

## ICASH-A38

# EFFECT OF TREE MARIGOLD (*Tithonia diversifolia* (HEMSL.) A. GRAY) LEAVES INFUSION AND ETHANOL EXTRACT ON BLOOD GLUCOSE LEVEL AND LIVER HISTOPATHOLOGY OF STREPTOZOTOCIN-INDUCED MALE WISTAR RATS

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### ABSTRACT

**Background:** Common Indonesians often use alternative therapy for Diabetes Mellitus (DM) due to its high prevalence. Tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves show its positive effect in decreasing blood glucose level. Thus, it is important to analyze the negative effect of consuming the proposing alternative including its toxicity level to vital organs. This study aims to observe the blood glucose level and liver histopathology in streptozotocin-induced diabetic male rats treated by infusion and ethanol extract of the tree marigold leaves.

**Methods:** Pretest and posttest with control group design experiment was conducted to analyze effect on blood glucose level and posttest only with control group design was for determining liver histopathology. Twenty-four male white rats (Wistar strain) were randomly divided into 4 groups: normal control group (KN), positive control group (K+) treated with 0.45mg/kg of glibenclamid, treatment group 1 (P1) treated by 100 mg/kg of tree marigold leaves ethanol extract, and treatment group 2 (P2) treated by 1373 mg/kg of tree marigold leaves infusion. The rats at the groups of K+, P1, and P2 were induced by streptozotocin to reach hyperglycemic state. Comparison of blood glucose level effect between infusion and ethanol extract was analyzed by One-Way ANOVA continued by post-hoc test, while comparison of liver histopathology effect was determined by Kruskal-Wallis continued by Mann-Whitney test.

**Results:** Significant differences of blood glucose level decrease were found in all groups. Group P2 significantly decreased blood glucose level more than P1 ( $p=0.000$ ) as much as 124.97 mg/dl and 101.46 mg/dl respectively. Significant differences of liver histopathology were obtained between KN vs K(+), KN vs P2, K(+) vs P2, and P1 vs P2. Group P2 showed more severe of liver histopathology changes than P1 ( $p=0.025$ ).

**Conclusion:** Tree marigold leaves extract is more effective in lowering blood glucose level and has less toxic effect to liver histopathology than its infusion.

**Keywords:** *Tithonia diversifolia* (Hemsl.) A. Gray leaves, blood glucose, liver histopathology

### INTRODUCTION

Hyperglycemia is a medical condition where blood glucose level increases more than normal. Hyperglycemia is one of characteristics of Diabetes Mellitus (DM) [1]. The increase of DM prevalence becomes one of global health problems [2] Basic Health Research (Riskesmas) by Ministry of Health in 2013 reported that DM proportion in Indonesian population is about 6,9% (females has higher number

than males) [3] In addition, International Diabetes Federation (IDF) report in 2015 revealed that Indonesia is the 7<sup>th</sup> country with highest DM prevalence (10 million for age rank 20 to 79 years old) and will increase to be 16.2 million in year 2040 [4].

Diabetes Mellitus can be generally treated by oral hypoglycemic drugs [4]. However, those medicaments have various adverse effects to certain people such as hypoglycemia, hepatotoxic, nephrotoxic, and many others. Thus, Indonesian people frequently seek and use alternative therapy for DM. They prefer to use traditional herbs or plants to lower blood glucose level in DM patients. One of those traditional plants often used is tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves.<sup>[5]</sup> The plant mostly grow wild in steep places. It is believed that the plant contains many compounds which are able to decrease blood glucose level. Previous studies have showed that tree marigold leaves extract and also infusion have hypoglycemic effect [5]. Nevertheless, in addition to its positive effect, it is important to also know whether the plant can cause toxic effect to vital organs [6]. Therefore, this research was conducted to compare the effect of tree marigold leaves extract and infusion to both blood glucose level and liver histopathology of streptozotocin-induced male Wistar Rats.

## MATERIALS AND METHODS

Ethical clearance approval No. 98/EC/FK/XI/2017 was obtained from Ethical Committee of Faculty of Medicine Universitas Swadaya Gunung Jati. This experimental research was conducted in the Laboratory of Food and Nutrition PAU and Laboratory of Pathology Anatomy of Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta from February to April 2018. Pretest and posttest with control group design was done for blood glucose level analysis and posttest only with control group design was for liver histopathology examination by using male white (Wistar strain) Rats as the research objects.

### *Animal protocol*

Twenty-four rats (of age three months old) whose weight 200-250 grams were divided randomly into 4 groups: group KN, group K(+), group P1, and group P2. After 7 days of adaptation (all rats were fed routinely with standard alimentation and the cages were cleaned daily) and randomized grouping, the rats were treated differently according to their group during 7 days. All groups received standard feeding and 5 ml aquadest. Group KN is normal control which was non-induced by streptozotocin-nicotinamide.. Group K(+) acts as positive control where the rats were induced by 65 mg/kg streptozotocin and 230 mg/kg nicotinamide intraperitoneal, and treated with 0.45 mg/kg glibenclamid and 5 ml aquadest [7,8]. Group P1 is treatment group which was induced by streptozotocin-nicotinamide and treated by 100 mg/kg of tree marigold leaves extract. Group P2 was also induced by streptozotocin-nicotinamide and treated by 1373 mg/kg of tree marigold leaves infusion. All the treatments were given one time daily in the morning.

### *Blood glucose level measurement*

Blood glucose level measurement was done twice: once after 3 days of streptozotocin induction before the treatment began (pretest) and once again after all treatment for 7 days was done (posttest). The blood was taken from retroorbital (ophthalmic venous plexus) by using hematocrit micropipette and the blood glucose level was measured by automatic glucometer.

### *Liver histopathology*

After 7 days of treatment, all the rats were cervically dislocated and the livers were taken. Liver histopathology slide preparation was done by fixating, washing, dehydrating, clearing, impregnating, embliding, cutting, staining, and mounting according to previous study. Six hematoxylin and eosin (H&E) stained liver sections were prepared for each rat in all groups [9]. Histopathology examination was analyzed by using binocular light microscope and counted in 5 viewing field with 400x objective lens. Liver histopathology was scored and graded using Manja-Roegnik score from 1 to 4. Score 1 for normal cells domination, score 2 if cells with parenchymatous degeneration are dominant, score 3 if hydropic degeneration is dominant, and score 4 if there are dominant necrotic cells [10].

*Statistical analysis*

Comparison of blood glucose level effect between ethanol extract and infusion was analyzed by One-Way ANOVA continued by LSD post-hoc test. Comparison of liver histopathology effect was determined by Kruskal-Wallis continued by Mann Whitney test.

**RESULTS**

*A. Blood Glucose Level*

*Blood Glucose Level Before (pretest) and After Treatment (posttest)*

Blood glucose level was measured before and after the treatment with tree marigold leaves. The difference between pretest and posttest blood glucose level of all groups was analyzed statistically with paired T-test. Table 1 reveals the average level of blood glucose from all four groups and that there is significant difference between pretest and posttest blood glucose level. KN is normal control group with no diabetic induction [11, 12].

Table 1. Mean decrease of blood glucose level

Group	n	Pretest ± SD (mg/dl)	Posttest ± SD (mg/dl)	Decrease ± SD (mg/dl)	p value
KN	6	76.64± 3.32	77.01± 3.43	-0.37± 0.29	0.025 <sup>a</sup>
K(+)	6	273.43± 6.82	141.63± 5.85	131.79 ± 1.32	0.000 <sup>b</sup>
P1	6	267.75± 4.68	166.29± 3.70	101.46 ± 3.06	0.000 <sup>b</sup>
P2	6	272.06± 5.88	147.08± 2.88	124.97 ± 5.64	0.000 <sup>b</sup>

<sup>a</sup> means significant increase

<sup>b</sup> means significant decrease

The highest decrease amount is shown by group K(+) with 131.79 ± 1.32 mg/dl, followed by P2 with 124.97 ± 5.64 mg/dl and P1 with 101.46 ± 3.06 mg/dl respectively. Decrease of blood glucose level presents in all groups except KN (Fig. 1).

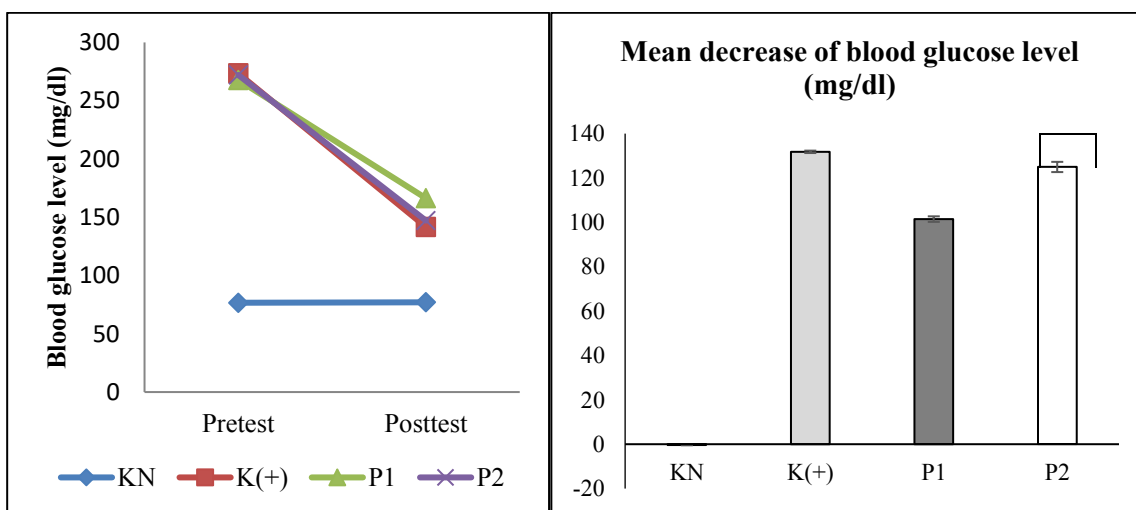


Fig. 1. Mean of Wistar Rats Blood Glucose Level. A. Graphic showing the lowering effect of administration of tree marigold leaves on blood glucose level (pretest and posttest). B. Mean decrease of blood glucose level of each group. (\* means significant decrease). KN is normal control, K(+) is positive control, P1 is group with tree marigold leaves ethanol extract, and P2 is group treated by tree marigold infusion.

*Comparison of Blood Glucose Level Decrease*

Blood glucose level decrease between each group was analyzed by using One-Way ANOVA test to know whether there is significant difference among them. One-Way ANOVA analysis showed that there are at least two groups whose average decrease is significantly different ( $p=0.000$ ).

All data have normal distribution and same variation, thus we performed LSD post-hoc test to see which pairs of groups have significant difference. Table 2 reveals LSD post hoc test result. It shows that significant difference presents in all pairs of groups with confidence interval (CI) 95%.

Table 2. LSD Post hoc analysis of blood glucose level average decrease comparison between groups

Groups	Difference of mean decrease	CI 95%		p value
		Minimum	Maximum	
K(+) vs P1	30.32	26.37	34.27	0.000
K(+) vs P2	6.82	2.87	10.76	0.002
P1 vs P2	-23.50	-27.45	-19.55	0.000

*B. Liver Histopathology*

All liver sections were scored from 1 to 4 according to its cells domination: normal cell, parenchymatous degeneration, hydropic degeneration, or either necrotic cell. Necrotic is the most severe degeneration. Histopathologic analysis of H&E stained liver sections revealed various types of degeneration in all groups except in group KN (Fig. 2).

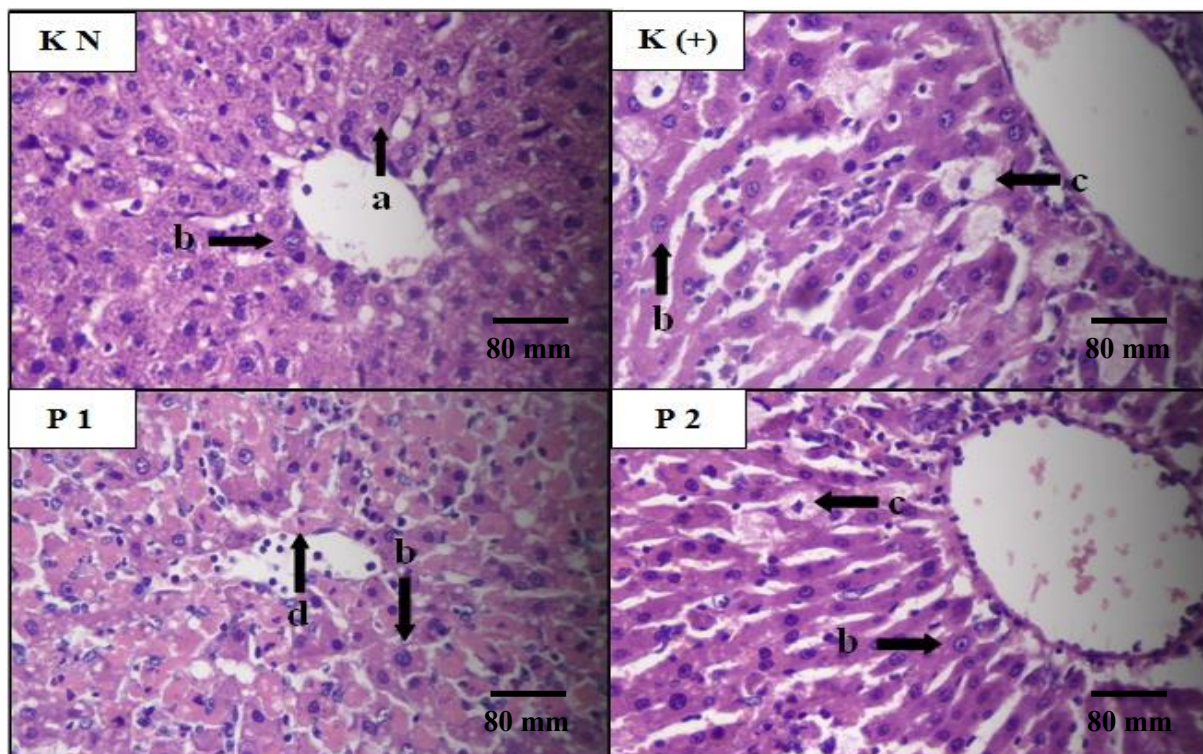


Fig. 2. H&E stained liver sections of four groups after 7 days of treatment in objective lens 400x. KN is normal control, K(+) is positive control treated by glibenclamid, P1 was administered by tree marigold extract, and P2 was administered by tree marigold infusion. Arrow (a) shows normal hepatocyte, (b) parenchymatous degeneration, (c) hydropic degeneration, and (d) is necrotic cells.

Grade 2 (parenchymatous degeneration) was the most frequent histopathology (46.7%) observed in group KN although grade 1 (normal hepatocyte) and 3 (hydropic degeneration) were also present (Fig. 3). Meanwhile group K(+) and group P2 have majorly grade 3 (hydropic degeneration) histopathology with percentage 63.3% and 86.7% respectively. Different from the others, group P1 is the only one which showed the present of grade 4 (necrotic cells) (26.7%), even though the grade 2 (parenchymatous degeneration) is the most frequent (60.0%) degeneration observed.

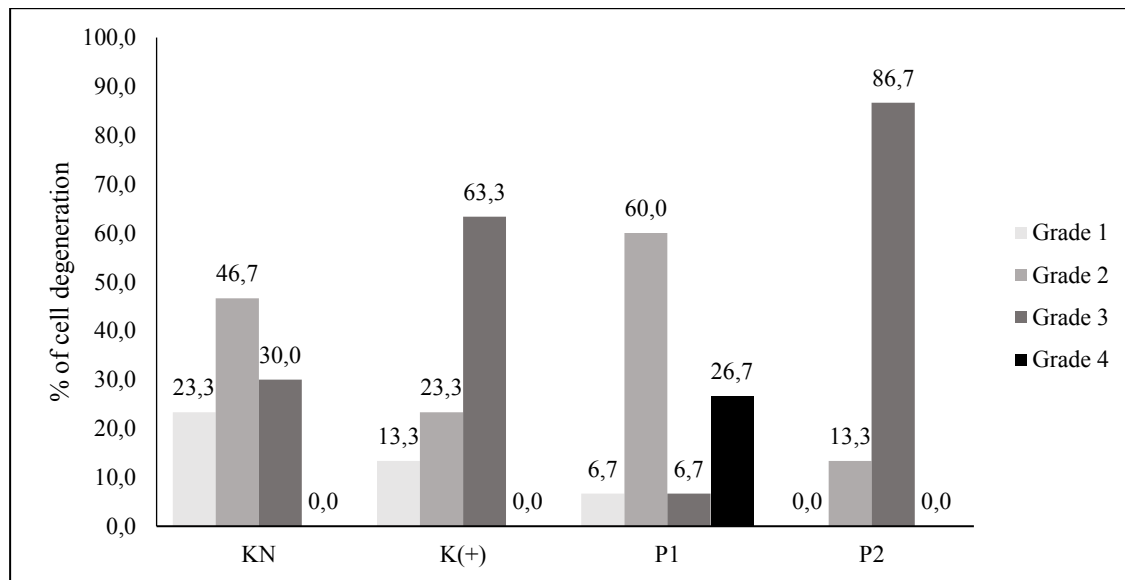


Fig. 3. Proportion of liver histopathology grade score in all group. Grade 1 is marked by domination of normal cells, grade 2 is parenchymatous degeneration, grade 3 is hydropic degeneration, and grade 4 is necrosis. KN is normal control, K(+) is positive control, P1 is group with tree marigold leaves extract, and P2 is group treated by tree marigold infusion

Distribution of scoring data of histopathologic analysis was not normal, thus non-parametric test was performed to know whether there is significant difference between all groups. Kruskal-Wallis test revealed  $p = 0.001$  which means among those groups there are at least two groups who showed significant difference in histopathologic scoring. We further performed Mann-Whitney post-hoc test to see which pairs of groups have the significant difference (Table 3). Mann-Whitney test result shows that there is significant difference in liver histopathology of group KN vs K(+), KN vs P2, K(+) vs P2, P1 vs P2.

Table 3. Mann-Whitney test

Group	KN	K(+)	P1	P2
KN	-	0.019*	0.106	0.000*
K(+)	0.019*	-	0.637	0.027*
P1	0.106	0.637	-	0.025*
P2	0.000*	0.027*	0.025*	-

\* p value < 0.05 which means significantly different

## DISCUSSION

### *Effect of Tree Marigold (Tithonia diversifolia (Hemsl.) A. Gray) Leaves on Blood Glucose Level of Male Wistar Rats*

This research has showed that tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves, both in the form of its ethanol extract or either infusion, can lower blood glucose level in male Wistar Rats induced by streptozotocin. Significant difference in blood glucose level decrease was observed each pair of

groups treated with glibenclamid, tree marigold extract. The highest decrease effect was observed in group treated by glibenclamid. Group P2 treated by tree marigold leaves has shown better effect in decreasing blood glucose level, compared to the group treated by its leaves extract (P1). However, the dose given to the rats should be considered. Since the dose of infusion (P2) was much (about 13 times) higher than extract dose, tree marigold leaves extract was considered to be more effective than the infusion.

Glibenclamid is one of hypoglycemic drugs classified as sulfonylurea which is frequently used to treat DM patients. Glibenclamid lower blood glucose level by stimulating pancreatic  $\beta$  cells to release insulin through its interaction with ATP-sensitive potassium channel on cells membrane. This interaction leads to membrane depolarization and open the sodium channel. The opening of sodium channel will cause  $Ca^{++}$  ions enter the cells and stimulate the granules containing insulin. Insulin secretion will decrease glucose level in the blood [13]. This research showed that tree marigold leaves also have decreasing effect to blood glucose level. Administration of 100 mg/kg of the leaves extract could lower blood glucose level as much as  $101.46 \pm 3.06$  mg/dl, while treatment with 1373 mg/kg of the infusion gave  $124.97 \pm 5.64$  mg/dl of decrease. Considering the decrease level and also the dose given to the rats, this study proved that tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves extract is more effective in lowering blood glucose level. Qualitative phytochemical analysis have shown that tree marigold leaves ethanol extract contains secondary metabolic compounds such as flavonoid, alkaloid, phenolic, steroid and saponin [5]. Flavonoid compound especially quercetin has antidiabetic and hepatoprotection activity. The compound regenerates and repairs damaged pancreatic  $\beta$  cells (Fig. 4). It also restores insulin receptors sensitivity of the cells leading to the increase of insulin production [14]. Besides, flavonoid also stimulates pancreatic  $\beta$  cells to release more insulin and increases peripheral glucose utilization by skeletal muscle [15].

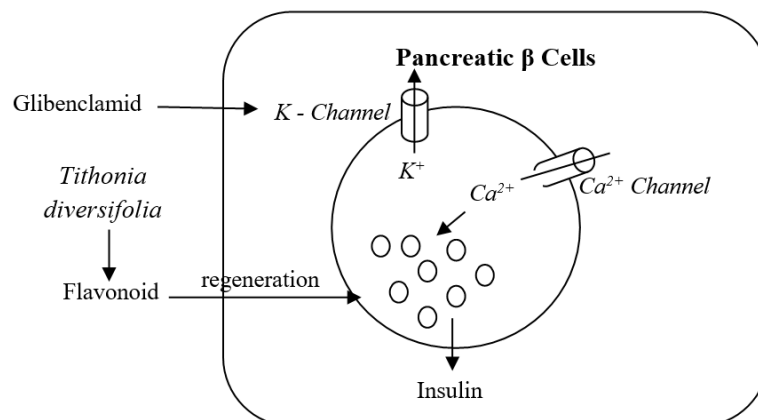


Fig. 4. Mechanism of glibenclamid and tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves in decreasing blood glucose level. Glibenclamid increases insulin secretion while tree marigold leaves have effect on repairing pancreatic  $\beta$  cells and also stimulates insulin secretion.

Different preparation of tree marigold leaves could influence its effect in lowering blood glucose level. Extraction with maceration method can lead to imperfect purifying process due to only 50% of active substances can be extracted, thus the effect might not maximal [16].

#### *Histopathological Changes in The Liver of Streptozotocin-Induced Male Wistar Rats Administered with Tree Marigold (Tithonia diversifolia (Hemsl.) A. Gray) Leaves Extract and Infusion*

This study has revealed that tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves administration in the form of extract and infusion can affect liver histopathology of male Wistar Rats induced by streptozotocin. Histologic changes observed are parenchymatous degeneration, hydropic degeneration, and necrosis. Effect of tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves administration in

the form of the extract or either infusion was compared to normal control and also positive control which was treated by glibenclamid. In H&E stained liver sections, parenchymatous degeneration (grade 2) was the most frequent histopathology observed from group KN, though normal cells (grade 1) and hydropic degeneration (grade 3) were also present. Meanwhile, positive control group and also group P2 (which was administered by the tree marigold leaves infusion) have majorly hydropic degeneration (grade 3) histopathology. Compared to other groups, most histopathology found in group P1, which was given the leaves extract, is parenchymatous degeneration (grade 2). However, it was also noticed that group P1 also has few necrotic cells which is the most severe among liver histopathologic changes.

Parenchymatous degeneration is the least severe because it occurs only on mitochondria and endoplasmic reticulum due to oxidation alteration. This type of degeneration is still reversible. If the pathologic process continues, more severe damage in hepatocytes can happen, which is hydropic degeneration. This is generally caused by metabolic disorders such as hypoxia or chemical toxicities. If the cell injury causes stay, it can tear up the plasma membrane and change the nucleus leading to cell necrosis or death (Fig. 5). All drugs or foreign substances could cause a pathologic process to hepatocytes [17]. Chemical compounds contained in tree marigold leaves such as sesquiterpene, lactone, and polyphenol allegedly cause hepatotoxic effect [18].

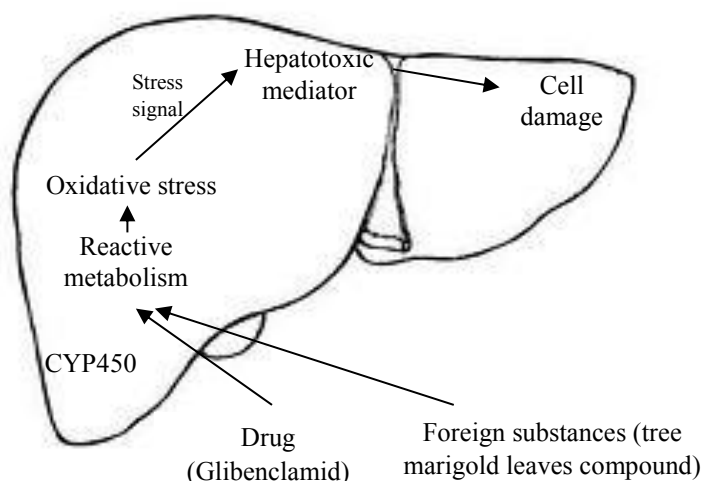


Fig. 5. Possible hepatotoxic mechanism

Liver damage caused by toxic substances is influenced by several factors such as types of the chemical substances, dose administered, and also the duration of exposure (acute, sub chronic, or chronic). The higher the concentration of chemical substances given, the more severe toxic effect. The longer the exposure of the substances, the more hepatocytes damaged and become irreversible.

## CONCLUSION

Tree marigold (*Tithonia diversifolia* (Hemsl.) A. Gray) leaves extract is more effective than its infusion in decreasing blood glucose level of streptozotocin-induced male Wistar Rats. Besides, the ethanol extract causes less damage to the rats' liver than the infusion. It is necessary to see further effect with longer duration of treatment and more samples. Besides, toxic effect analyses on other vital organs such as stomach and kidney are also needed.

## CONFLICT OF INTEREST DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this paper.

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