

## The survey of effect of Kombucha tea on activity of hepatic UDPGT enzyme in mice

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### ABSTRACT

**Background and aims:** Kombucha is an ancient food and healing source with Asian origin. Kombucha consists of a wide range of acids, including vitamin C, organic materials, enzymes, and B-group vitamins, which has provided it with immense value. This study was aimed to investigate the effects of Kombucha tea consumption on hepatic UDPGT enzyme in mice.

**Methods:** In this experimental study, 21 small male albino mice and CD-1 genus were used. Albino mice species were purchased from serum Institute in Karaj Hisarak. Mice with a weight between 18 to 25 g were selected. Animals were kept in triplex group in polycarbonate cages. Animal rooms were equipped with air-conditioner. Ambient temperature was retained at about 22°C, and humidity 50%. A light cycle was set at 12 hours brightness and 12 hours darkness. The intensive diet was used that produced by animal Pars feeds for mice feeding. Finally, UDPGA enzyme was measured.

**Results:** Consumption of kombucha tea for seven days of experiment caused a significant increase in enzyme activity in mice liver UDPGT compared to the negative control group, from the first day until the seventh day of experiment.

**Conclusion:** Kombucha tea induced the UDPGT enzyme; thus, it accelerated the detoxification of the body and should be cautioned about simultaneous administration of this beverage with some drugs (such as steroids, acetaminophen, cardio-vascular medicine, etc.) that they metabolize through conjugation. Medical community in this case must be justified and their opinion must be considered when people are using this tea. Finally, kombucha tea consumption did not increase the liver weight. So, UDPGT induction was not the reason of liver weight increase.

**Keywords:** Kombucha, Liver, UDPGT, Mice, Iran nonpharmacologic nursing care.

### INTRODUCTION

Currently, the use of herbs, fruits and vegetables have increased due to their protective effect against diseases such as cancer, hepatic and cardiovascular diseases.<sup>1,2</sup> In traditional medicine, many

materials have been used to treat various disorders and diseases. One type of materials that have therapeutic effect in traditional medicine is Kombucha. Kombucha has been used in traditional

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medicine to treat many diseases. Kombucha is an ancient food source with healing effects that has an Asian origin. First time was discovered in China and was used as habits such as coffee. This drink tastes like cider often was produced at home by fermenting tea, sugar and fungus.<sup>3,4,5</sup> Kombucha consists of special bacteria and yeasts living in symbiosis, of which *Acetobacter xylinum* is the most important one. This organism ferments sugar in tea form a drink with Kombucha. Kombucha has a wide range of acids, including vitamin C, organic materials, enzymes, and vitamins group B, which provided it with immense value. Kombucha is an abundant natural source of glucuronic acid, which is not found in nature easily. The main function of Kombucha is its detoxification abilities.<sup>6-12</sup> The Kombucha is consumed widely in parts of the former Soviet Union, and Central Asia. It is used in Europe and the United States of America as a deterrent to disease and is beneficial for human health.<sup>13</sup> Kombucha tea has some useful effects in AIDS treatment, reduction in hair loss, reduction the symptoms of diabetes, arthritis, prolonging life, losing weight, improving mental capacity, treatment of cancer, reduction of blood pressure, increasing of strength, eliminating acne, pain reduction, increasing amounts of T cells, removing wrinkles, cleaning the gallbladder, relieving constipation and even restore gray hair to its original color.<sup>14</sup> Recent studies have introduced Kombucha as hepatoprotective, anti-stress, nephroprotective, anti-oxidant hypocholesterolemic and anti-microbial<sup>15-20</sup>. Kombucha drink has been suggested in America for the treatment of cancer, enhancing immune system, decreasing blood pressure, and relieving arthritis.<sup>9</sup> The main metabolites identified in fermented beverages containing lactic

acid, acetic acid, glucuronic acid, gluconic acid, ethanol and glycerol. The metabolic composition and concentration is depends on the source of mushroom tea, sugar concentration and fermentation time, induces fermentation process and pH and folic acid synthesis. The amount of B-complex vitamins of Kombucha is reduced during the fermentation process followed by an increase in organic acid content. Green and black tea is the best substrate for the production of acetic acid and gluconic acid respectively which are proper for growing Kombucha. Tea polyphenols [epigallocatechin and (EC) epicatechin, (ECG) epigallocatechin, (EGCG) gallate organic acids are the active ingredients in tea Kombucha] have widespread and useful effects.<sup>21-23</sup>

Despite many health benefits of this beverage, and despite being widely used in many populations around the world; yet its beneficial effects and probable adverse side effects have not been adequately studied.<sup>24, 25</sup> So, other beneficial effects are necessary to investigate. With respect to the specific ingredients of Kombucha and its beneficial therapeutic effects, this study was aimed to investigate the effects of Kombucha tea consumption on hepatic UDPGT enzyme in mice.

## METHODS

In this experimental study, small male albino mice and CD-1 genus were used.<sup>26,27</sup> Albino mice species were purchased from Serum Institute in Karaj Hisarak. Mice weight was selected between 18 to 25 g. Animals were kept in triplex group and in polycarbonate cages. Animal's rooms were equipped with air-conditioning equipment. Ambient temperature was retained at about 22 °C, and humidity 50%. A light cycle was set at 12 hours in brightness and 12 hours in

darkness. The intensive diet was used that produced by animal Pars feeds for mice feeding.

In the present study, the enzyme provocateurs were used by the oral solution of phenobarbital. Pure phenobarbital was used as 0.1% weight-volume.<sup>28-30</sup> After preparing the solution, pH was adjusted by the pH meter model of corning on the number 7.

To prepare Kombucha beverage, a number of two-liter Becher and a Pyrex glass container with a capacity of about two liters with the loose mouth was washed and was placed for two hours at a temperature of 120 °C in the oven to sterilize. After sterilizing necessary equipment, a liter of distilled water to which was added 200 grams of white sugar was spilled within a beaker and boiled for five minutes. Then, the flame turned off and two numbers of tea bags were added to solution to take tea. After preparation of tea, beaker placed in a clean place to reach room temperature. In the next part, the beaker contents transferred into a glass Pyrex and 100 ml of Kombucha tea that made from previous step was added to the fermented reactions as starter. Then, Kombucha cellulose disk was placed from bright side to side to the outside of the container in a Pyrex glass. Clean cloth was placed on mouth of the jar and tightly closed to prevent contamination and the container was put in a quiet, clean place and away from sunlight at temperatures between 22-20 °C for 9 days. At the end of the ninth day, the cloth was removed from the container and then the brighter disk which was belongs to baby was taken from container. Dissolved inside Pyrex container which was Kombucha beverages was smoothing through a clean lace and transferred into clean glass bottles in the fridge for use of animals.

To prepare the Kombucha beverages, a number of beakers with a capacity of two liters were used and sterilized. Distilled water was poured and heated to boiled and then boiled for five minutes. Then two tea bags were added to it. The simple tea was prepared after 10 minutes and was poured in a glass jar and was kept in the refrigerator.

In this study, three groups of 21 mice were selected. The first group of 21 mice was put in series A, and was divided into seven subgroups of three mice in seven separate cages and was called A1-A7. These mice were selected as positive control groups that received phenobarbital. The same as group A, 21 mice were put in group B and into seven subgroups of three mice in seven separate cages and was called B1-B7. These mice received Kombucha beverage and were selected as test group. Other 21 mice were put in group C and were divided into 7 subgroups and were named C1-C7. The mice in group C received simple tea as negative control group.

Oral administration time of phenobarbital solution to experimental animals in order to enzymatic stimulation was between five to seven days.<sup>28-31</sup> So, in this study, a period of seven day is considered to get soluble. In the first day, 40 ml phenobarbital 0.1% was poured in container instead of drinking water for group A. After over time of 24 hours at 8 o'clock, the exact amount of phenobarbital consumption was measured and recorded. Then group A1 was transferred to the laboratory for subsequent work. After this action, 40 ml soluble phenobarbital 0.1% was poured instead of drinking water in a pot of water A2-A7 groups. Thus, after the each day, one group of animals was eliminated from phenobarbital consumption and the rest groups were received fresh phenobarbital solution and this practice continued until the seventh

day in this way. This method was done in the same way for all three groups with the difference that in group B which was the test group, Kombucha beverages was used instead of drinking water and also group C which was the negative control group received simple tea instead of drinking water.

Liver tissue harvested from the animal's body should be placed in cooled STKM solution. Preparation method of STKM solution is as follows:

Sucrose 85.6 g (250 mM), Tris acid chloric 6.05 g (50 mM), potassium chloride, 1.864 g (25 mM) and magnesium chloride 1.01 g (5 mM) were dissolved in one liter of distilled water and PH was adjusted on 7.4 by HCl 0.1 M.<sup>29</sup>

50 ml ethanol was added to 100 ml of coomassie blue G-250. Then 10 ml of phosphoric acid 85% was added to the previous solution and then super solution volume was completed with distilled water to one liter. After a smoothing the beta, Whatman filter paper No. 1 was used to measure protein.<sup>29</sup>

1. Tris acid chloridric 50 mM: 0.60 g in 100 ml of distilled water.
2. Tris acid chloridric 300 mM: 3.6 g in 100 ml of distilled water.
3. Ortho- Aminophenol 2 mM and Ascorbic acid 1.6 mM: 22 mg Aminophenol and 40 mg Ascorbic acid in 100 ml of distilled water.
4. UDPGA 20 mM: 0.63 g in 5 ml distilled water.
5. TCA acidic buffer and NAH<sub>2</sub>PO<sub>4</sub> with pH 2.25: 16.3 g TCA and 13.8 g NAH<sub>2</sub>PO<sub>4</sub> in 100 ml of distilled water.
6. Sodium nitrite 0.1% weight/volume percent: 100 mg in 100 ml of distilled water.
7. Ammonium sulfamate 0.5 weight/volume percent: 500 mg in 100 ml of distilled water.

8. N-NEDA 0.1 weight/volume percent: 100 mg in 100 ml of distilled water.<sup>28</sup>

9. Aniline 400 μM: 0.52 mg in 100 ml of distilled water.<sup>28</sup>

Animal was killed by Spinal cord injury and the liver was removed quickly and was placed in STKM solution. After removing the excess tissue, the liver was washed several times with STKM solution. Tissue dried with filter paper, and then weighed and 2 ml of STKM solution per gram tissue was added.<sup>30</sup> Then, using long-handled scissors, liver was chopped. Chopped liver suspension was homogenized in a 25 mL tube by using a homogenizer Polytron pt 2000 model four times, and each time for about 20 seconds at 1000-2000 rpm<sup>28-31</sup> Homogenized suspension was centrifuged for ten minutes at 680 g by Beckman refrigerated centrifuge model R5417. Then, the supernatant was removed and centrifuged again for 15 min at 12000 g. The supernatant containing microsomes and santusol was placed in freezer -20 ° C and was used as a source of enzyme UDPGT.<sup>32</sup> After preparation of the liver microsomes of mice, the amounts of protein content of the liver of each mouse should be measured accurately, until at the end of experiment, all microsomes diluted with a certain concentration and were examined.

Bradford method is a method for measuring protein concentration which measurements was done using a protein absorbance standard curve of BSA changes in the concentration (amount) of protein. This is done as follows (Figure 1).

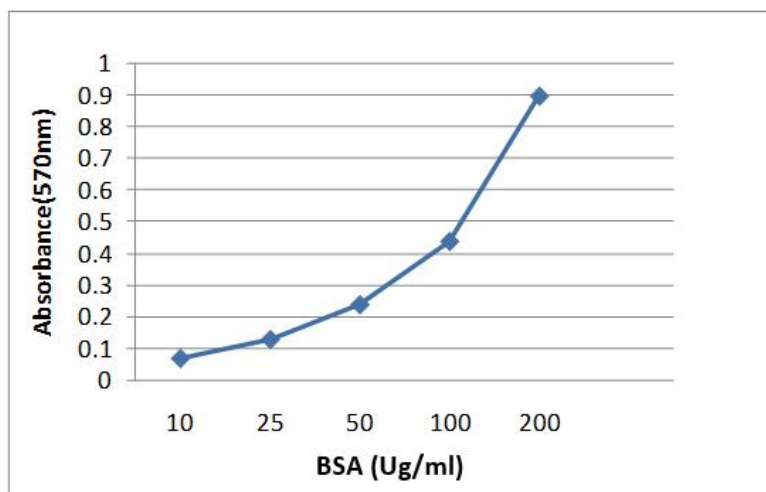


Fig 1: The absorbance level of BSA in various concentrations of protein

50  $\mu$ l from 10, 25, 50 and 100 BSA 200  $\mu$ g/ml was mixed with 200  $\mu$ l of coomassie blue and also in STKM buffer in ELISA plate tubes. After 5 minutes, light absorption was read at h 570 nm wavelength by a Microplate Reader device (model Dynex-MRX). STKM buffer was used as blank. Curve of changing Light- absorption at 570 nm and the concentration of BSA was obtained using the Linear Regression equation.<sup>29</sup>

To determine the amount of microsomal protein, it was performed the same as BSA protein. It means that prepared microsomes were diluted by adding STKM solution with amounts of 1000, 1200, 1400, 1600 and 1800 times, respectively. 50  $\mu$ l of each dilution and 200  $\mu$ l coomassie blue solution was spilled into ELISA wells micro plate and then mixed. After 5 minutes, absorption was read by a Microplate Reader at wavelength of 570 nm. Using optical absorption variation curves of STKM buffer as the blank, protein content of the microsomes was calculated.<sup>29</sup>

Aniline is similar to the 2-aminophenol in terms of chemical structure, thus because of an amino group in both combination, this two can act the same in formation reactions of diazonium salt.<sup>28</sup> 25, 50, 100, 200 and

400 mM Aniline concentrations were prepared in 50 mM Tris-HCl buffer and PH 6.7 saturated with Karbogen (O<sub>2</sub> 5% + CO<sub>2</sub> 95%). The amount of 112.5  $\mu$ l distilled water, 12.5  $\mu$ l Tris-HCl 300 mM with pH=7.4 and 25  $\mu$ l of each dilution which prepared from Aniline well mixed and then 25 ml of acidic buffer (NaH<sub>2</sub>po<sub>4</sub>-TCA) with pH 2.25 were added to it.<sup>33</sup> 50  $\mu$ l of sodium nitrite 0.1% weight-volume in 4 °C was added to 150  $\mu$ l of above mixture that was kept in 4 °C for 5 min. After two minutes, 50  $\mu$ l of cooled ammonium sulfamate 0.5% weight-volume was added to the reaction solution and was kept in 4 °C for three minutes. Finally 50 ml reagent N-NEDA 0.1 % was added to the above reaction. After 2 hours at ambient temperature, and away from light, the solution was read at 570 nm by Microplate Reader device.<sup>28</sup> Then with the help of absorbance of different dilutions of Aniline, Aniline effect was plotted in Diazuistion reaction.

Microsomes are saturated with 50 mM Tris-HCl buffer and pH 6.6. Then, they were diluted with carbogen gas up to 5.2 Mg/mL proteins. microsomes with 10 mg/ml of protein were used for the control group. 75  $\mu$ l UDPGA 25 mM, 20  $\mu$ l UDPGA 300 mM buffer with pH=7.4 were mixed with

distilled water in tube eppendorf. After placing the above combination in thermo mixer (model 5436) in 37 °C for 5 min, 25 µl ortho-aminophenol 2 mM was added to start the enzymatic reaction. Distilled water was used instead of aminophenol for blank. After mixing the reaction for 30 minutes in 37 °C 25 µl acidic buffer (NaH<sub>2</sub>PO<sub>4</sub>-TCA) 1 mM and pH=2.25 was added. The reaction was placed on ice for 5 min. After centrifuging in 10000 g for 10 min and at 4 °C, 150 µl of the supernatant was added into the wells of a micro plate containing 50 µl of sodium nitrite 0.1% w/v in 4 °C. After two minutes, 50 µl of cooled ammonium solution 0.5% w/v was added to reaction solution and 3 minutes was kept in 4°C. Then, 50 µl N-NEDA reagent was added to the above reaction and after 2 hours at

ambient temperature and away from light absorption of the solution was read at 570 nm by Microplate Reader device.<sup>28-30</sup>

## RESULTS

20 ul of each concentration moved to wells of micro plate then 180 ul from coomassie blue was added to content of each well and after 5 minutes, the absorption rate of the samples was read at 570 nm by Microplate Reader.

Various concentration of Aniline with 25 ul aminophenol 2 mM and 100 ul distilled water were used in reaction and the absorbance of each sample was read at a wavelength of 570 nm (Figure 2). The regression coefficient of above line is equal to diazotisation.

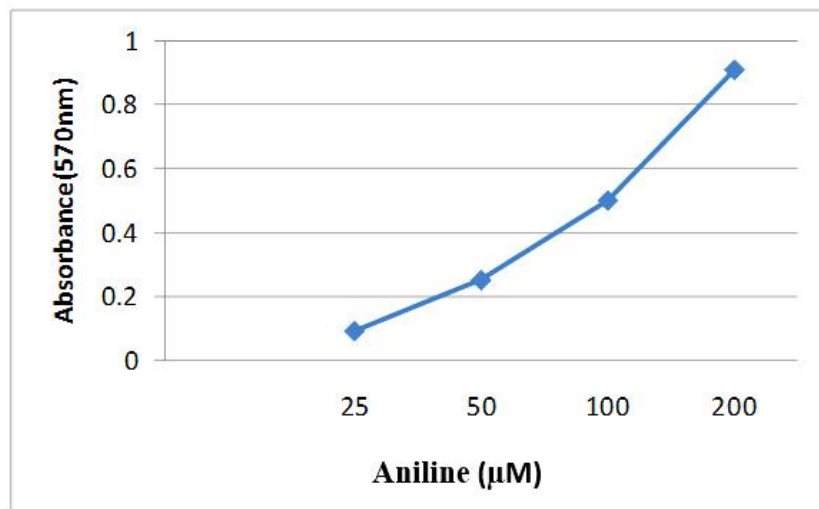


Fig 2: The absorbance level of Aniline in various concentrations of protein

25, 50, 100 and 200 uM concentrations of aniline in hydrochloric acid Tris buffer with pH 7.4 with an acidic buffer (NaH<sub>2</sub>PO<sub>4</sub>-TCA) with a pH of (1.2), (2.25) and (2.7) was used in formation of diazonium salt. Then, after 20 minutes rate of absorbance was read in 25 C and at wavelength of 570 nm by microplate

Reader. The optimal pH for formation of colored diazonium salt with free amino groups and regression coefficient was R<sup>2</sup>=0.9983 is 2.25. In another figure, a comparison of Aniline absorbance level in various concentrations of protein was showed (Figure 3).

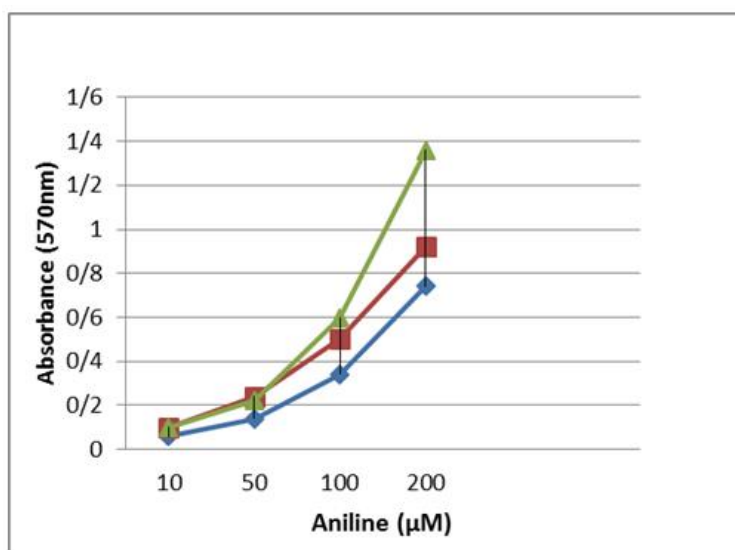


Fig 3: Comparison of Aniline absorbance level in various concentrations of protein

20 microsomes with UDPGA 2 µM and aminophenol concentration in 50 µM Tris-HCL and pH=7.4 were incubated in 37 C for 30, 45, 60, 90, 120 minutes. The reactions stopped by an acid (NaH<sub>2</sub>PO<sub>4</sub>-TCA) with pH= 2.25, then the steps of reaction were done

as follows in method and the amount of absorbance was read in wavelength of 570 nm by microplate Reader (Figure 4). Glucuronide aminophenol forms between 15 and 30 minutes are linear.

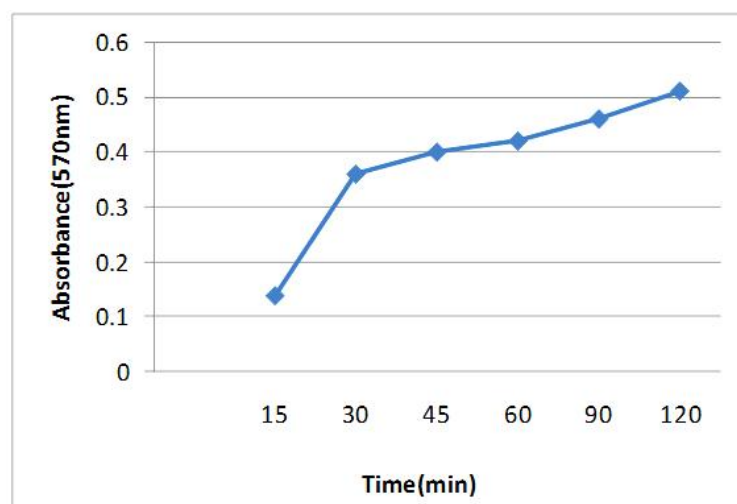


Fig 4: The absorbance level of aminophenol in different time

Various concentrations of microsomes with UDPGA 4 mM and aminophenol 1 mM in Tric-HCL 50 mM that its pH was 7.4 incubated in 37 C for 30 minutes. After that, the reactions stopped with an acid (NaH<sub>2</sub>PO<sub>4</sub>-TCA) that its pH was 2.25. The

diazotisation reaction was performed. Absorbance level of each sample at a wavelength of 570 nm was read by a Microplate Reader device (Mean ± SD). All points are belonging to three tests.

Various concentrations of aminophenol with UDPGA 4 mM and 20 mg/ml microsomes in Tric-HCL 50 mM that its pH was 7.4 incubated in 37 C for 30 minutes. After that the reactions stopped with an acid (NaH<sub>2</sub>PO<sub>4</sub>-TCA) that its pH was 2.25. The diazotisation reaction was performed. Absorbance level of each sample at a

wavelength of 570 nm was read by a Microplate Reader devices Mean  $\pm$  SD (Figure 5). All points are belonging to three tests. Between the 5.5–22 mM of ortho aminophenol concentration, rate of glucuronide formation in above enzymatic reaction is linear and rising bur from the 44 mM the reaction was reversed (Figure 6).

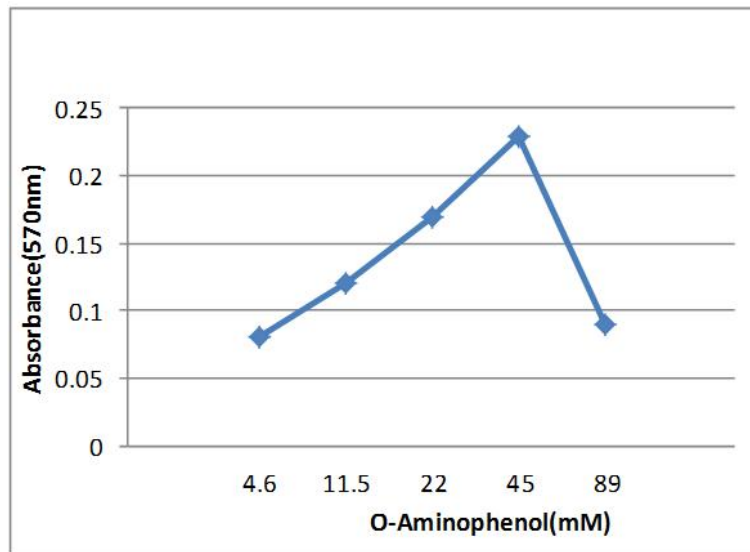


Fig 5: The absorbance level of O-aminophenol in various concentrations of protein

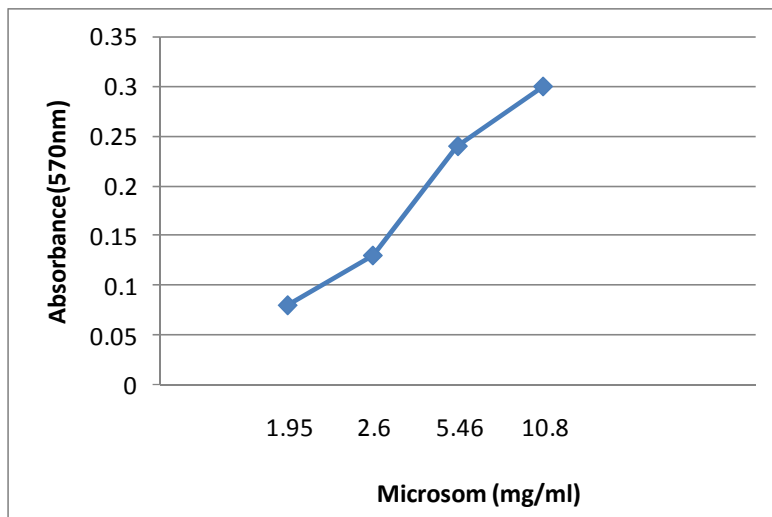


Fig 6: The absorbance level of microsomes in various concentrations of protein

Various concentrations of UDPGA with two 5 mg/ml and 10 mg/ml microsomes and aminophenol 2 mM in Tric-HCL 50 mM

that its pH was 7.4 incubated in 37 C for 30 minutes. After that the reactions stopped with an acid (NaH<sub>2</sub>PO<sub>4</sub>-TCA) that its pH



was 2.25. The diazotisation reaction was performed. Absorbance level of each sample at a wavelength of 570 nm was read by

Microplate Reader devices. The graph was linear between the 2 and 20 mM of UDPGA with 10 mg/ml microsomes (Figure 7).

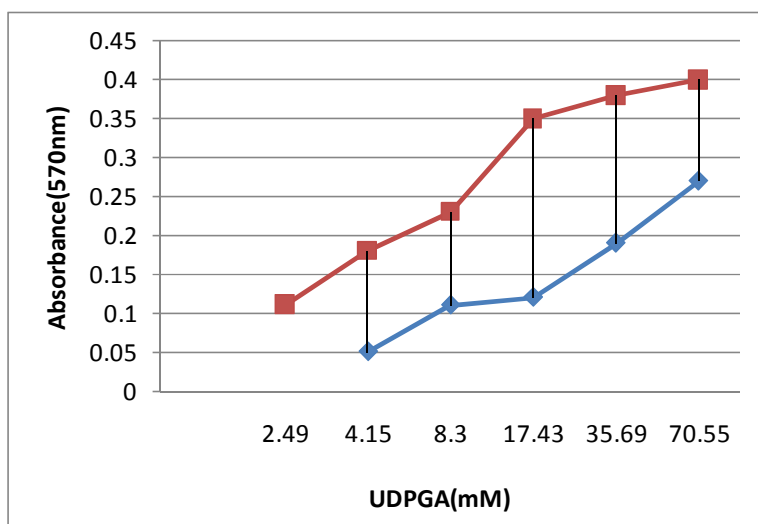


Fig 7: The absorbance level of UDPGA in various concentrations of protein

Mice received 40 ml of each of the three solutions instead of drinking water. Before killing the animal, separately weighed each day and then after killing its liver weighed too. All points in each day (Mean ± SD) were recorded for animals  $P < 0.05$ . Liver weight ratio to body weight

was increased in group receiving phenobarbital compared with the control group while appeared that this ratio in the group receiving kombucha tea had no increase compared to the control group (Figure 8).

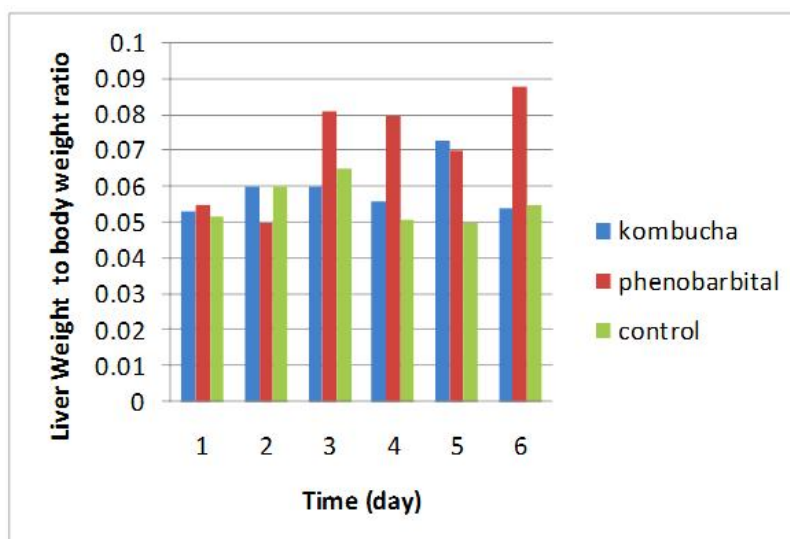


Fig 8: liver weight in different groups in different time

Phenobarbital, Kombucha simple tea was used by mice instead of distilled water. Three mice from each group were killed using colorimetric method was used to

measure the level of enzyme activity of hepatic UDPGT. All points in each of days Mean  $\pm$  SD are stated for animals  $P < 0.05$  (Figure 9).

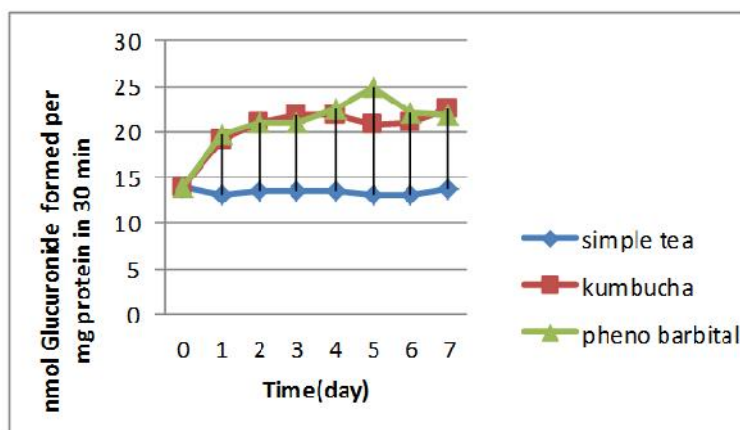


Fig 9: Formed Glucuronide in different groups in different time

## DISCUSSION

In this study, researchers tried to investigate the effect of Kombucha tea consumption in terms of pharmacokinetic on reactions of phase II metabolism. In this way the measurement of hepatic UDPGT enzyme activity in mice was selected as a response to change in phase II of conjugative reactions. Therefore, it was sought to examine the issue that whether the Kombucha tea had considerable effect on metabolism or not and whether or not increased in UDPGT enzyme activity.

The first result: Kombucha tea during a seven-day trial resulted in a significant increase in liver UDPGT enzyme activity in mice compared to the negative control group. This means that from the first day until the seventh day of the experiment, it was seen a significant increase in UDPGT activity. The positive control group that received orally a solution of 1% phenobarbital also caused a significant increase in liver UDPGT enzyme activity in these mice compared to negative control mice. Of course, this effect was expected and also was reported in previous researches

and the results of this study confirmed these reports. Thus, it can be concluded that the materials in simple tea such as theobromine, theophylline, and etc. have no effect on enzyme activity of hepatic UDPGT, but Kombucha tea at 81%  $\pm$  6.9 mL dose for each rat within 24 h caused a meaningful increase and was statistically significant in the rate of liver enzyme activity like phenobarbital, so kombucha tea is a inducer of UDPGT enzyme.

Thus, according to the reports from various sources, one of the most important ingredients in fermented Kombucha tea is GA and noted in the same sources that GA plays an important role in the process of liver detoxification and excretion xenobiotic, poisons and waste products from the body. Then, Kombucha tea, via increasing UDPGT enzyme activity is useful and effective in phase II detoxification reactions; it can help the liver to flash out toxins and waste products. Despite of above actions, it should be also understood that the excretion of many drugs which used by humans is also through the conjugation

phase II reactions. Medicine meanings such as morphine, diazepam, steroid hormone of contraceptive pills, antibiotics, etc. are excreted mainly through conjugation with GA. So, when an individual drinks kombucha tea with above drugs, he should see UDPGT enzyme the same as other inducers like phenobarbital and exactly pays attention to the interactions. The concomitant use of Kombucha tea and drugs that their metabolisms are through conjugation with GA, stimulate the metabolism of these drugs and decrease their half-life. So, they could not do their therapeutic effect. In these cases, users must use kombucha tea under their physician's opinion. So, some created modes resulting in kombucha tea consumption in different people attributed to increased excretion of toxins. It is caused by elevated levels of the enzyme UDPGT.

Another result of this study that was obtained was observation of the effect of phenobarbital on increasing liver weight in mice after the third day of testing compared to the negative and control groups. Changes in liver weight were considered. Since the first part of the project showed preparation of microsomal, the liver was weight due to adding the STKM solution; it was found that the liver of mice in positive control had a significant and meaningful increase from the third day of experiment compared to the negative and test control groups. However, this increasing weight has been mentioned in the credible references: Chronic administration of phenobarbital increased the amount of proteins, fats, enzymes, cytochrome 4-450 and GT enzymes. Increased liver weight due to phenobarbital consumption could be had several reasons that comment on this item is subject to the histopathology research in this regard. Due to the fact that carrying ahead the project in terms of toxicology of Kombucha tea like phenobarbital can increase liver weight, So

it can be concluded that increasing liver weight was not due to increasing enzyme UDPGT and kombucha did not such an increase in the liver like phenobarbital. Colorimetric diazotization method was chosen of the two mentioned methods for measuring GT enzyme activity. As mentioned in the introduction, Florimetric method is more sensitive, more accurate and less use of time and is also able to detect subtypes, and may also be induced, but the Colorimetric method was chosen because of:

1. In this method, all induced possible subtypes is measurable and also a pharmacology and experimentally test did not do on kombucha. Only this assumption was discussed about kombucha that this herb is useful in detoxification due to existence of GA, and at first the overall increase of GT enzymes should be evaluated in case of a positive result following induced subtypes.
2. Colorimetric method was used, because in Florimetric method, the diversity of inducers are high and also to measure the UDPGT enzyme by any inducer, several substrates are required for glucuronide formation, thus the UDPGA substrate consumption rate is high in this method, according to our country's economic problems.

Colorimetric method like any other method used for enzymatic measurement must set optimal conditions for measurement of enzyme at the first time. This work was done in two stages. First, according to diazotisation reaction, it was found maximum amount of colors occurrence in terms of concentration and which pH, so it was used aniline standard. Aniline at concentrations of 25 to 200  $\mu\text{M}$  in diazotisation reaction was completely linear and the slope of the obtained line was optimal.

The linear equation obtained from the graph is used to calculate the GT enzyme activity based on nmol. The effect of different pH on the rate of color

development in diazotisation reaction was performed by aniline standard and proper pH in this reaction was 2.25. The next step is to provide the best conditions for enzymatic reaction for glucuronide formation. In this step, the situation should be adjusted in terms of right time for maximum enzyme activity, and concentration. To compare the hepatic enzyme activity of animals, two works should be done: 1. Dividing total enzymatic activity of tissue to weight of tissue 2. Determining the protein concentration of the microsomes and expression of enzyme activity as the ratio of product to sample protein concentration that this way was better and more conventional. The advantage of the second method was to eliminate errors caused by broken down tissue during various stages of the experiment. Microsomes were prepared with a constant concentration of protein and enzyme reactions were tested at different times by forming glucuronide. 30-minute intervals of enzymatic reaction are a linear graph, and are determined the best time for enzymatic reaction of glucuronide aminophenol formation. It Means enzyme activity reached its peak and the highest possible level of glucuronide form appears in these time intervals. As it was mentioned, according to a protein concentration of microsomes different concentrations of microsomes contributed in constant concentration of UDPGA substrate to determine the enzyme activity of GT to identify in what concentration of microsomes, the graph is linear, which between 1.35– 5.5 mg of microsome protean is linear. This enzymatic reaction has two important components, amount of aminophenol and UPDGA. Figures 3-6 illustrated the gap between concentrations of 0.5 to 2 mM aminophenol; the reaction is linear and ascendant. Among these concentrations, 2 mM aminophenol is proper

for enzyme reaction. Figures 3-7 showed the application of different concentrations of UPDGA with two protein concentrations of the microsomes and suggested that the graph is linear in protein concentration of 10 mg/ml microsomes and in the interval from 2 - 20 mM UPDGA. Other important issues discussed in this study, was the use of kombucha tea and phenobarbital to laboratory animals. Due to the fact that the Kombucha tea used in traditional medicine orally and it was not possible to use Kombucha by injection, phenobarbital also can use in a period of five to seven days orally and as a solution in place of drinking water. However, it was decided that both solutions was placed instead of drinking water indefinitely.

## CONCLUSION

Kombucha tea induces the UDPGT enzyme; thus, it should be accelerated the detoxification of the body and should be caution about using of this beverage with some drugs (such as steroids, acetaminophen, cardio – vascular medicine, etc.) that they metabolized through conjugation. Medical community in this case must be justified and their opinions must be considered when people are using this tea. Finally, kombucha tea consumption does not increase the liver weight. So, UDPGT induction is not the reason of liver weight increase.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

## ACKNOWLEDGEMENT

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