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# Dual Application of Al-Kheriat of Removal of Arsenic from Aqueous Solution and Acting as Rodenticide

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**ABSTRACT**

Arsenic is a prevalent and pervasive environmental contaminant with varied amounts in drinking water. Arsenic exposure causes cancer, cardiovascular, liver, nerve, and ophthalmic diseases. The current study aimed to find the best conditions for eliminating arsenic from simulated wastewater and their effect on biomarkers of hepatic in mice. Adsorption tests including pH, contact duration, Al-kheriat dosage, and arsenic concentrations were evaluated. Seventy-two healthy albino mice (male) were accidentally allocated into nine groups ($n$ = 8), the first group was considered as healthy control, the second group (AL-Kheriat), and other groups received AL-Kheriat and arsenic 25, 50, 75, 100, 125, 150 and 175 mg/kg, respectively. Next 10 days, the following were examined: LD50 level, ALP (alkaline phosphatase), ALT (alanine aminotransferase), and AST (aspartate aminotransferase), besides the histological condition of the liver. The results showed that the best time for arsenic removal was 4 hours, pH 8, Al-kheriat dose 1 gram, and 50 ppm of pollutants. The level of alkaline phosphatase ALP, alanine transaminase ALT, and aspartate transaminase AST was increased to 150.96 (U/L), 143.1(U/L), and 32.8(U/L), respectively, in Al-Khriet and arsenic exposed population than the healthy control group, When the appropriate dose of Al-Khriet and arsenic mixture is used, it can aid in the selection of a safe way of disposing of the adsorbed residue. Additionally, it can serve as a low-cost rodent pesticide, increasing the commercial viability of this removal strategy.

**Keywords:** arsenic, bio-sorbent, rodenticide, green processes, Al-Kheriat, liver enzyme.

## INTRODUCTION

Pesticides are a broad category of chemicals that are frequently employed to counteract the negative consequences that pests might bring [1]. Arsenic inorganic compounds are considered toxic and carcinogenic chemicals which are mostly harmful to the wide ecosystem [2-6]. Arsenic Oxide as an example, can be found in aqueous waste media. Pharmaceutical, electronic, and metallurgical industries can be possible candidates for water pollution [7]. Adsorption is a common technology for separation that is extensively used in wastewater treatment. Its versatile, simple, and efficient use in addition to the minimal energy consumption had led it to possess a pronounced impact on the separation processes [8]. An added value of the adsorption process can be found in utilizing biomass adsorbents. Such adsorbate hosts can be good alternatives to the conventional types such as activated carbon, zeolites, and other synthesized adsorbents, due to their cost-effective merit. Waste tea [9, 10], orange waste [11], powdered cockle shell [12], exhausted coffee grounds [13], olive pomace [14], wheat bran [15] chitosan [16, 17] and many more, are used as bio sorbents for adsorbing different types of heavy metals where biosorption refers to living and dead organism [18]. Al-Kheriat is a sweet powder found in the legs of the agricultural natural plant namely "Typha domingensis" present in the southern part of

Iraq. Past studies had proven its effective use in the removal of lead and cadmium [19] and copper [20]. One of the drawbacks of the adsorption process is that it is only a phase change process that consists of replacing the pollutant from one phase to another normally from liquid media to a solid adsorbent. Batch processes sustain the pollutants in the adsorbents which should be regenerated on further action as in the two-bed continuous operation adsorption mode [18]. Therefore, regeneration of the adsorbent is an important step for continuous reuse and achieving an economic cycle. Strong acids, bases, and salts are common agents for desorption used in arsenic-contaminated adsorbents [21]. The fate of the contaminated adsorbent after use is an important concern. Even after many cycles of regeneration and consecutive use, the adsorbent reaches a point when the adsorption efficiency drops appreciably. The disposal of the timeworn adsorbent depends on the arsenic concentration according to USEPA 1992 where if the leaching solution contains below 5.0 mg/L then the adsorbent waste can be discarded in landfills [22]. Green processes are designed to produce the desired products with zero-waste products, no toxic by-products, or with no use of reagents and toxic solvents. It is also, expected to reduce greenhouse emissions and lower energy consumption. All the issues if present are generating risk and adverse impacts on human health in a particular manner and on the environment in general. Studies in the past focused on how to make the engineering processes economically feasible by increasing the profit outcomes but the need for eco-friendly processes has become a more valuable aim[23-25]. Ahuja [26] proposed solutions to the contamination of water from the point or nonpoint sources using the principles of green chemistry. Some are minimizing wastes, using catalysts and/or safe solvents, increasing the efficiency of energy by operating under normal conditions, and designing chemicals that are degradable after their use. The concept of E factors describes the relationship between the waste products and desired product from a certain process. As this factor gets close to zero, the process gets greener with a neglected amount of waste which reflects a negative impact on the environment [24, 27]. Recent animal and human epidemiological investigations have found a link between exposure to arsenic (As) and unfavorable reproductive and developmental consequences [28–30]. Many studies [31, 32] show that sodium arsenate alters the tissue architecture of the heart and liver. In the liver, lungs, kidney, nails, and hair, elevated arsenic concentrations have been widely documented [33]. Epidemiologic investigations have demonstrated a relationship between arsenic exposure and severe liver damage and kidney problem [33]. The arsenic exposure that contributes to organ damage is not detected yet. A human's liver is one of many internal organs that is adversely impacted by long-term exposure to arsenic. In epidemiological studies, persistent arsenic exposure has been linked to liver illness, including hepatomegaly and hepatic fibrosis, and liver failure. Chronic arsenic exposure is associated with aberrant liver functions, such as acute digestion issues and significant increases in liver enzymes in the blood (ALP, ALT, and AST) [34-37]. As a consequence of being subjected to arsenic through drinking water, mice have elevated levels of enzymes lever in their blood [38] and liver sinus endothelial capillarization [39]. Because the liver is the central location for arsenic metabolism [40], exposure to arsenic induces liver damage in persons who have been subjected to it [34]. Arsenic-mediated mammalian toxicity cannot be fully understood without first gaining a firm understanding of the toxin's organ-specific histological effect. The gold standard for determining the extent of organ damage caused by chronic metal exposure is organ-specific histology examination. Histological degeneration alters markers of organ function, which is unusual. For example, in arteries and brains, arsenic has yet to be studied in detail, while other organs such as kidneys and liver are still a mystery to researchers [41]. The focus of the current research is on the adsorption of arsenic compounds onto Al-Kheriat agricultural powder as a means of removing these chemicals from wastewater. The utilization of the contaminated Al-Kheriat in the preparation of rodenticide is examined to produce low-cost rodenticide from low-cost adsorbent in a simple no waste green process, then examine the risk of chronic arsenic environmental contamination on the liver tissue architecture of mice. Understanding how arsenic affects target organs and how it affects tissue architecture in important places would help define a mechanism of efficacy for arsenic-stimulated cytotoxicity in animals and reduction the misunderstanding in hazard evaluation for this heavy metal.

## METHODOLOGY

### Materials

Al-Kheriat biomass was obtained from marshes in the southern region of Iraq, cleaned extensively, dried at 110 degrees Celsius, sieved, and then kept in a desiccator for subsequent usage. For the purpose of determining the identities of certain functional groups, a technique known as Fourier-transform infrared spectroscopy (FTIR) was utilized on a Shimadzu IRPrestige-21 from Japan. The spectra of the adsorbent were analyzed at wave numbers ranging from 400 to 4,000 $cm^{-1}$, respectively. Arsenic oxide ($As_2O_5$) was purchased from BDH Chemicals (UK), and it was put to use in the process of preparing simulated solutions with varying concentrations from 50–200 ppm. The chemical composition of Al-Khriet is 40% carbon, 34% silica, 12% hydrogen, 3.24% cellulose, 3% nitrogen, 2.25% hemicelluloses, 2% silver, 1.35% lignin, and the remaining components are lipids and proteins. The impact of pH on arsenic adsorption was investigated at several pH values, ranging from 4 to 10, using HCl (with a concentration of 35–38 percent from the BDH laboratory in the United States) and NaOH (99.9 wt. percent, Sigma- Aldrich, Germany).

### Experimental work

#### *Adsorption tests*

The adsorption studies were initially done in a batch system at 25 degrees Celsius, which corresponds to room temperature. In the beginning, 0.5 g of Al- Kherait was put into a conical flask that contained 25 ml of solution, and then the flask was shaken at a 250-rpm rate. During this time, samples were taken every 30 minutes, and an atomic emission spectrophotometer was used to determine the concentration of arsenic in the samples (Perkin- Elmer 5000, UK) until the concentration of Arsenic remain constant so the time needs to reach equilibrium (4 hr) was investigated. The other experiments were conducted by making the different concentrations of Arsenic (50–200 ppm), amount of Al-Kherait dose (0.2–1 g), and pH (4–10). In these experiments, flasks were agitated at a rate of 250 rpm. for 4 hours then the solutions were measured after being filtered to find the quantity of Arsenic remaining [42]. The quantity of arsenic that Al-Kherait absorbs at equilibrium ($q_e$ mg/g), and the deletion percentage of Arsenic (%$R$) were determined in accordance with Eqs. 1 and 2, respectively.

$$q_e = \frac{(C_0 - C_e)V}{W} \tag{1}$$

$$\%R = \frac{(C_0 - C_e)V}{C_0} \times 100 \tag{2}$$

where: $C_0$ and $C_e$ – the starting and equilibrium arsenic concentrations (mg/L);
$V$ – the solution volume (L);
$W$ – the Al-Kherait adsorbent weight (g).

#### *Experimental animals*

72 male mice, weighing 23–25g, and ages 6–8 weeks and are used in this investigation. divided into nine groups (n=8 each), the control group was given distal water, the groups serving as the experiments were given plant extract and the other groups receive arsenic with plant extract in different concentrations. Different concentrations of the mixture (AL-Kheriat with arsenic) were prepared from the stock (Al- kheriat dose 1 gram, and 50 ppm of pollutants). In Baghdad, Iraq, Al-Nahrain University's Biotech Research Group purchased the mice for use in this study. There was a constant supply of food pellets and drink for the animals, who were housed in cages. They were split up into the following nine categories: The first group received distal water treatment, the second received plant extract treatment, the third received 25 mg/kg of mixture treatment, the fourth received 50 mg/kg of plant treatment, the fifth received 75 mg/kg of treatment, the sixth received 100 mg/kg of treatment, the seventh received 125 mg/kg of treatment, the eighth received 150 mg/kg of treatment, and the ninth received 175 mg/kg of AL-Kheriat combined with arsenic. A single dose each day (0.1 mL) was administered to every of the study groups via oral gavage of the examined material for ten days. As of day 10, all the animals had been slaughtered for laboratory measurements [43].

#### *Biochemical measurements*

For all tests (biochemical, enzymatic), Blood was obtained via heart puncture and transported to an Eppendorf tube, where it was allowed to

coagulate for 15 minutes at ambient temperature, then centrifuged for 10 min at 3,000 for each of the tests under investigation (biochemical and enzymatic analyses). The serum that was obtained was used for the evaluation of all parameters linked to liver function. Quantification of ALP, ALT with AST, and enzymes was performed with commercial kits along with a spectrophotometer (UV–VIS Record 2401, PC, Japan) [44].

### *Histological study*

These samples were sliced, treated with 10 percent neutral formalin liquid, dried in successively higher concentrations of alcohol, and fixed in paraffin wax. Hematoxylin and eosin were used to stain paraffin slices (5 m thick) for routine histological analysis (H&E). Each segment was analyzed for histopathological alterations using a light microscope (×40) to select eight field areas according to Noman [41, 43–45].

### *Statistical study*

These findings were reported as the average standard deviation. GraphPad Prism 6.01 statistics software (GraphPad software 6.01, USA), The data was investigated by means of one-way variation assessment (ANOVA). When the variation between the means was less than 0.05 ($P \leq 0.05$), the difference was found significant.

## RESULT AND DISCUSSION

### FTIR interpretation

Arsenic adsorption on the agricultural crop Al-Kheriat was studied using Infrared spectroscopy. Figures 1 and 2 represent the pre-adsorption and post-adsorption processes respectively.

The parent plant (Fig. 1) revealed a typical example of a carbonaceous source. The wavenumbers 2924 and 2854 $cm^{-1}$ can be linked to alkanes and stretching modes of distinct $CH_3$, $CH_2$, and CH groups [46–49]. The absorption band in the region of 3344–3502 $cm^{-1}$can indicate the presence of a hydrogen bond. If refers to hydroxyl ions, there should be extra peaks present in 1000–1200, 1300–1600, and 600–800 $cm^{-1}$ regions [50]. Different peaks around 1000 $cm^{-1}$ are linked with the C-O stretching mode [49, 51]. The peak located at 1635 $cm^{-1}$ can refer to the adsorbed water [52]. Comparing Fig 1 and 2 reveals the presence of two peaks of 802 and 918 $cm^{-1}$ which could be attributed to the arsenic present in the host Al-Kheriat. Roddick-Lanzilotta et al reported the appearance of broad bands in the region of 792,800, 820, 858,908 $cm^{-1}$ at different pH values [53]. Also, Cowen et al displayed their results for the characterization of significant arsenic compounds. They observed frequencies at 797 and 823$cm^{-1}$ that were identified as belonging to the $AsO_3^{-4}$ stretching vibrations [54].

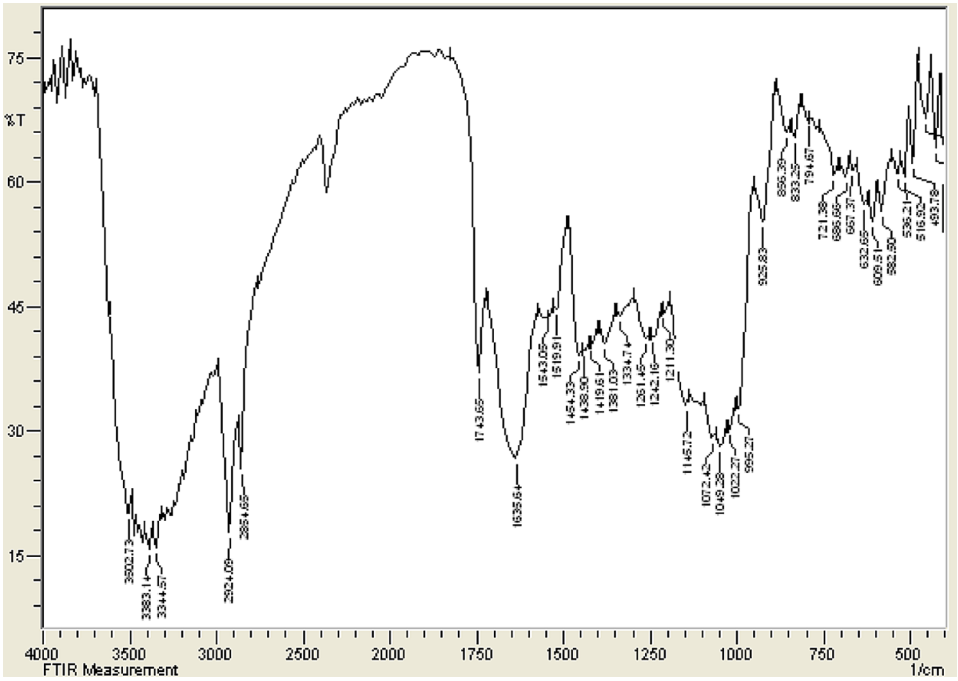


**Figure 1.** FTIR Spectrum of Al-Kheriat before arsenic adsorption

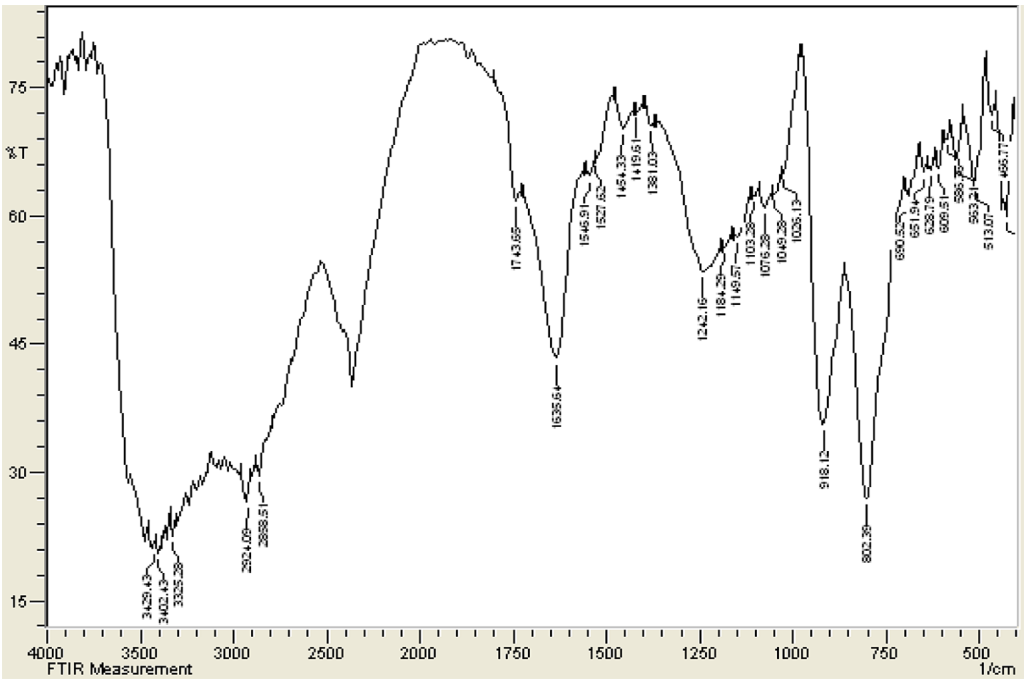


**Figure 2.** FTIR Spectra of Al-Kheriat after arsenic adsorption

## Adsorption analysis

### *Influence of initial pH concentration*

The effect of pH on arsenic deletion was examined using 0.5 g of Al-Kheriat in 50 ppm of arsenic solution. Mixing lasted for 6 hours. Results as appeared in Figure 3 show that the removal was maximized at pH 8. This confirms that pH performs an essential role in desorption when the host surface varied between acidic to basic media. Polowczyk et al concluded that the best arsenic removal on fly ash was at high basic media [55]. Khamkure et al found out that magnetic ferric oxide got different pH values by the varying catalyst of xerogel monoliths. The greatest amount removed was at pH 5 using MC50, while it was between the neutral and acidic region using MC100 [56]. In a similar study using modified ash, it was found that the ideal removal was at pH 7 [57].

### *Contact time influence*

The contact duration influence on the adsorption of Arsenic was studied using 0.5 g of Al-Kheriat in 50 ppm of arsenic solution and pH 8. Figure 4 reveals that increasing the contact time enhanced the removal. A sharp increase in the initial 30 min was observed as a result of the enormous area of Al-Kheriat and the large concentration gradient between Al-Kheriat and the solution.

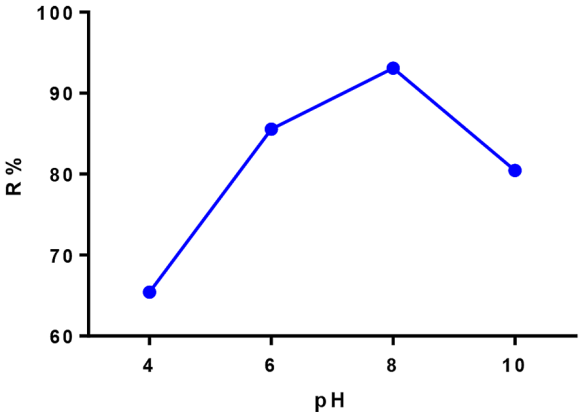


**Figure 3.** Influence of pH on arsenic deletion using 0.5 g in 50 ppm concentration after 6 h

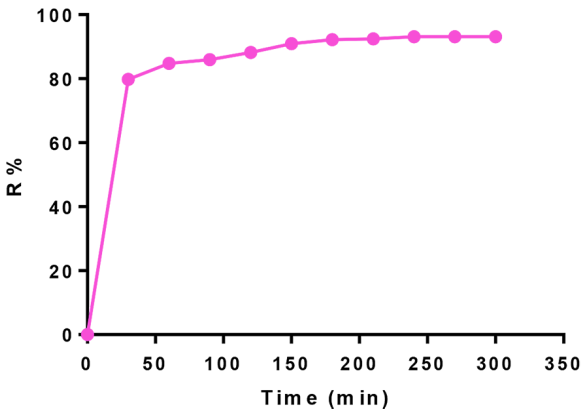


**Figure 4.** Influence of contact duration on arsenic deletion using 0.5 g in 50 ppm concentration and pH 8

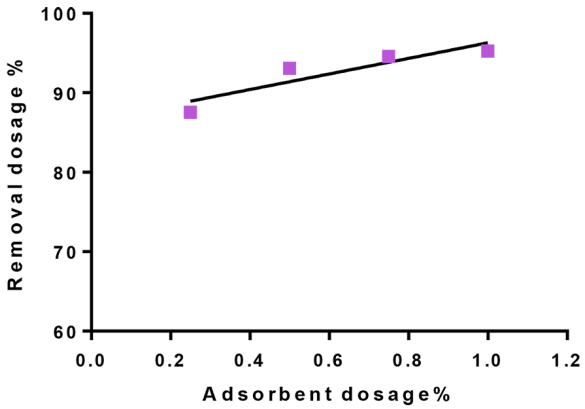


**Figure 5.** Influence of Al-Kheriat dosage on removal of arsenic at pH 8, 50 ppm concentration, and 4 hrs

Then a slight increase continued until reaching 240 min and this is due to the low concentration gradient. After that the removal was constant and this indicates that equilibrium was reached [58].

*Effect of Al-Kheriat Dosage*

It can be deduced from Figure 5 that there is a minimal increase in the removal of arsenic for rising in the dosage of Al-Kheriat. With increasing adsorbent content, arsenic absorption was shown to rise. The maximum removal was reached at pH 8 using 1 g of Al-Kheriat in 50 ppm arsenic concentration after 4 hours. The same result was stated by Mondal et al [59].

*Effect of arsenic initial concentration*

Different concentrations were investigated (50, 100, 150, 200) at pH 8 using 0.5 g of Al-Kheriat with continuous stirring for 4 hours. As clearly shown in Figure 5 that increasing arsenic concentration had a diverse effect on the removal where about 93% of the pollutant was removed for a starting concentration of 50 ppm then was reduced to 80.5% at the highest concentration (200 ppm). The increase in concentration can offer the driving force for adsorption until there aren't enough active sites in the host adsorbent to occupy the increased amount of the pollutant [60].

## Biosorption isotherms

Experimental data were compared to three equilibrium models using biosorption isotherms: Langmuir and Freundlich. Arsenic adsorption to adsorbent weight under constant temperature, pH, and starting concentration is described by a biosorption isotherm.

Langmuir isotherm can be revealed in Eq. 3 [58]:

$$\frac{C_e}{q_e} = \frac{1}{bq_0} + \frac{C_e}{q_0} \tag{3}$$

Arsenic ion concentration (mg/L) and arsenic adsorption (mg adsorbate/g adsorbent) at equilibrium are $q_e$ and $C_e$, respectively [61]. $C_e/q_e$ plotted versus $C_e$ shows that the highest capacity of adsorption $q_0$ (mg/g) and the constant of Langmuir b (L/mg) may be obtained as demonstrated in Figure 7. Table 1 shows the values of $q_0$ and $b$.

The isotherm of Freundlich is provided by Eq. 4 [42]:

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \tag{4}$$

where: $K_f$ (mg adsorbate/g adsorbent) and n are the constants of Freundlich. The $K_f$ values and $n$ are obtained from the plot of slope and intercept of $q_e$ against $C_e$ in Figure 8. Table 1 shows how much $K_f$ plus $n$ are. The value of n indicates that the adsorbate is easily adsorbed [42].

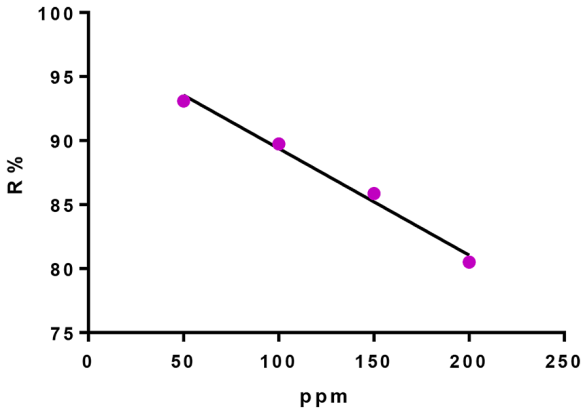


**Figure 6.** Effect of arsenic initial concentration at 0.5 g Al-Kheriat, pH 8, 50 ppm concentration and 4 hrs

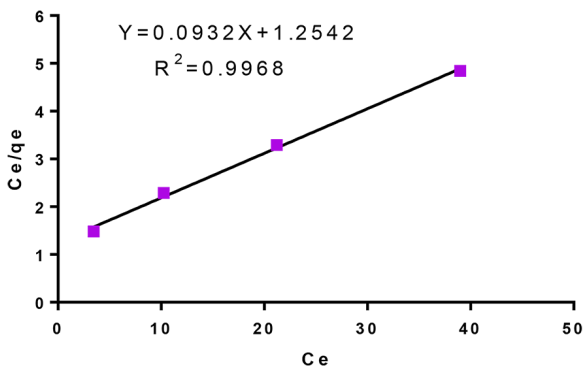


**Figure 7.** Isotherm plots of Langmuir for arsenic ion adsorption, pH 4, Al-Kheriat (0.5g), and arsenic (50 ppm)

**Table 1.** Equilibrium isotherms

| Isotherm type | Equation | Parameters | % $R^2$ |
|---|---|---|---|
| Langmuir | $\frac{C_e}{q_e} = \frac{1}{bq_0} + \frac{C_e}{q_0}$ | $q_m$ (mg/g)=10.7296<br>b (L /mg)=0.0743 | 99.68 |
| Freundlich | $\ln q_e = \ln K_f + \frac{1}{n} \ln C_e$ | $K_f$ (mg/g) = 1.2743<br>n= 1.9316 | 99.01 |

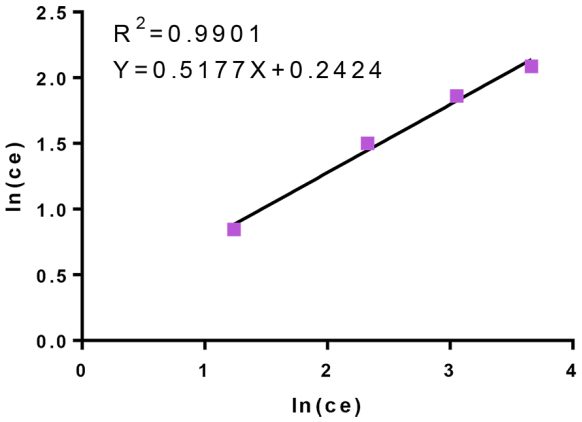


**Figure 8.** Freundlich isotherm plots for arsenic ion adsorption, pH 4, 0.5 g of Al-Kheriat, and 50 ppm of arsenic

## Toxicity of a mixture of Al-Kheriat and arsenic as a rodenticide

Results of rodenticide tests showed that mice given Al-Kheriat saturated with arsenic died in substantial numbers (As). According to the mice's consumption of rodenticide, different ratios and periods of death occurred. Both control groups were found to be free of fatalities when the median fatal dose (LD50) was computed which is the toxin dose that is needed to kill half of the tested samples, mice in this case. The LD50 is a measure of the amount of toxin (in mg) per kilogram (kg) of dead animals, expressed as a percentage. The LD50 (mg/kg) was determined to be 175 (mg/kg), which agreed with the literature [62].

## Acute liver damage biomarkers induced by arsenic

Normal mice were given oral doses of arsenic (175 mg/kg/day) along with AL-Kheriat, and all serum biochemical parameters of the liver differed from those of mice in normal control fractions. ALT, AST, along with ALP activities were used to assess the severity of liver injury in the participants in our research (Figure 1, 2, 3). Liver function abnormalities were found in those exposed to prolonged arsenic exposure, indicating liver injury and malfunction.

As shown in Figure 9, In AL-Kheriat-treated mice, serum ALP activity was close to the control group about 31.7 (U/L) and 31.5 (U/L), respectively. In contrast, in arsenic-treated mice and co-treatment with arsenic and AL-Kheriat, the ALP activity was increased to 121.2 (U/L) and 143.1 (U/L), respectively. Mice exposed to AL-Kheriat with arsenic (175 mg/kg/day) produced a substantial liver injury in blood enzyme activity ALP levels that were comparable to the control.

As revealed in Figure 10, In AL-Kheriat -treated mice, serum ALT activity was similar to the control group about 23.1(U/L) and 23 (U/L), respectively. While arsenic (As) treated mice and co-treatment with AL-Kheriat and As, the ALT activity was increased to 31(U/L) and 32.8 (U/L) respectively. Mice exposed to AL-Kheriat with arsenic (175 mg/kg/day) caused significant hepatic injury, with blood enzyme activity (ALT) at comparable levels to control.

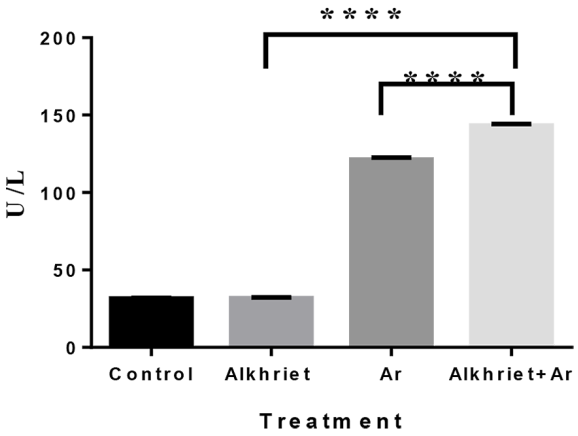


**Figure 9.** ALP enzyme activity in mice following treatment with Al-Kheriat combined with AS 175mg/Kg. the Data show the mean and standard deviation (± SD) of three repetitive experiments. One-way analysis (Anova) utilizing distal water serves as a control for statistical data measurement of each period individually (****p≤ 0.0001)

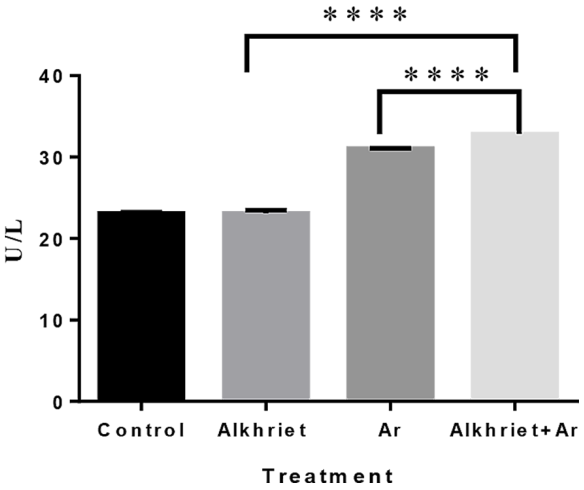


**Figure 10.** ALT enzyme activity in mice following treatment with Al-Kheriat combined with As 175 mg/Kg. the Data show the mean and standard deviation (± SD) of three repetitive experiments. One-way analysis (Anova) utilizing distal water serves as a control for statistical data measurement of every period individually (****p≤ 0.0001)

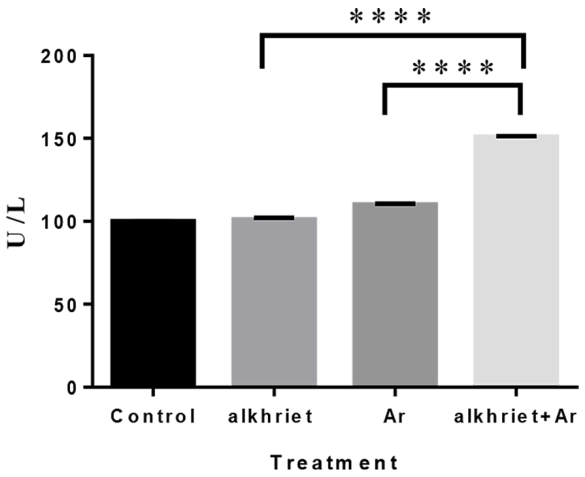


**Figure 11.** AST enzyme activity in mice following treatment with Al-Kheriat combined with As 175mg/Kg. the Data show the mean and standard deviation (± SD) of three repetitive experiments. One-way analysis (Anova) utilizing distal water serves as a control for statistical data measurement of each period individually (****p≤ 0.0001)

As exposed in Figure 11, In AL-Kheriat-treated mice, serum AST activities were close to the control group about 101.1 (U/L), and 99.96(U/L) respectively. While As treated mice and co-administration of arsenic and Al-Kheriat, the AST activity was increased to 110(U/L) and 150.96 (U/L), respectively. Mice exposed to Al-Kheriat with arsenic (175 mg/kg/day) produced substantial liver damage in blood enzyme activity AST levels compared to controls.

As a result, the activities of mice blood enzymes (ALP, AST, and ALT) were affected by arsenic treatment. The rise in blood serum hepatic enzymes is possibly attributed to liver malfunction and an alteration in the permeability of a hepatic membrane [63]. When AL-Kherait and As were administered together, the above-mentioned parameters in the mice were significantly elevated (AST, ALT, and ALP).

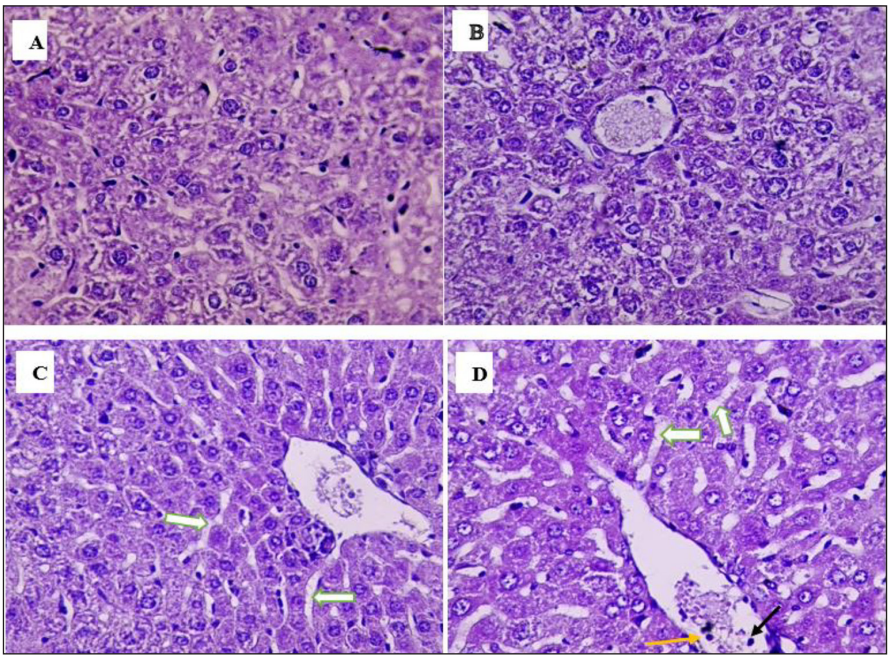


**Figure 12.** A microscopic image of the liver's internal structure H&E 40X A-control group. B-al-kheriat treated mice. C-arsenic treated mice. D-AL-Kheriat and As treated mice

Figure 12 depicts representative images of the histological investigation of liver tissue (a-d). Liver slices from mice in the control group and mice treated with AL-Kheriat both exhibited normal hepatic cytoarchitecture. They were generated by hepatocytes that spread out from the middle vein to the peripheral of certain lobules (Figure 4a and b). The lobules of the liver in As-received mice demonstrated dilation of the portal vein, as well as inflammatory cells in the entrance area and sinusoidal dilatation (white arrow) (Figure 4c). Liver slices from mice treated with AL-Kheriat+ As (Figure 4d) revealed slight to moderate dilatation (white arrow) of the central vein, as well as an inflammatory infiltration (orange arrow) and necrosis (black arrow).

## CONCLUSIONS

Using Al-Khriet as an arsenic removal method was shown to be successful and cost-effective due to its abundance in the natural environment. As a consequence of this study, adsorbent saturated AL-Kheriat with arsenic was tested on albino mice and compared with the median lethal dosage (LD50) for those mice. AL-Kheriat, as demonstrated in this study, enhanced the activity of ALP, ALT, and AST enzymes in a way that might raise their serum levels. It's safe to conclude that the combination of AL-Kheriat and As can harm the liver in terms of both biochemical and histological alterations in mice. In terms of key metabolic organs, the liver was the primary target. Toxicological effects can be induced by a combination of plant extract with individual heavy metals. The performance and severity of injuries are influenced by both the type of heavy metals and experimental animals. This residue had an impact on the mice, according to the test results. Additionally, a low-cost rat insecticide will boost the economic viability of this type of elimination by establishing a safe method of disposing of adsorbed residue.

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