Possible Efficacy of Agarose Powder Compared with Activated Charcoal in Treatment of Acute Valproic Acid Overdose in Rats

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KEYWORDS
Agar,
Adsorbent,
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Activated charcoal,
Enterohepatic reabsorption.

ABSTRACT

Acute toxicity is a critical medical emergency that needs urgent and effective treatment. Debates about the effectiveness of activated charcoal have been raised last years, and toxicologists have started searching for alternative adsorbents. This experimental study assesses the efficacy of agar as an adsorbent to drugs with enterohepatic reabsorption, like valproic acid, in comparison with activated charcoal. Method: Randomized controlled trial was designed using thirty-two non-pregnant female adult albino rats, which were divided into four groups at random. Groups I, II, III, and IV represented the negative control, positive control, overdose, and treated groups, respectively. Group III received valproic acid (200mg/kg) only, while group IV was subdivided into three groups that received the same dose of valproic plus activated charcoal (1g/kg), agar (1 g/kg), and both activated charcoal and agar in groups IVa, IVb, and IVc, respectively. Results: The mean serum valproic acid levels in the treated groups (IVa, IVb, and IVc) were statistically significantly decreased in comparison with the overdose group. In comparing the three treated groups, group (IVb) showed the least mean of valproic acid, but the difference with group (IVa) was statistically insignificant. Liver enzymes were lower in groups treated with agar only or agar and activated charcoal than in the group treated with activated charcoal only. Conclusions: Agar reduces the serum level of valproic acid, which may be due to its possible adsorptive effect and interference with enterohepatic circulation. Further studies are needed on a broad spectrum of drugs whether they have enterohepatic circulation or not.

Introduction ·

Acute toxicity is a global problem. It ranks as one of the most prevalent medical emergencies and raises the population's rates of morbidity and mortality (Wahba et al., 2021). According to estimates from the World Health Organization (WHO), acute poisoning accounts for about 45,000 fatalities per year (Celegen, 2021).

There are significant regional differences in the annual incidence of acute poisoning referrals to emergency departments, which range from 0.076% to 0.7%. (Kaya et al., 2015). Since the majority of these occurrences aren't really reported and prior epidemiological studies have mainly focused on local data, it's difficult to determine the precise number of poisoning incidents that happen annually in Egypt (Abdelhamid, 2021).

The information released at Ain Shams University Hospitals, the Poison Control Center of (PCC-ASUH), which is the major poison control center in Egypt, poisoning is

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mostly a trigger for increasing threats challenging the community (Tawfik and Khalifa, 2017).

In cases of acute poisoning, activated charcoal (AC) is recommended for the toxin's primary elimination (Barnes et al., 2021). However, there are no globally recognized guidelines for the use of AC in the treatment of acutely intoxicated patients. (Zellner et al., 2019).

Randomized controlled trials using AC are virtually impossible due to ethical issues; hence, the majority of the data come from in-vitro investigations, animal experiments, studies with volunteers, case reports, clinical case series, or observational studies. The only significant human research on the use of AC was conducted in developing nations, with relatively contradictory results (Eddleston et al., 2008).

Activated charcoal should be given to alert and cooperative patients as soon as possible. The toxin must exhibit adequate binding to AC, which is not the case for acids/bases, glycols, organic solvents, metals, or alcohols. Adverse effects of AC include mainly GIT upset in the form of vomiting and nausea, in addition to constipation or diarrhea, the urge to defecate, and anal discomfort, especially with regular use (Zellner et al., 2019). Aspiration of charcoal leading to pulmonary failure is a rare but serious complication with a potentially fatal outcome (Golej et al., 2001). So, there is a need for using new adsorbent agents for acutely intoxicated cases, such as agar.

Agar is a seaweed-derived gelatinous material that is accessible, affordable, safe, and simple to feed. It can bind to bilirubin in the colon and lessen its enterohepatic circulation, making it useful for hyperbilirubinemic newborns (Radwan et al., 2023), so agar acts as a trapping agent in the intestinal lumen. From this point, the authors suggested that agar can bind other toxic substances in intestine and prevent their absorption into the systemic circulation, especially those that enter the enterohepatic circulation, such as valproic acid (VA).

Valproic acid is an anticonvulsant widely used for the treatment of bipolar disorder and epilepsy (Löscher, 2002). It is reabsorbed at a later time after being excreted in the bile and then transported via the hepato-biliary/ gastrointestinal tract (Pollack and Brouwer, 1991).

It is significant to remember that VA toxicity can be a medical emergency, and if toxicity is suspected, immediate medical assistance should be sought. This enables quick assessment, suitable management, and monitoring to assure the affected person's wellbeing (Ghodke-Puranik et al., 2013).

To our knowledge, no attempt has been made till date to evaluate the possible adsorptive effect of oral agar in cases of acute poisoning. The present study aimed to evaluate the possible adsorptive effect of oral agar in acute valproic acid overdose and compare it with that of activated charcoal in rats.

Methods:

Chemicals:

1. Activated charcoal powder (molecular weight: 12.01g/mol, CAS number: 7740-44-0), was purchased from Piochem Laboratory Chemical Company, Egypt.
2. Agar powder (molecular Formula: $C_{14}H_{24}O_9$, molecular weight:336.337 g/mol) was purchased from Premier International Pharmaceutical Company, Cairo, Egypt.

3. Sodium valproate (Depakine ®) 200 mg/ml, (molecular formula: $C_8H_{15}NaO_2$, molecular weight: · 166.19 g/mol) was purchased from Sanofi Company, France.
4. Valproic acid level Enzyme Linked Immunosorbent Assay (ELISA) kits were purchased from Glory science company, Ltd., China.
5. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), Blood Urea Nitrogen (BUN), creatinine (Cr), and lactate colorimetric method kits were purchased from the Egyptian Company of Biotechnology, Cairo, Egypt.
6. Sodium (Na), and potassium (K) colorimetric method kits were purchased from Biodiagnostic Company, Giza, Egypt.

Study type:

Experimental animal study (Randomized controlled trial). The rats were randomly assigned into 8 equal groups (total number of groups and subgroups) using a computer-generated list of letters that were masked in sealed envelopes and opened before the experiment.

Sample Size Calculation:

Sample size is calculated by using the resource equation method (Arifin and Zahiruddin, 2017) where n= (DF/k) +1, where n is the number per group, DF is the minimum (10) and maximum (20), and k is the number of studied groups. Therefore, the minimum number of rats that can be used in the present study is four, and the maximum number is six per group.

Animals and experimental design:

Thirty-two non-pregnant female adult albino rats were used; their weight was about 150-170 g. Rats were purchased from Assiut University's faculty of medicine's animal breeding facility. According to the referenced authority (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011), the study was carried out in accordance with the protocol that was approved by the Medical Ethics Committee (04-2024-300355), Faculty of Medicine, Assiut University, and with the application of all directions with regard to dealing with animals. In accordance with standard laboratory procedures, rats were kept in groups of four in sanitary, acceptable cages (LWH; 35 20 25cm3) in an aerated room with a suitable temperature (25±2C°) and a 12-hour light/dark cycle. There was free access to water and the typical rodent diet of bran and ground maize. Rats were placed into four groups at random after acclimation for one week:

Group I (N= 4): (Negative control) rats received 2ml of distilled water (DW) through a gastric tube.

Group II (N=12): (Positive control) rats were subdivided into three subgroups: -

Group IIa (N=4): rats received AC once at a dose of 1g/kg (Silberman, Galuska et al. 2022, Lu and Xue 2019) dissolved in 2ml of distilled water through a gastric tube.

Group IIb (N=4): rats received agar once at a dose of 1g/kg (equal to that of AC) dissolved in 2ml of distilled water through a gastric tube.

Group IIc (N=4): rats received both agar and AC once at a dose of 1g/kg for each dissolved in 2ml of distilled

water through a gastric tube. Activated charcoal was given first, and then after 60 minutes, agar was given.

Group III (N=4): (overdose group) rats received VA overdose (200mg/kg) (Peter et., al 1998) once through a gastric tube. The concentration of valproic acid in the used preparation is 200mg/ml, thus the dose is adjusted according to weight.

Group IV (N=12): (treated groups) rats were subdivided into three subgroups: -

Group IVa (N=4): rats received a VA overdose (200mg/kg) then received AC after half an hour once at a dose of 1g/kg dissolved in 2ml of distilled water through a gastric tube.

Group IVb (N=4): rats received a VA overdose (200mg/kg) then received agar after half an hour once at a dose of 1g/kg dissolved in 2ml of distilled water through a gastric tube.

Group IVc (N=4): rats received a VA overdose (200mg/kg) then received both agar and AC after half an hour, once at a dose of 1g/kg for each dissolved in 2ml of distilled water through a gastric tube. Activated charcoal was given first, and then after 60 minutes, agar was given.

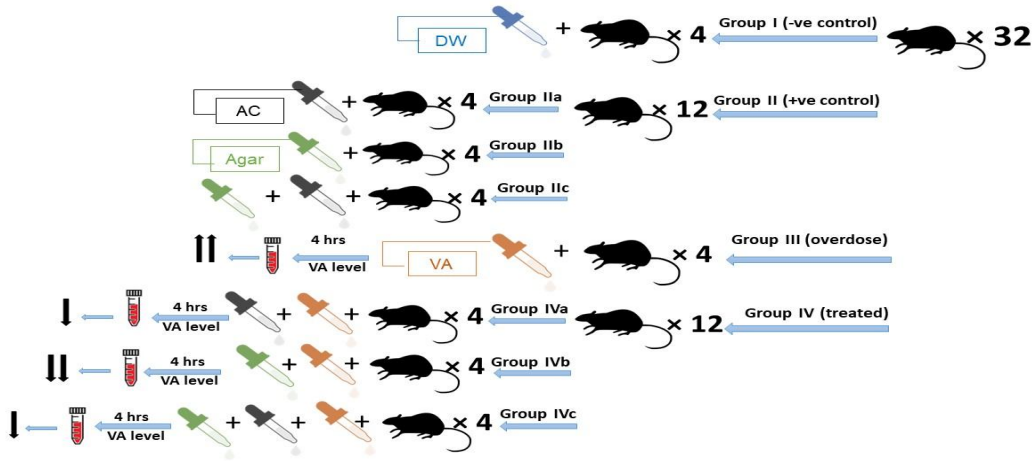

Fig (1): Schematic draw for the studied groups and main findings of the study.

Laboratory investigations

1. Blood sample collection: Approximately 3 ml of venous blood samples were drawn from each rat (retro-orbital) into plain tubes. Samples were coded and sent to the lab to be processed on an icebox. In the laboratory, samples were left for half an hour at room temperature to coagulate then centrifuged for 10 minutes using a Rotofix centrifuge (32A, Germany).
2. Valproic acid level measurement:

   The serum level of VA was measured after four hours of administration according to (Sztajnkrycer, 2002) by Enzyme-Linked Immunosorbent Assay (ELISA) according to the protocol suggested by the manufacturer. Optical densit (OD) of samples was measured at 450mn wavelength using a microplate reader (Pharmacia LBK Spectrophotometer, Biochrom, England).

The VA level was calculated by a standard curve. Assay range: 5ng/L -350 ng/L.

3. Liver and renal functions, lactate, sodium and potassium levels:

   Aspartate aminotransferase (AST), alanine aminotransferase (ALT), Blood Urea Nitrogen (BUN), creatinine (Cr), lactate, sodium (Na), and potassium (K) were analyzed by the colorimetric method according to the protocol suggested by the manufacturer.

   The reference ranges are as follows; AST: 7- 89 U/L, ALT: 4 - 94U/L, Urea: 15-50mg/dL, Cr: 0.7-1.3 mg/dL, lactate: 4.5 – 19.8 mg/dL, Na: 135 – 150 mmol/L, and K:3.6- 5.5 mmol/L.

Statistical analysis

SPSS version 21.0 (Statistical Program for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows was used for data entry and analysis. The Kolmogorov-Smirnov and Shapiro Wilk tests for normality were used to test normality of the distribution of data. Regarding parametric and normally distributed data (VA level, urea, creatinine, and Na), analytic statistics in the form of descriptive analysis (mean ± SD) and ANOVA were used and post hoc pairwise comparison was conducted by Tukey's HSD. Non-parametric data (lactate, AST, ALT, K levels) were analyzed using the Kruskal Wallis test and post hoc pairwise test was conducted by Bonferroni correction. P- values that are equal to or less than 0.05 were considered significant.

Results

Valproic level measurement results

The mean serum levels of VA in the treated groups (IVa, IVb, and IVc) were statistically significantly decreased in comparison with the overdose group (III). In comparing the three treated groups, group VA+ agar (IVb) showed the least mean of VA, but the difference with group VA+ AC (IVa) was statistically insignificant. Notably, the level in group VA+AC + agar (IVc) was statistically significantly higher than in groups VA+ AC and VA+ agar (see table 1).

Liver and renal functions, lactate, sodium and potassium levels results

The mean difference in urea level between the studied groups was statistically significant with a p value of ≤0.001, but the level of urea was within normal in all groups. The highest level was observed in the overdose group, which was statistically significant in comparison with all treated groups. In comparing the groups VA+ AC (IVa) and VA+ agar (IVb), there was a statistically insignificant difference. Conversely, creatinine levels didn't show a significant difference between the study groups (see table 2).

Regarding lactate level, there was a statistically significant difference among the investigated groups, with a p value of 0.006. Treated groups showed a lower lactate level than overdose, but the difference wasn't statistically significant. Analyzing the electrolyte level results, potassium and sodium levels showed statistically significant differences between the groups with p values of 0.05 and ≤0.001, respectively. The highest level of sodium was observed in the treated group VA+AC, with a statistically significant difference from all other groups (see table 2 and figure 1).

Liver function assessment in the form of investigating the mean AST and ALT levels revealed a statistically significant difference between the study groups with p values of 0.009 and 0.004, respectively. Group VA+AC

was statistically significantly higher than the negative control group in the AST level, with a p- value of 0.024. On the other side, group VA+AC was statistically significantly higher than group VA+ agar and VA+ AC+ agar in the ALT level with p- values of 0.019 and 0.004, respectively (see figure 1).

Table (1): The mean difference in four hours post valproic acid overdose level between the studied groups (n: 32).

	Group III Overdose Group	Group IVa Treated VA+ AC	Group IVb Treated VA+ agar	Group IVc Treated VA+ AC+ agar	P- value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Valproic acid level (ng/l)	42.1± 2.8	32.2±1.4[a]	31.1±1.7[ab]	35.7±1.3[abc]	**≤0.001**

SD: Standard deviation, p-value < 0.05 considered statistically significant. ANOVA test was used to compare the mean difference between groups and post hoc pairwise comparison was conducted by Tukey's HSD. Letter a indicates statistically significant difference with group III, b indicates statistically significant difference with group IVa, and c indicates statistically significant difference with group IVb.

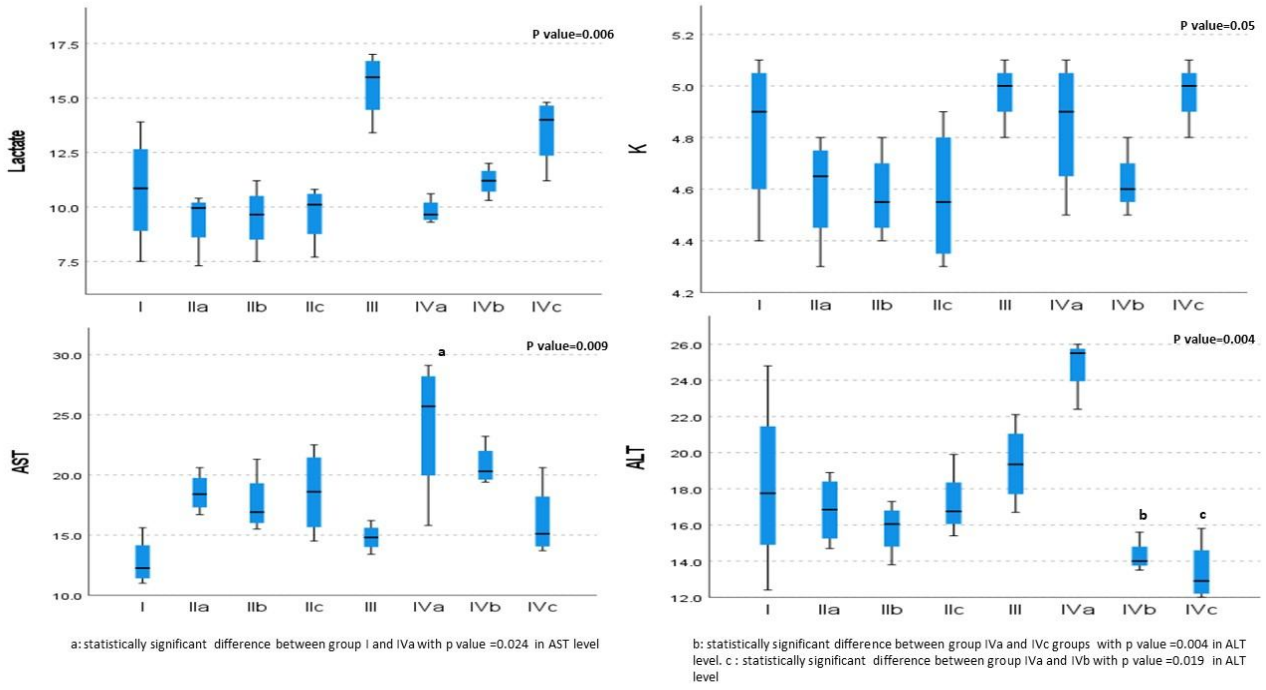

Fig. (2): Box and whisker plot showing the mean lactate, potassium, AST, and ALT levels among the studied groups. Kruskal Wallis test and post hoc pairwise test was conducted by Bonferroni correction. **P-value** < 0.05 considered statistically significant. From left to right: group I "negative control", group IIa "activated charcoal positive control", group IIb "agar positive control", group IIc "both activated charcoal and agar positive control", group III "valproic acid overdose", group IVa "valproic acid + activated charcoal", group IVb "valproic acid + agar", and group IVc "valproic acid + activated charcoal + agar". Whiskers represent standard errors.

Table (2): The mean difference in liver and renal functions, lactate, sodium, and potassium levels between the studied groups (n: 32).

	Group I -ve control	Group IIa +ve control (AC)	Group IIb +ve control (agar)	Group IIc +ve control (AC+ agar)	Group III Overdose Group	Group Iva Treated VA+ AC	Group IVb Treated VA+ agar	Group IVc Treated VA+ AC+ agar	P-value
Urea (mg/dl)	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**≤0.001**
	28.9± 3.4	24.8±4.1	24.6±4.4	24.4± 4.5	36.2± 3.0^{abcd}	33.1±2.3^{bcd}	29.6±0.9^{bcde}	25.8±1.9^{ef}	
Creatinine (mg/dl)	0.75±0.05	0.72±0.0	0.72±0.02	0.72±0.04	0.78±0.1	0.75±0.04	0.69±0.03	0.79±0.06	0.195
Na level (mmol/L)	141.7±5.2	140.7±2.1	139.4±2.7	141.1±3.6	146.1±2.7^{bcd}	155.0±2.6^{abcde}	146.0±4.2^{bcf}	143.9±1.8^{f}	**≤0.001**

SD: Standard deviation, **p-value** < 0.05 considered statistically significant. **ANOVA** test was used to compare the mean difference between groups post hoc pairwise comparison was conducted by Tukey's HSD. Letter a indicates statistically significant difference with group I, b indicates statistically significant difference with group IIa, and c indicates statistically significant difference with group IIb, d indicates statistically significant difference with group IIc, e indicates statistically significant difference with group III, f indicates statistically significant difference with group IVa.

Discussion

Acute poisoning usually requires immediate or urgent management for the best outcome. Prevention of the absorption of the toxin is still a pivotal part of acute poisoning management, especially in the absence of effective antidotes. Adsorbents may be used in place of, or in addition to, emesis or lavage to stop further toxicants' systemic absorption. These agents act by adsorbing a chemical or toxicant in the gastrointestinal tract (GIT) and facilitating its fecal excretion. The most popular adsorbent used, according to Olson (2010) and DeClementi (2018), is activated charcoal.

In order to address the significance of immediate treatment, the rationale of this study was to evaluate the effectiveness of agar as an adsorbent in overdose and poisoning cases, particularly in light of recent concerns about the ritual use of AC. The present study assessed the effect of using AC alone, agar alone, or both together on serum VA levels, renal and hepatic functions, lactate, sodium, and potassium levels following VA overdose.

Valproic acid, a well-known anticonvulsant, is reabsorbed when bacterial enzymes deconjugate the VA glucuronide, releasing free VA again, which can be reabsorbed into the bloodstream through the intestinal wall (Pollack and Brouwer, 1991). Moreover, during VA overdose, an increased concentration of VA can saturate the protein-binding sites, leading to an increase in the fraction of free VA. The increased levels of free VA contribute to its toxicity (Wallenburg et al., 2017). Hence, VA overdose serves as a good example of poisoning to assess the adsorptive properties of the tested agents that might be determined by measurement of the VA-free serum level, which reflects its toxicity.

The current study demonstrated that treatment with either oral agar at a dose of 1 g/kg alone or oral AC at a dose 1 g/kg alone and concurrent treatment with both agar and AC at the same mentioned dose resulted in a

significant reduction in VA serum levels in overdosed rats. The decline in the serum VA was the most evident in the agar-only-treated group. The basis for the proposed mechanism by which agar enhances the clearance of VA is the high binding affinity of enteral agar for VA glucuronide and sequestration from enterohepatic circulation. However, the results of this study showed that, compared to agar-only or AC-only regimens, oral agar administration in combination with AC had less influence on the mean serum level of VA overdose. This can be explained by the narrow time window used in this study to separate both agar and charcoal. Authors postulated that 60 minutes could be adequate. Another considerable factor was that adsorbents' effectiveness increases when administered within one hour of intoxication. Thus, a one-hour separation between both adsorbents was suitable (Zellner et al., 2019).

Several studies have reported the ability of charcoal to decrease the effectiveness and serum levels of various medications given with it, but no definite time window has been approved (Windrum et al., 2000). The obvious evidence was not to give any drug concomitantly with AC. Additionally, another factor influencing the extent of drug interaction administered concomitantly with AC is the presence of food in the stomach, as reported by (Imaoka et al., 2019).

Peak serum levels of valproic acid can be reached after one to four hours of oral ingestion (Sztajnkrycer, 2002). The present study assessed VA level after 4 hours of administering the VA overdose since it gave an adequate time to assess the reabsorption potential of the adsorbents and their effect on peak level.

Activated charcoal is primarily used as an adsorbent and detoxifying agent, and it is generally considered tasteless but may have a slight earthy flavor and black color, while agar, on the other hand, has an advantage for its neutral taste, gelatinous texture, and white color.

As an adsorbent, agar possesses a porous structure that can trap and retain certain substances. The agar-gel matrix provides a favorable environment for adsorption due to its high surface area and hydrophilic nature. It could also adsorb several organic compounds that couldn't be adsorbed by AC, including dyes, pigments, and heavy metals (Chen et al., 2021).

Activated charcoal is widely used as an adsorbent due to its large surface area and porous structure. But it is well known that activation is needed to create a network of tiny pores and increase the available surface area, enabling AC to effectively adsorb a wide range of substances (Husien et al., 2022). Concerning the complications addressed by patients after AC administration, vomiting is the most reported side effect. Constipation and diarrhea are reported at high doses. The serious complications of either small intestinal pseudo-occlusion or charcoal stercoliths perforating the colon or aspiration of charcoal leading to pulmonary failure are rare conditions (Menzies et al., 1988; Chyka et al., 2005; Olson 2010). Accordingly, Zellner et al. (2019) reported that a precise analysis of the risks and benefits is needed for each administration.

Activated charcoal is employed not only in acute intoxication management but also in the management of other medical conditions like neonatal jaundice, gout, hypercholesterolemia, and porphyria (Windrum et al., 2000). Agar could as well bind bilirubin in the intestine, decreasing its enterohepatic circulation, and therefore be proposed to be used in neonatal hyperbilirubinemia too (Abdel-Aziz et al., 2022).

Substantial features of VA overdose involve CNS depression, hepatotoxicity, thrombocytopenia, hemodynamic instability, electrolyte abnormalities, hyper-ammonemia, and acid-base imbalances (Spiller et al., 2000; Muñiz, 2017). The possible protective effects of AC and agar on liver, kidney, and acid base and electrolytes balance were investigated in the current study. Although normal ranges among all studied groups were observed through the studied investigations, significant differences between groups were still detected.

Concerning the urea level in this study, the lowest urea value was observed in the positive control group treated with both AC and agar, followed by the positive control group treated with agar alone. In comparing the serum urea levels among the three treated groups (group IV), the lowest level was in the group treated with both AC and agar, followed by the group treated with agar. Therefore, regimens that included agar either alone or with AC were linked to lower urea levels. The liver plays a crucial role in the production and metabolism of urea. Liver dysfunction can impair the liver's ability to metabolize urea, potentially leading to elevated urea levels in the blood (Meseguer et al., 2021). Additionally, propionic acid, which is a VA metabolite, inhibits mitochondrial carbamoyl phosphate synthetase, an enzyme necessary for ammonia elimination, resulting in hyperammonemia observed in VA overdose. The interaction of VA with carnitine may be another attributable cause of hyper-ammonemia (Murty, 2019). On the other hand, creatinine levels were insignificantly different between the tested groups.

Lactic acidosis, hypernatremia, hypocalcemia, and hypophosphatemia are the potentially serious metabolic complications linked to VA overdose. Lactic acidosis is the acid-base imbalance resulting from the buildup of lactic acid in the body (Ge et al., 2017). This study demonstrated that the mean lactate blood level was elevated in the overdose group in comparison with other groups. The previously mentioned effect of VA metabolites is the depletion of intramitochondrial coenzyme A and carnitine, which in turn inhibits the b-oxidation of fatty acids, impairing ATP production (Raskind and El-Chaar, 2000). Metabolic acidosis is an important consequence of acute VA intoxication because profound acidosis after massive ingestions confers a poor prognosis (Judge, 2005). The lactate level in the group treated with both AC and agar was higher than the lactate level in groups treated with either adsorbent alone. The difference between the groups treated with agar-only and AC-only regimens wasn't significant.

Electrolytes play a vital role in body hemostasis. Valproic acid toxicity-related metabolic acidosis and renal damage have an impact on electrolyte homeostasis. Moreover, electrolytes have an impact on each other's.

The current study showed a significant elevation in the sodium level between the overdose group and the controls. Concerning the difference between the overdose and treated groups, it was not statistically significant except for the AC-only group, which was the highest. Hypernatremia is simply due to VA's composition as sodium valproate (Crudup et al., 2011). It could be accounted for by the fact that AC is a poor ion and inorganic salt binder (Zellner et al., 2019).

Valproic acid is primarily metabolized in the liver (Meseguer et al., 2021). The results of the presenting study revealed that the groups treated with AC-only were associated with higher levels of ALT and AST in comparison with agar only or AC-and-agar groups. Agar has been used in

hyperbilirubinemia cases for the last few years and may be associated with hepatic protection properties that make it a promising adjuvant in hepatotoxic compound poisoning management. Direct hepatotoxicity is a well-known VA overdose sequel. This frequently shows up as a slight rise in blood transaminase levels, but it can also happen as an idiosyncratic reaction that causes hepatic failure. There aren't many examples of hepatic failure after acute VA intake, although other clinical features such as elevated transaminases, hepatic dysfunction, and hyperammonemia are frequently seen (Russell, 2007). The lower urea levels in groups using the agar regimen may be due to the proposed hepatoprotective effect, as the liver is important for urea metabolism.

Conclusions

Ultimately, giving agar orally to rats which have taken a valproic acid overdose has greatly reduced their serum level and decreased the likelihood of hepatic injury. Reviewing the mechanism of action, activated charcoal could be more helpful in adsorbing drugs in the upper GIT, but agar could act on the re-absorption of the drugs in the intestine. Agar could be specifically effective for hepatotoxic and lipophilic drugs. Therefore, agar can't replace activated charcoal; they can augment the effects of each other. Gastrointestinal decontamination can be done with activated charcoal and agar with longer separation time.

Therefore, agar can be tested as an adsorbent for drugs with enterohepatic reabsorption properties. Additionally, the side effect profile of agar is favorable (doesn't cause constipation nor vomiting as it has an acceptable taste). Thus, its use may be a beneficial adjuvant in the treatment. Further randomized controlled studies are needed before the use of agar as an adsorbent becomes a true standard of treatment.

Recommendations and limitations

We recommend using repeated doses of agar in the forthcoming studies. Trials with other drugs rather than valproic associated with enterohepatic circulation might be considered. Additionally, conduction of randomized controlled clinical trials is required to support the result of the animal studies. We advise augmenting the effect of activated charcoal with agar by increasing the time interval between them. We think that charcoal could act earlier than agar, and both have different mechanisms of action that can complement each other. Besides, repeated measures of valproic acid can be addressed as a limitation in the current study.

Acknowledgement

Not applicable.

Conflict of Interest

Authors declare no conflict of interest.

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