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# GENOMIC DNA EXTRACTION FROM MICE TISSUES BY USING TWO METHODS (FAVOR PREP™ TISSUE GENOMIC DNA EXTRACTION KIT AND RAPID METHOD)

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**ABSTRACT : This study aimed to evaluate two methods for DNA recovery from animal tissues (livers, Kidney, Spleen and Tail) focusing on the best DNA yield in term of quality, quantity, and integrity for use in several downstream molecular techniques. Six male and female Balb/c mice were sacrificed (liver, kidney, spleen and Tail) tissues (n=20) were then harvested and stored. The conditioned animal tissues were used for DNA extraction by Rapid method or Favorgen Tissue kit. The extracted gDNA was visualized on 1.5% agarose gel electrophoresis to determine the quality of gDNA and analyzed spectrophotometrically to determine the DNA concentration, Both methods Rapid and Favorgen Tissue kit found to be appropriate for yielding high quantity of gDNA, with Rapid method yielding a greater quantity (P < 0.045) than the kit. Rapid method is cheap, fast, effective, and is a crucial tool for yielding DNA from animal tissues (livers, Kidney, Spleen and Tail) exposed to harsh environment with little limitations. And this study its useful in forensic science to detection and helpful to investigate for any guide to crime resolve.**

***Key words :*** Favorgene tissue kit, rapid method, concentration of DNA from (liver, kidney, spleen and tail) tissues.

## INTRODUCTION

DNA isolation is an important first step in DNA analysis for biomedical and forensic purposes (Comey *et al*, 1994). The obtainment of great amounts of high quality DNA from small quantities of tissue is often a laborious task (Pereira *et al*, 2011). Whether the DNA is extracted from a plant or animal tissue or from a bacterium, the product obtained has to be pure or free from contaminants (proteins, carbohydrates) to be used in numerous applications in molecular biology including polymerase chain reaction (PCR), genotyping, DNA sequencing (Magana-Arachchi and Wanigatunge, 2011). In forensic science, purification of high quality and suitable quantity of DNA from challenged biological samples is a key tool for subsequent successful forensic DNA profiling (Al – Griw *et al*, 2011). A wide variety of protocols are found in the literature to extract and purify genomic DNA from different tissues. All protocols start with cell lysis as the first step, followed by deproteination and precipitation of DNA (Magana-Arachchi and Wanigatunge, 2011).

Upon the death of an organism, internal nucleases contained within the cells cause autolysis, cellular organelles and nuclear DNA degradation over time" (El-Harouny *et al*, 2008). Determining the quantity and quality of DNA may provide precise way to estimate the post mortem interval (Liu *et al*, 2001). Therefore, it is important to know which organ is most reliable for DNA extraction, and also to know the effect of post mortem interval on DNA degradation (El-Harouny *et al*, 2008).

Favor Prep Tissue Genomic DNA Extraction Mini Kit are an excellent tools offering a speedy and economic method to purify total DNA from several types of animal tissue. This technology first lyses cells and degrades protein by using a chaotropic salt and Proteinase K then binds DNA to silica based membranes, washes DNA with ethanol contained wash buffer and then elutes purified DNA by low salt elution buffer or $ddH_2O$ (Dhaliwal, 2013). Compare with other harmful and time-consuming procedures, this kit shortens the handling time within one hour (lysis time varies depending on the type of tissue processed.). After using this kit, the high quality total DNA can be used directly for the down-stream applications.

The Rapid Method (RM) a nonorganic extraction method, has been optimized for human blood samples

(Lahiri and Schnabel, 1993; Paikara *et al*, 2014). In an effort to minimize sample size without compromising the quality or quantity of DNA, the (RM) can be used for the isolation and purification of small quantities of DNA from mouse tissues (Hofstetter *et al*, 1997). The main advantage of the RM is that it is a nonenzymatic and inorganic procedure a fast, safe, and economical procedure.

**The aim of this study**

1. Extraction DNA from several tissue including (Liver, Kidney, Spleen and tail) of mice (Balb/c) Males and females and compare between them.
2. Compare the efficiency of two extraction methods, the first its favorgen kit (Colomun spin) and rapid method.

## MATERIALS AND METHODS

**Animal and tissue recovery**

All efforts were made to fulfill the ethical experimentation standards such as minimizing the pain during animal handling and experiments as well as reducing the number of animals used. Six (Balb/c) mice of both gender (male, female) with an age range of five to eight weeks and weight range of 12 g to 25 g, were used in this study. They were bred in the animal house of the Zoology Department, College of Science, University of Babylon, (Babylon, Iraq) and housed under natural conditions of light (12-hour cycle), temperature (24 ± 2°C) and 55 ± 5% relative humidity. At the age of eight weeks, mice were sacrificed by cervical dislocation and the intended tissue samples were removed (Liver, Kidney, Spleen and Tail), n=80, 40 per each extraction method, 20 for male, 20 for female). The tissue samples were cut into small pieces (~ 1gm) and stored immediately in sterile Eppendorf tubes at 8ºC.

**gDNA extraction**

**Favorgen Purification Kit For (Tissue)**

The DNA was extracted from tissue according to the instructions of Favorgen Purification Kit as follows:

**Step (1) sample preparation**

A weight of 10 mg of tissue sample was transferred into a 1.5 µl micro centrifuge tube. Then use Micropestle to grind the tissue sample.

Add 200 ìl FATG1 Buffer and mix well by Micro pestle or pipette tip.

Proteinase K (20 µl of 10 mg/ml) was added to the sample mixture and mixed thoroughly by vortexing,, then incubated at 60°C until the tissue is lysed completely (1~3 h).

4. RNase A (4 ìl of 100 mg/ml) was added to the sample mixture Mix thoroughly by vortexing and incubate at room temperature for 2 min.

**Step (2) Cell Lysis**

1. A volume of 200µl of FATG1 buffer was added to the sample mixture and mixed thoroughly by pulse-vortexing
2. The reaction was incubated at 70°C water bath for 10 min to lysis the cells.
3. The required elution buffer (for step 5 DNA Elution) was preheated in a water bath at 70ºC.

**Step (3) Binding**

1. A volume of 200µl of ethanol (96-100%) was added to the sample mixture. Mix thoroughly by pulse-vortexing. Briefly spin the tube to remove drops from the inside of the lid.
2. The FATG Mini Column was placed in a 2ml collection tube, transferred the sample mixture (including any precipitate) carefully to FATG Mini Column, centrifuged for 1min at full speed (~18,000 x g) then place the FATG Mini Column to a new Collection Tube.

**Step (4) Washing**

1. The FATG Mini Column was washed with 400 µl (W1) buffer, centrifuged at full speed for 1 min and discarded the flow-through.
1. Ethanol added into W1 Buffer when first open.
2. The FATG Mini Column was washed with 750 µl of wash buffer, centrifuge at full speed for 1 min and discard the flow through. Ethanol added
3. Centrifuge at full speed for an additional 3 min to dry the column.
4. Important Step, This step will remove the residual liquid.

**Step (5) Elution**

1. The dry FATG column was placed in a new 1.5ml micro centrifuge tube.
2. A volume of 100 µl of the preheated elution buffer or ddH2O (pH 7.5-9.0)
3. was added to the membrane of the FATG Mini Column, the membrane absorbs stand FATG column for 3-5min or until the buffer.
4. Important Step, for effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
5. The column was Centrifuge at full speed for 2 min to elute DNA.

**Table 1 :** Sequences of the primers to detect Serine /Threonine-protein kinase D1 isoformX4.

| Primer name | Primer Sequence5' -3' | Product size/bp |
|---|---|---|
| **Serine / Threonine-protein kinase D1 isoform X4** | Forward: AGAGTCGCGCTGTAAGAAGC | 196 bp |
| | Reverse: TGGTCTTGTCACTTCTCATC | |

6. The purified DNA extract was stored at - 20°C until analysis.

**Rapid method : Tissue samples**

**Step (1) Sample preparation**

Tissues (15 mg each) were minced with a sterilized razor blade on an ice-cold glass plate and homogenized at 47C for about 30 s in 0.5 ml of TKM buffer (2 mM EDTA, 4 mM $MgCl_2$), tissue sample was transferred into a 1.5 µl micro centrifuge tube. Then use Micropestle to grind the tissue sample.

**Step (2) Cell Lysis**

The homoge nized tissues were suspended in 0.5 ml of TKM buffer containing 2.5% NP-40, NP-40 can be substituted by Triton X 100, Sigma (Lahiri and Schnabel, 1993).

The cells were lysed by inverting several times. The suspension was centrifuged at 1000g for 10 min at room temperature (RT).

**Step (3) Wash**

The pellet was washed two more times with TKM and the final pellet was resuspended in 0.1 of TKM buffer containing 0.75% SDS. The suspension was mixed vigorously and incubated for 5 min at 55C, the tube was mixed well after saturated NaCl was added, and centrifuged at 12000g for 5 min.

The supernatant contained DNA, which was precipitated using ethanol.

**Step (4) Elution**

DNA pellet was washed with 70% ethanol, air dried, and final DNA was resuspended in 0.5 ml of 10 mM Tris-Cl, pH 7.4 and 1 mM EDTA, pH 8.0 (TE), at $37^0$C.

**Quantity of g DNA**

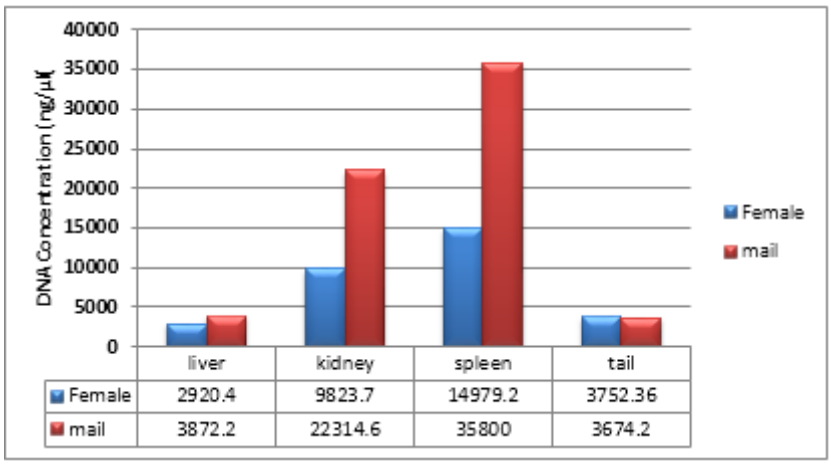
The gDNA concentration was assessed by optical density measurements using the Nano Drop Spectrophotometer. For this purpose. DNA was quantified by measuring UV absorbance at 260 nm.

**Gel Electrophoresis Protocol**

1. The gel- infusion dish was put in plastic infusion, examined the teeth of the reed are approximately 0.5mm above the gel lower.
2. A 500ml of TAE (1X) was prepared by adding 50ml of TAE (10X) stock solution to the last volume of 500ml of deionized water.
3. A100ml of the buffer into a 500ml ûask was mixed with 1g of agarose and heated with mixing until completely agarose dissolved until no agarose particles were apparent.
4. The agarose solution was cooled to approximately 60°C and 2-3 µl of the ethidium bromide stock solution was added, then slow cast the agarose into the gel-infusion tray with removing any air blisters by the yellow tip.
5. Let the agarose solidify for approximately 20–30 min. After the agarose has solidiûed remove the comb with a soft back and forth motion, taking care not to split the gel.
6. The gel-infusion plate was removed and placed the tray on the centric supporting platform of the gel chest.
7. The electrophoresis buffer was added into the buffer chamber until it scopes a level of 0.5–1cm above the surface of the gel.
8. Firstly, 5µl of ladder molecular weight marker was loaded to each side of the gel (flanking the sample line) and 5 µl of DNA samples in the other wells.
9. The cover was sat on the gel box and connected the electrodes. DNA will travel towards the positive (red) electrode positioned away from the well. Turn on the force equipping.
10. The electrophoresis was done until the tracking dye moves at least 10 cm of the gel tallness.

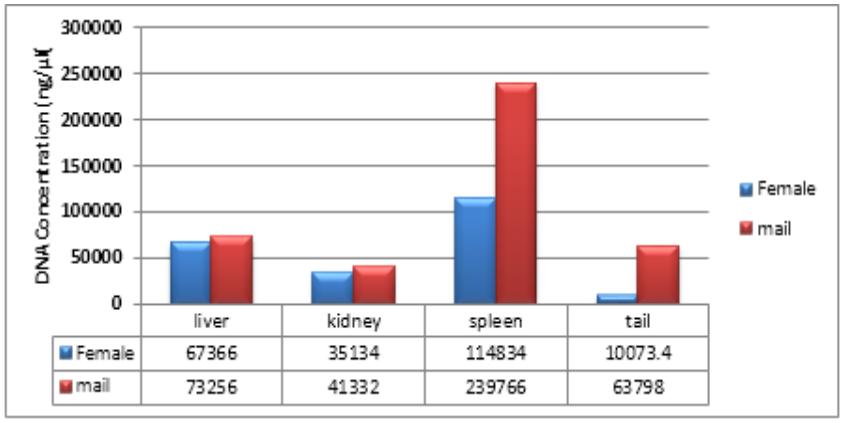
**Agarose Gel electrophoresis**

The detection of amplified PCR product by agarose gel electrophoresis with dye ethidium bromide, after that the product visualization. The gel tray placed in electrophoresis chamber. Added 0.5x TBE buffer to the chamber until covering the gel surface completely. PCR products were loaded into the agarose gel wells, 7µl from a single product to single well in known sequence, followed by 3µl from the suitable ladder to one of the wells in each row. The electric current was performed at 75 volts for 1 hour.




| | liver | kidney | spleen | tail |
|---|---|---|---|---|
| Female | 2920.4 | 9823.7 | 14979.2 | 3752.36 |
| mail | 3872.2 | 22314.6 | 35800 | 3674.2 |

**Fig. 1 :** Average concentration of DNA measured in ng/µl. DNA extracted from mice tissues (liver, kidney, spleen and tail) for females and males using Favorgen kit. Data are expressed as Mean ± S.D, P ≤ 0.05 significant.




| | liver | kidney | spleen | tail |
|---|---|---|---|---|
| Female | 67366 | 35134 | 114834 | 10073.4 |
| mail | 73256 | 41332 | 239766 | 63798 |

**Fig. 2 :** Average concentration of DNA measured in ng/µl. DNA extracted from mice (liver, kidney, spleen and tail) for females and males using Rapid Method. Data are expressed as Mean ± S.D, P ≤ 0.05 significant.

### Gel documentation

The agarose gel was visualized by UV trans illuminator provided with the gel registration unit, in which the agarose gel was placed above the UV trans illuminator device, then the gel was open to UV light and the photos were taken using the Canon Digital camera.

### Amplification of Serine/ Threonine-protein kinase D1 isoform X4 by conventional PCR

### Primer

The primer that used in this study shown in Tables 1-3.

### DNA Amplification by Polymerase Chain Reaction Protocol

1. Template DNA (about 50 ng) was added into PCR master mix tubes.
2. Forward and reverse primers (about 10 Pico moles) were added into PCR master mix tubes.
3. Distilled water was added to PCR Premix tubes to a total volume of 20 µl.
4. The lyophilized blue pellet was dissolved by vortex, and spinning down briefly.
5. Reactions were placed in a thermal cycler (Eppendorf–Nexas Gradient) that had been preheated to 95°C and previously set up to the desired cyclic conditions.

### Preparation of Stock Solution

The Stock solution was prepared as shown in Tables 2-3.

### PCR Program

The Serine /Threonine-protein kinase D1 isoformX4 was detected by PCR program as mentioned in Table 3.

### Statistically analysis

Use program IBM SPSS Statistics, version. 23, One –Way-ANOVA, Least Significant Differences LSD, Independent Sample T test.

## RESULTS AND DISCUSSION

### Evalution of the genomic DNA quantity extracted from mice tissues

The resulst showed from Fig. 1 the quantity of DNA that extracted from (females and males) of mice tissue samples (liver, kidney, spleen and tail) by using first method (Favorgen kit).

For females tissue samples (liver, kidney, spleen and tail) the quantities of the DNA extracted were 2920.4±1778.4 ng/µl, 9823.7±9567.7 ng/µl, 14979.2±10714.6 ng/µl and 3752.3±3792.2 ng/µl, respectively. The highest value of DNA quantity which was obtained from spleen, whereas the lowest value from liver. There are significant + respectively.

For males tissue samples (liver, kidney, spleen and tail) the quantities of the DNA extracted were 3872.2±1451.0 ng/µl, 22314.6±20758.9 ng/µl, 35800.0±26258.3 ng/µl and 3674.2±2013.5 ng/µl, respectively. The highest value of DNA quantity which was obtained from spleen, whereas the lowest value from tail. There are significant difference between spleen, liver and spleen, tail samples (P- value LSD = 0.01and 0.01) respectively.

The results showed from Fig. 2 the quantity of DNA that extracted from (females and males) of mice tissue samples (liver, kidney, spleen and tail) by using second method (Rapid method).

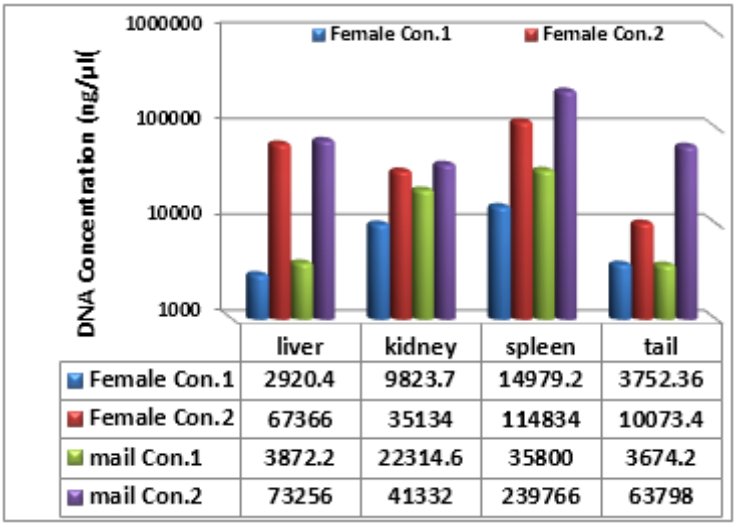



**Fig. 3 :** Average concentration of DNA was extracted from animal tissues (liver, kidney, spleen and tail) for females and males using The Favor Prep™ Tissue Genomic DNA Extraction kit (Favorgen) kit (1) and Rapid Method (2). Data are expressed as Mean ± S.D, P ≤ 0.05 significant.

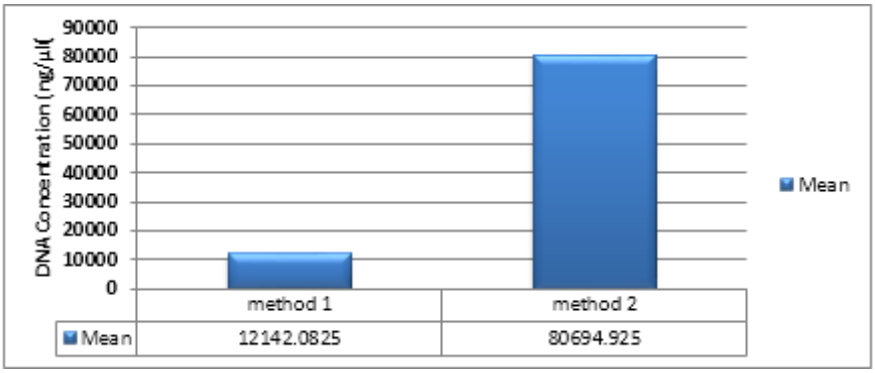



**Fig. 4 :** Average concentration of DNA measured in ng/µl. DNA was extracted by using The Favor Prep™ Tissue Genomic DNA Extraction kit (method 1) and Rapid method (method 2). Data are expressed as mean ± S.D, P < 0.05 significant.

For females tissue samples (liver, kidney, spleen and tail) the quantities of the DNA extracted were 67366.0±25719.0 ng/µl, 35134.0±8106.1 ng/µl 114834.0±29468.2 ng/µl and 1007.4±9535.7 ng/µl, respectively. The highest value of DNA quantity which was obtained from spleen, whereas the lowest value from tail. There are significant difference between (liver, kidney), (liver, spleen), (liver, tail), (kidney, spleen) and (spleen, tail) samples (P- value LSD =0.025, 0.002, 0.000, 0.000 and 0.000), respectively.

For males tissue samples (liver, kidney, spleen and tail) the quantities of the DNA extracted were 73256.0±21872.1 ng/µl, 41332.0±19772.7 ng/µl, 239766.0±57458.4 ng/µl and 63798.0±20557.0 ng/µl, respectively. The highest value of DNA quantity, which was obtained from spleen, whereas the lowest value from kidney. There are significant difference between (spleen, liver), (spleen, kidney) and (spleen, tail) samples (P- value LSD =0.000, 0.000and 0.000), respectively.

The result showed from Fig. 3, the quantity of DNA extracted from (females and males) mice tissues samples (liver, kidney, spleen and tail) by Using The Favor Prep™ Tissue Genomic DNA Extraction kit method and Rapid method. The highest value of DNA quantity which was obtained from males spleen in Rapid method 239766.0±57458.4, the lowest value from females liver in Favor Prep™ Tissue Genomic DNA Extraction kit 2920.4±1778.4.

In many forensic cases, the identification of a perpetrator, victim or missing person may be complicated, samples often include biological specimen that contain only minute amounts of DNA or they have suffered environmental stress so the samples are decomposed, due to these reason there is strong necessity for introduction of fast and reliable methods for forensic DNA analysis. (Jakovski *et al*, 2015). This study is to compare two methods of extraction of DNA from different tissues of males and females mice (liver, kidney, spleen and tail) with favorgen kit and rapid method to confirm which of this two methods produce better results. The two methods were compared for DNA extraction from the animal tissues according To the following criteria: quantity, purity, quality, environmental safety, time and cost efficiency.

As shown in Figs. 1, 2, 3; the greatest amount of DNA was obtained from spleen tissue samples, whereas the lower from liver and tail tissue samples in first method and second method, respectively.

This results agreed with previous studies such as Chomczynski *et al* (1997); Hofstetter *et al* (1997) and Sheaff and Hopster (2005) they said that the blood or splenic tissue are the best specimens for DNA analysis, and should be frozen if there is likely to be any delay before reaching the laboratory, a larger quantity of genomic DNA can be recovered from a testis and spleen but another studies such as Paikara *et al* (2014) were found the concentration of DNA to be lowest from spleen.

This results can explained that, the spleen is lymphatic tissues in the mice, and its strategic position in the blood circulation, the spleen is able to filter, phagocytize, and mount immunological responses against blood-borne antigens. The spleen contains all the components (B and

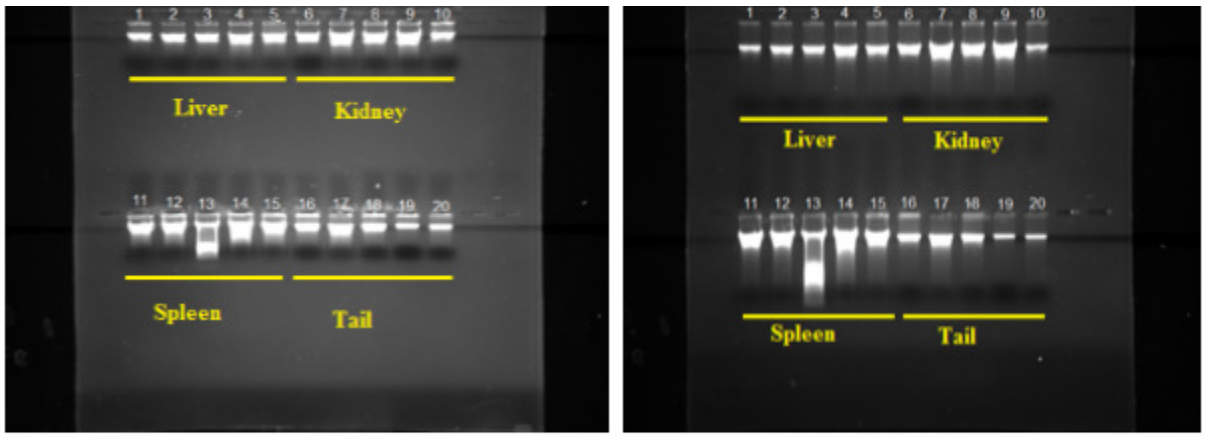



**Fig. 5, A, B :** Agarose gel electrophoresis of extracted DNA from Mice (A: Female, B: Male) Tissue samples by using the Favorgen Kit. Lanes1-5 liver. 6-10 kidney, 11-15 Spleen, 16-20 tail, at70 volt for one and half hour.

T lymphocytes, APCs and phagocytic cells) necessary for this function, the white pulp of the spleen is an important production site of lymphocytes, which then migrate to the red pulp and reach the lumen of the sinusoids, where they enter the blood circulation. Inert particles are also intensely phagocytized by spleen macrophages and these cells contain high quantity of DNA concentration and the fact that the spleen is a large lymphoid organ contains many immune cells and many types of white blood cells in addition to the large numbers of cells containing nuclei in the pulp red and white, the two components of the spleen and this means to obtain large amounts of DNA obtained in the current study.

In the present study, the liver result is lower that agree, with Piasecka –Pazik *et al* (2000) successfully extracted DNA from blood and decomposed tissue, particularly from the heart and kidney, DNA extraction from the liver was poor. Lin *et al* (2000) observed that the DNA degeneration rate of liver cells had a linear relationship to early postmortem period in rats. Johnson and Ferris (2002) reported that in tissues such as liver and kidneys, enzymes tend to be more active and accelerate DNA decomposition (El-Harouny *et al*, 2008) the used method was useful in detection of fragmented DNA in the liver up to 24 hours PM.

But in study of Hofstetter *et al* (1997) liver produced in the proteinase k method (PM) higher yield of DNA with less degradation than the rapid method (RM), (Biase *et al*, 2002). Higher concentration was obtained from the liver (Ebuehi *et al*, 2015) the study suggested that at a later PMI, the brain, followed by the liver were preferred organs for forensic studies than the heart and kidney, also in study of Al –Griw *et al* (2011) the quantity and the integrity of gDNA extracted from liver tissues which was prominent than the ones extracted from muscle tissues. According to the results of tail, agree with Hofstetter *et al* (1997) for tail the PM produced a higher yield of DNA but the RM resulted in a higher yield of PCR product.

Study of Picazo and Garcia-Olmo (2015) detectable amounts of DNA were obtained from samples in adult mice, the largest amounts were obtained from blood and tail samples. This result could be explained by the structure of tail have more cartilage and bone, the tail is an extension of the vertebral column that projects out the back side of the animal. The tail is a long cylinder consisting of three concentric layers. The innermost core of the tail is bone (vertebrate). The bone is surrounded by a layer of tendons and the tendons are surrounded by a layer of skin. Blood vessels run the length of the tail between the tendons.

**Comparison between first method and second method for (DNA quantity/ ng) in all tissues**

The result showed from Table 4 and Fig. 4 the quantity of DNA extracted using The Favor Prep™ Tissue Genomic DNA Extraction kit (method 1) and Rapid method (method 2). The quantities of the DNA extracted by method 1 and method 2 were 12142.0825±16226.15992, 80694, 9250±72168.02890, respectively.

As shown in Table 4, Fig. 4 the two techniques were found to be very different in quantity of DNA recovered from tissues samples. Our results showed the Rapid method resulted in much higher quantity of DNA compared to favorgen method which produced lower quantity of DNA samples.

The reason for poor quantity of gDNA produced from tissue samples when The Favor Prep™ Tissue Genomic DNA Extraction kit was used for gDNA extraction could

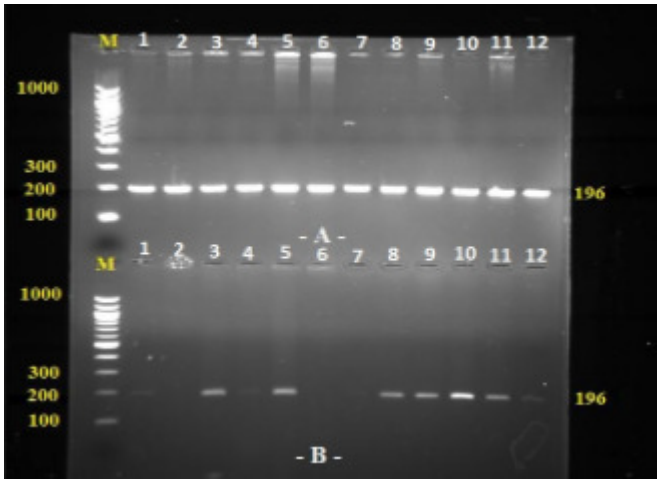



**Fig. 6 (A, B) :** Agarose gel electrophoresis of PCR products of Serine/threonine–protein kinase D1isoform X4 gene on 2.5% Agarose at 70 volt for one and half hour. A: Favorgen kit, B: Rapid method.

be explained by the fact that the use of silica columns for the binding of DNA is the possibility of overloading the column with DNA, resulting in a wash-through of non-adsorbed DNA and reducing the overall yield of DNA also the technique failed to incorporate a vital step that takes into account the special feature of animal cells. When using animal tissue, enzymatic lysis step or mechanical disruption of the tissue should be proceeding the separation of DNA from other cell components (Hofstetter *et al*, 1997; Dhaliwal, 2013).

### Evalution of the genomic DNA integrity by agarose gel electrophoresis

The integrity and quality of genomic DNA extracted by the two methods was also analysed on 1% agarose gel electrophoresis. This technique has been widely used for determining the size of DNA samples (Zimmermann *et al*, 1998) (Fig. 5A, B).

Agarose gel electrophoresis analysis of DNA isolated using the rapid method showed the patterns consistent with the presence of degradation DNA, with low quality DNA preparation and the staining of bands was less intense (Fig. 5B).

### PCR detection

Polymerase chain reaction (PCR) was used to detect Serine /threonine –protein kinase D1isoform X4 gene, PCR product for this gene approximate 156 bp in size, this step was conducted to ensure that the extracted DNA is pure, the results of PCR product was as shown below.

Electrophoresis PCR product in Fig. (6-A) all band of DNA clear, (6- B): thin band appeared in band 1, 3, 7, 8, in lane2, 4, 5, 6 no band appeared. Group C no band appear at all except Lane 2.

**Table 2 :** Preparation of Stock Solution.

| Component | Volume | Concentration |
|---|---|---|
| PCR master mix | 10 ìl | 1X |
| Forward primer | 1.0 ìl | 10 pmol/ ì l |
| Reverse primer | 1.0 ìl | 10 pmol/ ì l |
| Template DNA | 2.0 ìl | 50 ng |
| $ddH_2O$ | 6.0 ìl | |
| Final volume | 20.0ìl | |

**Table 3 :** PCR program of PCR.

| Step | Temp. (°C) | Time | No. of cycles |
|---|---|---|---|
| Initial denaturation | 94 | 5 min | ×35 cycles |
| Denaturation | 94 | 30 sec | |
| Annealing | 60 | 40 sec | |
| Extension | 72 | 30sec | |
| Final extension | 72 | 5 min | |
| Holding | 4 | Indefinite | |

**Table 4 :** Totally comparison between first method and second method for (DNA quantity/ng) in all tissues.

| First method | Second method | P-value (LSD) |
|---|---|---|
| **M ± S.D.** N=40 | **M ± S.D.** N=40 | |
| 12142.08±16226.15 | 80694.92±72168.02* | 0.000 |

*Significant differences, P ≤ 0.05, M = Mean, S.D. = Standard deviation, N= Sample number.

### Environmental safety, time and cost efficiency

The RM method required approximately 2-3 h, compared to the favorgen kit which required 3.5 to 4 hr., mostly for incubation time. The cost of isolating DNA from animal tissues was calculated, the most economical method was the Rapid method, while the Favor Prep™ Tissue genomic DNA extraction kit was considerably more expensive (Oliewi and Al-Hamairy, 2016; Manji and Al-Hamairy, 2015). The Rapid method is quicker, more cost effective and economical, the two method is a safe. It is not necessary to treat the sample with harmful organic solvents such as phenol and chloroform (Al-Dulaimi *et al*, 2015; Abbas and Al-Hamairy, 2016; Al-Hamairy, 2016; Chabuk *et al*, 2016).

## CONCLUSION

In present study concluded that the spleen gives highest quality of DNA concentration by two methods that used, followed it's the liver, kidney and finally the tail and in the same time the second method (Rapid method) appear to be acceptable regarding the quantity of DNA product from first method (Favorgen kit).

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