

1 **A novel green approach for treatment of immature Schistosomiasis**
2 **Mansoni infection in mice; Arabic gum (Acacia Senegal)**
3 **antischistosomal properties**

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25 **Abstract**

26 Schistosomiasis is one of the most socioeconomically exhausting parasitic
27 infection in tropical and subtropical areas. Praziquantel (PZQ), the only common
28 schistosocidal drug in use, is not efficient enough for treatment of immature infection.
29 Arabic gum (AG) is a complex polysaccharide acts as anti-oxidant which modulates the
30 inflammatory and/or immunological processes. This study explores for the first time, the
31 antischistosomal properties of AG in mice infected with the immature stage of
32 *Schistosoma mansoni*. Mice were divided into four groups: control group (infected non-
33 treated), AG treated group, PZQ treated group, and AG+PZQ treated group. Oral
34 administration of AG in a dose of 1gm/kg body weight, daily for 3 consecutive weeks
35 post-infection (p.i.) resulted in a statistically significant lower worm burden in both AG
36 group and AG+PZQ group compared to PZQ and control groups. AG+PZQ group always
37 showed the best performance when compared with other groups regarding tissue egg load
38 and oogram pattern. AG, both alone and in combination with PZQ, decreased the
39 number, diameter; increased the cellularity and the number of degenerated Schistosoma
40 eggs inside granulomas. Results obtained by this work elucidated a promising AG
41 bioactivity against *S. mansoni* immature stages and provided a platform for subsequent
42 experimental studies to illuminate the academia more about this novel and " green"
43 antischistosomal agent.

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45 **Author summary**

46 Schistosomiasis is a major public health threat in many parts of the world, it
47 affects more than 240 million people in more than 70 countries and almost 800 million
48 people are at risk of acquiring this disease. Serious consequences and disabilities might

49 result from untreated schistosomiasis such as hepatosplenic fibrosis with portal
50 hypertension, gastrointestinal hemorrhage and death.

51 Schistosomiasis control is focused on periodic treatment with praziquantel
52 (PZQ). However, PZQ has only moderate action against young developing stages of
53 schistosomula. Recently, resistance has emerged to PZQ. Therefore, chemotherapy
54 alone is unlikely to reduce infection levels of schistosomiasis. Several practical
55 approaches have been suggested to augment treatment programs. Of course, the
56 development of a complementary treatment would contribute enormously to the
57 reduction of schistosomiasis. Recently, natural products have been popular and
58 attracted most of the attention as it could offer new effective therapy against
59 schistosomiasis. Arabic gum (AG) is an edible, dried sticky exudate from *Acacia*
60 *Senegal*, which is used in this study to assess the AG antischistosomal properties. Our
61 study revealed that AG has an excellent statistically significant effect against immature
62 murine schistosomiasis, both alone and in combination with PZQ. This approach may
63 point to novel targets for treatment of schistosomiasis.

64 **Key words:**

65 *Schistosoma mansoni*, Immature stages, Arabic gum, Antischistosomal properties, Mice.

66 **Introduction**

67 Schistosomiasis is the most common disease caused by parasitic worms, known as
68 blood flukes, it affects over 240 million people around the world with almost 800 million
69 are at risk of infection [1]. Serious consequences and disabilities might result from

70 untreated schistosomiasis such as chronic malnutrition, anemia, organ scarring and
71 fibrosis [2].

72 Control of such long-term morbidity is a priority of the World Health
73 Organization (WHO), it adapts a preventive strategy via mass drug administration
74 campaigns [3]. Praziquantel is the drug of choice for treating all species of *Schistosoma*.
75 Unfortunately, some strains have developed a resistance against it making their treatment
76 a challenge [4-7]. Although praziquantel is highly effective against adult schistosomes
77 and very early stage of schistosomula just few hours after penetration into the host's skin,
78 it is much less effective against young developing stages of schistosomula [8], Thus, it is
79 essential to develop a new irresistible alternative lacking the aforementioned drawbacks
80 [9].

81 Arabic gum (AG) is a dried exudate obtained from stems and branches of *Acacia*
82 *senegal* (Leguminosae), consisting of calcium, magnesium, and potassium salts of the
83 polysaccharide Arabic gum acid [10]. It has been used in Arabic folk medicine to treat
84 patients suffering from chronic renal failure as it decreases the requirements as well as
85 the frequency of hemodialysis [11]. US Food and Drug Administration have listed AG as
86 one of the safest dietary fibers [12].

87 Different studies showed that AG can modulate TGF- β 1 generation and function
88 [13], stimulated mouse dendritic cells [14], control chemical plaque in subjects with
89 gingivitis [15], and exert a cytoprotection against Hg-induced nephrotoxicity [16].

90 Other studies reported several favorable renal effects including reduced plasma
91 phosphate concentration, blood pressure, proteinuria, as well as extra renal effects such as
92 slowing of intestinal glucose transport, which might be of value in the prevention and

93 treatment of obesity and diabetes [17, 18]. It has been also reported to induce fetal
94 hemoglobin in sickle cell anemia [19], prevents and enhances healing of gastric ulcers
95 [20], influences the expression of murine ovarian oxidative stress gene [21] and improves
96 semen quality and oxidative stress capacity in alloxan-induced diabetes in rats [22].
97 As antimicrobial agent, AG was reported to be an efficient antimicrobial agent, of a
98 natural origin, against many buccal microorganisms such as *Prophyromonas gingivalis*
99 and *Prevotella intermedia* [23] fungi as *Candida albicans*, *Aspergillus niger* and
100 *Microsporium canis* [24] bacteria as *Staphylococcus aureus*, *Staphylococcus epidermidis*,
101 *Streptococcus pneumoniae*, *Salmonella typhi*, *Ps. aeruginosa*, [25]. As far as we know,
102 only one published parasitological study has investigated the antimalarial effect of AG, it
103 stated that AG slightly, yet, significantly decreased the parasitaemia and significantly
104 expanded the life span of the infected mice [26].

105 The aim of this study was to explore and evaluate the antischistosomal properties
106 of AG in mice infected with *Schistosoma mansoni* at the immature stage.

107 **Materials and methods**

108 A pilot study was done on about 30 mice 2 months before the main experiment to
109 assess if there is any antischistosomal activity of AG which has been given as 10% in the
110 drinking bottles for 3 weeks starting from the day of infection. The results of AG on the
111 scale of total worm load was remarkable and encouraging to launch the main experiment.

112 **Parasites and animals**

113 Fifty laboratory-bred male Swiss albino mice, CD1 bred, were used in this study,
114 as this was the minimum required number to guarantee statistically reliable and
115 reproducible results. Cercariae of *S. mansoni* were obtained from infected *Biomphalaria*

116 *alexandrina* snails, which were reared and maintained at Schistosome Biological Supply
117 Program (SBSP), Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Each mouse
118 was infected with 80 *S. mansoni* cercariae suspended in 0.2 ml water via subcutaneous
119 injection [27].

120 **Ethics Statement**

121 This study was conducted in accordance with legal ethical guidelines of the
122 medical ethics committee of the Theodor Bilharz Research institute (TBRI) , Giza, Egypt.
123 Approval no. 4013/2016.

124 **Experimental design**

125 Mice were divided into 4 groups, 10-13 mice each, representing: AG treated
126 group, PZQ treated group, AG+PZQ treated group and untreated infected control group.
127 AG group mice were treated daily starting from the 1stday post-infection (p.i) till the 21st
128 day using a dose of 1gm/kg body weight (AG is a powdered material obtained from local
129 conventional herbal medicine market, suspended in water as a solvent reagent at a
130 concentration of 100mg/ml). This dose was similar to that of Nasir *et al.* 2012 [17], but
131 given individually to each mouse orally using a syringe with a curved end. On the 21st&
132 22nd day PZQ (Alexandria Company for Pharmaceuticals and Chemical Industries, Alex.,
133 Egypt) was freshly suspended in 13 ml of 2% cremophore-EL (Sigma Chemical Co.,
134 USA) and orally administered to mice at a dose of 500 mg/kg body weight for two
135 consecutive days [28]. Three weeks later (6 weeks p.i) all animals were scarified to assess
136 AG antischistosomal efficacy.

137 **Evaluation of AG antischistosomal effect**

138 **1. Worm Burden**

139 Schistosomes recovery was done by porto-mesenteric perfusion technique, 3
140 weeks post-treatment, according to the method of Duvall and DeWitt, [29]. Drug efficacy
141 was measured by percent reduction of worms according to the formula of Abdel Salam et
142 al., [30]: $R\%$ (percent reduction) = $C-T/C \times 100$, where C is the mean worm burdens in
143 control infected animals and T , mean number of worms in infected treated animals.

144 **2. Tissue egg load (hepatic and intestinal)**

145 Segments of liver and intestine were blotted between two filter papers, weighed,
146 transferred each to a test tube containing 5 ml 5% potassium hydroxide solution [31], and
147 left overnight at room temperature to facilitate tissue digestion without egg destruction.
148 Next morning, tubes were incubated at 37°C for 1 h to finish the tissue clearance [32].
149 Ova in homogenous emulsions were counted after being spread on slides, and the number
150 of ova/mg tissues was calculated. To detect the egg load in the hepatic and intestinal
151 tissue, the average number of eggs in 1 ml sample was multiplied by the total volume of
152 potassium hydroxide, then divided by the weight of tissue to yield the number of
153 eggs/gram tissue [33]. Percentage reduction was accordingly calculated. $R\%$ (percent
154 reduction) = $C-T/C \times 100$, percentage reduction was calculated using the aforementioned
155 equation [30].

156 **3. Oogram pattern**

157 After mice perfusion, three segments, one cm in length of the small intestine were
158 cut longitudinally, rinsed in saline, slightly dried on filter paper, compressed between two
159 glass slides and examined under microscope for oogram pattern that may reflect the
160 direct drug action on ova development [34]. Eggs of *S. mansoni* were classified in the
161 current study in three types immature, mature and dead [35].

162 **4. Histopathological examination**

163 Mice livers were fixed for 48 h in 10% buffered formalin and then embedded in
164 paraffin. Haematoxylin and eosin were used to stain sections [36] for granuloma counting
165 while Masson trichrome stains [37] were used to demonstrate collagen fibers. Lesions
166 containing single ova in their centers were selected for measurement [38]. The granuloma
167 diameter of each case was measured using the ocular micrometer [39]. For each section,
168 granulomas were counted in five successive fields (10x10).

169 **Statistical analysis**

170 Gathered data were tabulated and analyzed using SPSS statistical software (IBM
171 Corp., Armonk, NY, USA). Data were expressed as mean \pm SD or SE. Analysis of
172 variance between groups was done using ANOVA test and when significant, post hoc
173 Bonferroni test was applied for pairwise comparison between groups. P value <0.05 was
174 considered statistically significant. All statistical tests were two-sided. Chi square test
175 was used to assess if there was a significant difference between granuloma types in
176 various study groups.

177 **Results**

178 Regarding the worm load, the AG group demonstrated the best reduction rate
179 (75.6%) followed by the AG+PZQ group (72.5%) while the PZQ group had the lowest
180 rate (28.7%). The difference between all groups was statistically significant (P-value
181 <0.001). Comparing each group with the control group, the difference was significant
182 except for the PZQ group (P-value 0.151). While comparing the PZQ group with the AG
183 group and with the AG+PZQ group the difference is statistically significant (P-value
184 0.002 and 0.008 respectively), Table 1.

185 **Table 1. Performance of AG, PZQ and combined AG+PZQ therapeutic regimens on**
 186 ***Schistosoma mansoni* total worm burden after treatment of infected mice during the**
 187 **immature infection stage.**

Group		Control	AG	AG+PZQ	PZQ	P value*
Result						
Total worm count	Mean±SD	16±5.2	3.9±2.5	4.4±2.6	11.4±1.8	<0.001
	(range)	(11–24)	(0–8)	(2–8)	(9–13)	
	Reduction rate (R.R)	-	75.6%	72.5%	28.7%	
	<i>P</i> versus control		<0.001*	<0.001*	0.151	
	<i>P</i> vs AG			1	0.002*	
	<i>P</i> vs AG+PZQ				0.008*	

188 AG: Arabic gum.

189 PZQ: Praziquantel.

190 About egg count in the liver, the AG+PZQ had the lowest number (950±498.8), followed
 191 by the PZQ group (1964.8±909), then the AG group (2315.8±252.7) and the highest
 192 number belonged to the control group (8507.4±915.2), with statistically significant
 193 difference (P-value <0.001). Comparing each group with the control group, the difference
 194 was significant (P-value <0.001). The AG+PZQ group demonstrated the best hepatic egg

195 load reduction rate (88.8 %) followed by the PZQ group (76.9%) while the AG group had
196 the lowest rate (72.7%). Comparing the result of the AG group with that of the AG+PZQ
197 group revealed a statistically significant difference (P-value 0.010) while comparing it to
198 that of the PZQ group revealed a non-significant difference (P-value1). Similarly,
199 comparing the result of the AG+PZQ group to that of the PZQ group revealed a non-
200 significant difference (P-value 0.226).

201 A similar pattern was noted for the intestinal egg load as the AG+PZQ had the lowest
202 number (961.1±387.2), followed by the PZQ group (1121.8±629), then the AG group
203 (3168.8±1016.7) and the highest number belonged to the control group (7205.1±1049.6),
204 with statistically significant difference (P-value <0.001). Comparing each group with the
205 control group, the difference was significant (P-value <0.001). The AG+PZQ group
206 demonstrated the best intestinal egg load reduction rate (86.6 %) followed by the PZQ
207 group (84.4 %) while the AG group had the lowest rate (56%). Comparing the AG group
208 to either AG+PZQ or PZQ group yielded a statistically significant difference (P-value
209 <0.001 and 0.001 respectively), while comparing the PZQ and AG+PZQ groups yielded a
210 non-significant difference (P-value 1). Table 2.

211 **Table 2. Comparison between the reductive effect of AG, PZQ and combined AG+**
212 **PZQ therapeutic regimens reductive effect on *Schistosoma mansoni* liver and**
213 **intestinal egg count after treatment of infected mice during the immature infection**
214 **stage.**

Group	Control	AG	AG+PZQ	PZQ	P value between all
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Result						groups
Liver egg count	Mean±SD	8507.4±915.2	2315.8±252.7	950±498.8	1964.8±9097	<0.001
	(range)	(7830–10434)	(1418–3724)	(392–1818)	(963–2750)	
	R. R	--	72.7%	88.8 %	76.9%	
	<i>P</i> versus control		<0.001*	<0.001*	<0.001*	
	<i>P</i> vs AG			0.010*	1	
	<i>P</i> vs AG+PZ				0.226	
	Q					
Intestinal egg count	Mean±SD	7205.1±1049.6	3168.8±1016.7	961.1±387.2	1121.8±629	<0.001
	(range)	(5809–8600)	(19787–4851)	(484–1720)	(528–1818)	
	R. R	--	56%	86.6%	84.4%	
	<i>P</i> versus control		<0.001*	<0.001*	<0.001*	
	<i>P</i> vs AG			<0.001*	0.001*	
	<i>P</i> vs				1	

	AG+PZQ					
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215 AG: Arabic gum.

216 PZQ: Praziquantel.

217 Concerning the oogram pattern, the AG+PZQ presented the best results demonstrating
218 the lowest immature egg count (45 ± 1.7), followed by the PZQ group (51.8 ± 1.8), then the
219 AG group (54.9 ± 6.4) and the highest number belonged to the control group (51.1 ± 4.6),
220 yet the difference was insignificant (P-value 0.015). Comparing each group with the
221 control group, the difference was also insignificant. Comparing the result of the AG
222 group with that of the AG+PZQ group revealed a statistically significant difference (P-
223 value 0.009) while comparing it to that of the PZQ group revealed a non-significant
224 difference (P-value 1). Similarly, comparing the result of the AG+PZQ group to that of
225 the PZQ group revealed a non-significant difference (P-value 0.306).

226 The mature egg count was (40.4 ± 4.9) in the AG+PZQ group, (42 ± 2.1) in the PZQ group,
227 and (40 ± 6.7) in the AG group. The difference between each group and the control group
228 was statistically insignificant. Comparing the AG group to either AG+PZQ or PZQ
229 group, as well as comparing the AG+PZQ groups yielded non-significant differences (P-
230 value 1, 1 and 1 respectively).

231 While regarding the dead egg count, the highest number was detected in the AG+PZQ
232 ($5-25$, Mean \pm SD 14.6 ± 6.8), followed by the control group ($4-10$, Mean \pm SD 6.3 ± 2.1),
233 then, the PZQ group ($5-8$, Mean \pm SD 6.2 ± 1.3). When each group was compared to the
234 control group the difference was statistically insignificant (P-value 1 and 1) except for the
235 AG+PZQ group (P-value 0.001). While comparing the AG group with the AG+PZQ

236 group the difference is statistically significant (P-value <0.001) and statistically
 237 insignificant when compared with the PZQ group (P-value 1). On the other side, the
 238 difference between the PZQ and the AG+PZQ groups was statistically significant (P-
 239 value 0.003), Table 3.

240 **Table 3. Oogram pattern of AG, PZQ and combined AG+PZQ therapeutic regimens**
 241 **after treatment of infected mice during the immature infection stage.**

Group		Control	AG	AG+PZQ	PZQ	P value between all groups
Immature eggs	Mean \pm SD	51.1 \pm 4.6	54.9 \pm 6.4	45 \pm 7.1	51.8 \pm 1.8	0.015
	(range)	(42–55)	(45–65)	(35–55)	(50–54)	
	P versus control		1	0.320	1	
	P vs AG			0.009*	1	
	P vs AG+PZQ				0.306	
Mature eggs	Mean \pm SD	42.6 \pm 3.2	40 \pm 6.7	40.4 \pm 4.9	42 \pm 2.1	0.715
	(range)	(40–48)	(30–50)	(33–45)	(40–45)	

	P versus control		1	1	1	
	P vs AG			1	1	
	P vs AG+PZQ				1	
Dead eggs	Mean \pm SD	6.3 \pm 2.1	5.1 \pm 0.9	14.6 \pm 6.8	6.2 \pm 1.3	<0.001
	(range)	4–10	4–7	5–25	5–8	
	P versus control		1	0.001*	1	
	P vs AG			<0.001*	1	
	P vs AG+PZQ				0.003*	

242 AG: Arabic gum.

243 PZQ: Praziquantel.

244 The histopathological assessment of the granuloma diameter revealed that the smallest
 245 diameter belonged to the AG+PZQ (214.23 \pm 12.18), followed by the PZQ group
 246 (272.22 \pm 11.2), then the AG group (297.28 \pm 7.5) and the largest diameter belonged to the
 247 control group (353.15 \pm 12.4). Comparing each group with the control group, the
 248 difference was significant (P-value 0.0010, 0.0010 & 0.00010 for the AG, PZQ and
 249 AG+PZQ groups respectively).

250 On the other side, the AG+PZQ group demonstrated the lowest granuloma number
251 (3.32±1.21), followed by the AG group (3.9±1.13), then the PZQ group (5.4±1.82), and
252 the control group presented the highest granuloma number (10.62±1.97). Comparing each
253 group with the control group, both the AG and AG+PZQ showed a significant difference
254 (P-value 0.0064&0.0064 respectively) while the difference between the PZQ group and
255 the control group was insignificant (P-value 0.09).

256 While results of the granuloma type revealed that the AG group had the highest cellular
257 and the least fibro-cellular and fibrous types among all groups (80%,20% &0%),
258 followed by AG+PZQ group (65%, 30%&5%) and the last in order was the PZQ group
259 (55%,43%&2%). Only AG and AG+PZQ had significantly different granuloma types as
260 compared to the control group (P-value <0.001&0.022 respectively), while the types
261 distribution in the PZQ group was insignificantly different than that of the control group
262 (P-value 0.247).

263 The State of *S. mansoni* eggs demonstrated a different pattern as the lowest number of
264 intact eggs and the highest number of degenerated eggs was detected in the AG group (17
265 &83 respectively), while the AG+PZQ group had (23) intact eggs and (77) degenerated
266 eggs, and the PZQ group had (45) intact eggs and (55) degenerated eggs. Comparing each
267 group with the control group, the difference was significant (P-value <0.001). Table 4
268 and Fig 1.

269 **Table 4. Effect of AG, PZQ and combined AG+PZQ treatment regimens on**
270 **Schistosoma mansoni induced hepatic granulomas parameters as compared with the**
271 **control group**

	=0.0001*	0.0083*	0.022*	<0.001*
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272 AG; Arabic gum.

273 PZQ; Praziquantel.

274 **Fig 1.:** Liver histology at six weeks after *S. mansoni* infection of CD1 bred mice with 80
275 cercariae by subcutaneous injection (hematoxylin & eosin stain: 100x magnification)

276 1-A:liver infected sections non treated control mice groups 6 weeks p.i. showing large
277 number of fibrocellular granulomas stained with H&E (x100)

278 1-B:liver infected sections non treated control mice groups 6 weeks p.i. showing large
279 number of fibrocellular granulomas stained with masson trichrome stain (x100)

280 1-C: Liver of infected mice group treated PZQ
281 showing less number of fibrocellular granuloma

282 1-D: Liver of infected mice group treated PZQ showing decrease in granuloma size
283 showing small fibrous granuloma

284 1-E: Liver of infected mice group treated with AG and PZQ showing less number of
285 cellular granuloma

286 1-F: Liver of infected mice group treated with AG and PZQ showing decrease in the
287 granuloma size showing small granuloma with degenerated eggs

288 1-G: Liver of infected mice group treated with AG showing decrease in size of
289 granulomas

290 1-H: Liver of infected mice group treated with AG showing decrease in size of
291 granulomas degenerated eggs

292

293

294 **Discussion**

295 Schistosomiasis control programs are based mainly on a single drug which is
296 praziquantel tablet [40]. Despite the fact that patients could tolerate PZQ well, it has
297 some drawbacks including the emergence of drug resistance [4, 5], the poor efficacy on
298 the immature stages [41], the large, bitter tablets, and the unavailability of a pediatric
299 formula [42]. Recently, natural products and natural product-derived compounds have
300 been popular and attracted most of the attention as it could offer new effective therapy
301 against schistosomiasis. Arabic gum (AG) is an edible, dried sticky exudate from *Acacia*
302 *Senegal*, which is rich in soluble dietary fiber [43].

303 In this study assessment of AG antischistosomal properties revealed an excellent
304 statistically significant effect against immature murine schistosomiasis, both alone and in
305 combination with PZQ demonstrated in parasitological parameters; worm load, egg
306 count, oogram pattern and histopathological results; granuloma metrics (diameter,
307 number. and state of *Schistosoma* eggs within them).

308 In all parasitological parameters, apart from the worm load, AG+PZQ treated
309 animals showed the best results as compared to monotherapy groups, denoting a
310 considerable synergistic effect of AG+PZQ on both female fecundity, egg maturation and
311 ability to elicit its immunopathological effect. The best reduction rate of *Schistosoma*
312 worms was demonstrated in the AG monotherapy group, nevertheless, the difference

313 between AG and AG+PZQ treated mice worm load was negligible. On the contrary, the
314 PZQ treated mice demonstrated the worst results among all studied groups regarding total
315 worm load, however, such results were expected as PZQ is less effective against
316 immature schistosomiasis, the stage targeted in this experiment.

317 Regarding the histopathological parameters, the AG+PZQ group showed the least mean
318 granuloma diameter, while the largest diameter was demonstrated in the AG group. This
319 could be explained by the fact that the granuloma of that group is the highest cellular, the
320 least fibrocellular and fibrous granuloma types, lacking adequate fibers amount
321 diminishes its contraction and permits large sizes. Another explanation is based on the
322 highly significant difference in *S. mansoni* intact -degenerated eggs distribution within
323 the examined granulomas, as the cellularity that dominated granulomas of AG treated
324 animals might eliminate the physical barriers which would be created by fibrous tissue
325 and hampers the action of the host immune system. Concerning the mean granuloma
326 number, AG was significantly effective, both alone and in combination with PZQ,
327 followed by the combination of AG+PZQ and the least effect belonged to the PZQ
328 monotherapy. These results could be attributed to the destructive effect of AG on
329 fecundity which in turn decreases the number of evolving granulomas.

330 The AG therapeutic effect on immature murine schistosomiasis in this experiment could
331 be attributed to its immunomodulatory effect, as it stimulates the dendritic cells [14]
332 which are antigen-presenting cells responsible for triggering both innate and adaptive
333 immunity [44].

334 Also, it might be attributed to the antioxidant properties of AG in many tissues
335 like renal tissue [16], RBCs in sickle cell anemia (SCA) disease [19] and hepatic tissue as

336 mentioned by Ahmed *et al.*, [45] who stated that AG significantly decreased the level of
337 hepatic enzymes, lipid peroxidation, antioxidant enzymes as well as the expression of
338 oxidative stress genes. Activities of superoxide dismutase (SOD), catalase (CAT) and
339 glutathione peroxidase (GPx), which may contribute to the alleviation of *Schistosoma*
340 *mansoni* infection consequences similar to what has been reported in many other
341 antioxidants like gold nanoparticles *Ceratonia siliqua* pod extract [46], limonin [47].
342 Another theoretically potential mechanism of AG action relies on the fact that its
343 administration enhances butyric acid production in the bowel and hence rising its serum
344 concentration [13].

345 Butyric acid is a short chain fatty acid (SCFA) that synthesized via the
346 fermentation of otherwise non-digestible fiber by bacteria in the colon [48]. It has four
347 actions; first, its raises IL 10 serum level [14, 49], second, it increases serum levels of IL-
348 1 receptor antagonist (IL-1RA), third, it suppresses synthesis of transforming growth
349 factor (TGF- β 1) [13] and fourth, it fosters the expression of fetal hemoglobin in
350 erythrocytes [26]. Each of the aforementioned actions has a direct effect on
351 schistosomiasis infection outcome; IL10 regulates not just the intensity of egg-induced
352 inflammatory responses, but also the coherence of granuloma structure, particularly
353 deposition of collagen by fibroblasts around the periphery [50]. It also downregulates B7
354 MHC II, costimulatory molecules on APC [51], leading to hyporesponsive state through
355 induction of T cells energy [52]. IL-1RA was reported before to cause *in vivo* depletion
356 of exacerbated granuloma size and augmented regional cytokine production [53].

357 The effect of both IL 10 & IL-1RA might be manifested in this experiment in decreased
358 granuloma diameters, fibrosis, increased cellularity and deteriorated *Schistosoma* eggs
359 status inside the lesions.

360 Transforming growth factor (TGF- β 1) is one of the strongest factors that lead to
361 liver fibrosis. TGF- β 1 promotes hepatic stellate cell (HSC) proliferation and collagen
362 synthesis in the activated HSC [54] or modulates deposition of extracellular matrix
363 (ECM) components and immune functions [55]. Furthermore, a number of researchers
364 have recognized TGF- β 1 inhibition as one of the factors that can be used to evaluate the
365 antifibrotic effects of drugs on hosts infected with *Schistosoma japonicum* [56].
366 Consequently, possible suppression of TGF- β 1 by AG could reverse the
367 immunopathologic effect induced by *Schistosoma* eggs in the affected tissues as seen in
368 in the current study.

369 Blood-feeding parasites, including schistosomes, hookworms, and malaria
370 parasites, make use of aspartic proteases to produce initial or early cleavages in ingested
371 host hemoglobin. Although phylogenetically distinct, these parasites all have the same
372 food source; they are obligate blood feeders, or hematophagous. Hb from ingested or
373 parasitized erythrocytes is their major source of exogenous amino acids for growth,
374 development, and reproduction; the Hb, a 64-kDa tetrameric polypeptide, is broadly
375 catabolized by parasite enzymes to free amino acids or small peptides [57].

376 The fact that fetal hemoglobin has been shown to slowdown hemoglobin
377 degradation depriving *Schistosoma* worms of its food source [58], has inspired many
378 researchers to evaluate the effect of increasing its production on murine malaria

379 parasitemia [26], they reported that the administration of Arabic gum significantly
380 decreased the parasitaemia and extended the lifespan of infected mice.

381 The present study demonstrated that AG was highly effective against the immature form
382 of *S. mansoni* which resists PZQ, and using both agents together yielded the best results
383 owing to their synergetic effect.

384 To recapitulate, the study in hands shed the light on a novel and “green”
385 management approach of *Schistosomiasis mansoni*, being one of the safest dietary fibers,
386 and perceptibly effective in treating immature forms which entails the abortion of
387 reinfection in endemic areas. Further studies are on a larger scale are required to evaluate
388 the feasibility of using AG as an effective treatment of immature schistosomiasis *mansoni*
389 and for prophylaxis against reinfection, particularly in endemic areas where the control
390 programs are continually hampered by many socioeconomic, topographic and cultural
391 obstacles that are not currently anticipated to be defeated in the near future.

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