice compared to the control (administered normal saline). This suggests liver impairment or hepatocellular damage compared to the control group. Increase in the serum levels of AST and ALT (especially ALT) are reported to be associated with liver injury or damage (Mukherjere, 2003). The ratio of AST to ALT is useful in differentiating between causes of liver damages (Nyblom, *et al*., 2004; Nyblom, *et al*., 2006). Liver injury is characterized as hepatocellular when there is predominant elevation of the ALT, while AST is a mitochondria enzyme whose increase activity in plasma reflects severe tissue damage (Martins, 2006). The liver injury suggested by the elevated AST and ALT levels correlates with the inflammation observed in the liver cell photomicrograph of the extract treated mice (Figure 1). This could be because of prolonged (one week) administration of the extract which contained pyrollizidine alkaloids-intermedine and lycopsamine (Adumanya, *et al*., In press) with established hepatotoxic effects (Zalkow, *et al*., 1985; Helferich and Winter, 2001; Gomes, *et al*., 2007; FAO/WHO, 2011).

There was significant decreases (P>0.05) in the serum total protein levels of the extract treated albino mice (Table 2) compared with the control. This may be due to the reduction in protein synthesis exerted by the extract (Momoh, *et al*., 2014). Hypo-proteinaemia is a common finding in liver damage (Larrey, 2002). This was observed in the present study.

The weight of the mice (Table 3) were not significantly changed (P<0.05). This may indicates that the extract did not impose any acute weight loss, or gain in the animals (Alberti and Zimmet, 1998).

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Conflict of interest: None

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Appendices

Table A1: Conversion Table for alanine aminotransferase (transaminase) assay

Absorbance	U/L	Absorbance	U/L	Absorbance	U/L	Absorbance	U/L
0.025	4	0.150	25	0.275	48	0.400	72
0.050	8	0.175	29	0.300	52	0.425	77
0.075	12	0.200	34	0.325	57	0.450	83
0.100	17	0.225	39	0.350	62	0.475	88
0.125	21	0.250	43	0.375	67	0.500	94

Table A2: Conversion Table for aspartate aminotransferase assay

Absorbance	U/L	Absorbance	U/L	Absorbance	U/L	Absorbance	U/L
0.020	7	0.060	19	0.100	36	0.140	59
0.030	10	0.070	23	0.110	41	0.150	67
0.040	13	0.080	27	0.120	47	0.160	76
0.050	16	0.090	31	0.130	52	0.170	89

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